

“Enhancing Buffalo Production for Food and Economy”



Proceedings of International Buffalo Symposium 2017

November 15-18
Chitwan, Nepal



Faculty of Animal Science, Veterinary Science and Fisheries
Agriculture and Forestry University
Chitwan, Nepal



MICHIGAN STATE
UNIVERSITY



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Prof. Manaraj Kolachhapati, PhD	Registrar, Agriculture and Forestry University, Chitwan, Nepal
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Dr. Bimal Kumar Nirmal	Director General, Department of Livestock Services, Nepal
Prof. Nanda P. Joshi, PhD	Michigan State University, USA
Prof. Naba Raj Devkota, PhD	Director, Directorate of Research & Extension, Agriculture and Forestry University, Chitwan, Nepal

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Faculty of Animal Science, Veterinary Science and Fisheries
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Chitwan, Nepal

November, 2017



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New Baneshwor, Kathmandu

November 15, 2017

Ref No- 205/074/075

Message

It is my pleasure to write few words in the "Proceedings" published on the eve of International Buffalo Symposium which is going to be held on November 15 to 18, 2017. I want to congratulate the Organizing Chair, Organizing Secretary and Committee members for their untiring efforts to bring this Proceedings in final shape.

Agriculture and Forestry University (AFU), since its establishment in 2010 has been gradually developing its functions of teaching, research and extension. This symposium acts as the platform for networking the scientists working on various aspects of buffaloes. It explores the regional networking and multidisciplinary collaborative work to improve breeding and reproduction, forage production and nutrition, health and socioeconomic condition of the buffaloes to assure nutritional and food security for the smallholder buffalo farmers.

The research outcome presented by internationally renowned academicians and eminent scientists are highly valuable and believe that these findings will be the guidelines for developing strategic plan for the establishment of Buffalo research hub in Chitwan. The journey of academic and research excellence at AFU has been possible due to our outstanding faculties and students with international partners. I am proud to say that world's most renowned academicians and scientists collaborating with us turn this vision in to reality.

The Government of Nepal has planned to make the country self-reliant in milk by the next two years. Effective strategies should be focused on genetic studies and improvement of buffaloes, feed resource development, reproductive biotechnology, and management of infertility and mastitis problems. The collaborative research and capacity building program through consultations with our University partners will help establishing a buffalo research hub, a Centre of Excellence for multi-disciplinary buffalo research in the AFU, Rampur, Chitwan, Nepal.

On the behalf of the AFU, I would like to thank Michigan State University (MSU) for initiation and encouragement to organize this symposium. I would like to extend my sincere thanks to Dr. Nanda Prakash Joshi for his continuous support to strengthen AFU. I also would like to take the opportunity to inform you that USAID's initiation and continuous support has been instrumental in the development of this university. AFU has been one of the implementing partners for some USAID's supported Feed the Future (FTF) initiatives, such as, Innovation Labs and AVDRRC project. Dr. Adesogan, Adegbola Tolulope from University of Florida is highly acknowledged for supporting this symposium and capacity development programs at AFU.

I hope, this proceedings will eventually serve as reference guidelines to the faculty members, scientists, undergraduate and post graduate students of the universities, policy makers and other stakeholders. The success of such initiative would certainly upgrade quality research and academic standard of the university. I understand that proceedings of this symposium will be kept on AFU website and in print. This will ensure that the informed deliberations at this gathering will reach a global audience and facilitate the enquiry into the important issues on buffaloes.

Prof. Ishwari Prasad Dhakal, PhD

Vice Chancellor

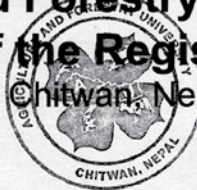
Vice Chancellor

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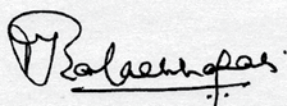
MESSAGE FROM THE REGISTRAR

It gives me an immense pleasure to write few words for the “**Proceedings of International Buffalo Symposium 2017**”. This symposium is being held from 15th to 18th November , 2017 in the beautiful city, Sauraha, Chitwan with the theme “Enhancing Buffalo Production for Food and Economy”.

I would like to take this opportunity to congratulate the organizing team of International Buffalo Symposium 2017 (IBS2017) for successfully publishing this "Proceedings of International Buffalo Symposium 2017 " including the information of symposium, technical papers, abstracts and many more.

Buffalo farming plays a significant role in the national economy of Nepal as a major producer of milk and meat in the country. I believe that the technical and scientific papers published in this proceeding will provide valuable information to the policy makers, researchers, academicians, development workers, entrepreneurs and those who are directly or indirectly involved for the development of this commodity by taking appropriate strategies and interventions to enhance buffalo production and productivity, globally.

Finally I'd like to express my heartfelt gratitude and sincere appreciation to all the co-organizers, supporters, keynote speakers, oral and poster presenters from different countries, local and national media representatives, and many other seen and unseen supporting hands for their continuous effort to make this event historically successful.



Prof. Mana Raj Kolachhapati, PhD
Registrar

November 15, 2017



WELCOME TO IBS2017

Respectable chief guest, special guests, distinguished Nepalese and foreign delegates, participant scientists and media representatives!

I am pleased to welcome you all here on behalf of the Organizing Committee of International Buffalo Symposium 2017 (IBS2017). I want to extend my sincere felicitation and wish you for a contributing and most memorable time in the IBS2017, and the beautiful touristic hub of the region, Sauraha!

Agriculture and Forestry University (AFU) is the first state-owned technical University in Nepal. It was established in 2010 by the Act of Government of Nepal. AFU envisions being a preeminent university for making Nepal into food-secure, economically vibrant, environmentally sustainable and socially equitable nation. We are committed to improving quality of life for the people of Nepal through agriculture including livestock and fisheries, and forestry education, research and extension.

AFU, Faculty of Animal Science, Veterinary Science and Fisheries in its history of establishment, has been proudly involving as an organizer of this International Symposium whereas the co-organizers are- Department of Livestock Services, Ministry of Livestock Development; Nepal Agriculture Research Council; and Michigan State University, USA. This symposium is also supported by Feed the Future Innovation Lab (USAID) of University of Florida and other several organizations across the globe.

The theme of symposium is "Enhancing Buffalo Production for Food and Economy". It is devoted to five thematic areas such as Policy and Socioeconomic; Genomics and Biotechnology; Nutrition and Production management; Buffalo Reproduction and Breeding, and Buffalo Health management.

There are 14 keynote addresses; 34 oral presentation and 35 posters display. It is my immense pleasure to state that about two hundred participants are taking part in the symposium including foreign delegates from 11 countries including USA, India, Pakistan, China, Bangladesh, Thailand, Philippines, Italy, UK and Canada.

I strongly believe that next four days of deliberation, interaction and discussion on the different aspects of buffalo rearing during the Symposium will bring concrete suggestions and recommendation related to policy formulation and broadly cover the pertinent areas for future research on buffalo production and management, breeding, health and other pertinent and allied issues. I hope the symposium will be a valuable and stimulating experience to you all. I also hope that you will get a chance to enjoy the natural beauty and wildlife of Sauraha where the Symposium is being held.

I sincerely acknowledge the contribution of the co-organizers and supporters. Finally, I would like to take this opportunity to thank and congratulate the organizing secretary, sub-committee chairs, members, colleagues and faculties who have put in tireless effort into preparation for this symposium, to make it a grand success. This would not have been possible without your hard work.

Once again I extend my warm welcome to all of you. Thank You !!

Sharada Thapaliya
IBS2017 Organizing Chair



EDITORIAL

Welcome to Chitwan, and the International Buffalo Symposium 2017 (IBS2017). The IBS2017 has been organized from November 15-18, 2017 by Agriculture and Forestry University, in collaboration with Ministry of Livestock Development, Nepal Agriculture Research Council and Michigan State University, USA. The symposium is the first of its kind organized in Nepal. The Feed the Future Livestock Systems Innovation Lab, University of Florida, USA is supporting the symposium. The theme of the symposium is “Enhancing Buffalo Production for Food and Economy” and the overall objective is sharing and generating knowledge among national and international scientists working on this field. The mission of the symposium is developing AFU as a “Center of Excellence” for multi-dimensional buffalo research activities.

We are highly encouraged to see that the symposium has attracted many foreign professional dignitaries, scientists and participants from India, Pakistan, China, Bangladesh, Thailand, Philippines, Italy, UK, USA and Canada. Similarly, there are many Nepalese scientists and professionals participating in the symposium. We have four days program, first day for registration and inauguration, second and third full days for plenary sessions, and closing and the last day a post-symposium tour.

Altogether, we have 5 special papers and 14 keynotes, 34 oral and 35 poster presentations/papers in six different thematic areas of buffalo health, nutrition and management, reproduction and breeding, genomics and biotechnology, policies and socio-economics, and buffalo as a source of food and nutrition. The papers are all good quality papers selected through a thorough blind review process. The technical committee of the IBS2017 has been solely responsible for the review of the abstracts and full papers contained herein. The editorial team is responsible for editing and formatting of the papers.

The Editorial Committee would like to thank the advisors, chair and other members of the Organizing Committee for their valuable contribution. We also thank the authors for their great contribution with papers, the session chairs, the keynote lecturers, oral and poster presenters and the participant scientists/dignitaries of the symposium. We are indebted to the reviewers for valuable evaluation and contribution to enhance the quality of the papers. We offer our special thanks to our co-organizers and supporters. We duly acknowledge Heifer International Nepal for supporting the proceedings publication. The contribution of Mr. Niranjana Karki, Ms. Indira Dhungana, Dr. Deepak Lamsal, Dr. Shatrughan Shah, Mr. Manoj Tamang and Ms. Sabina Tiwari Ghimire is highly appreciated who helped the editorial board while formatting the papers. Finally, we also thank all that by service minded efforts and specific skills have made this publication possible.

Editorial Board

15th November, 2017



SYMPOSIUM INFORMATION

A. General Information

Location:

The symposium will be held at Hotel Jungle Crown, Sauraha, Chitwan, Nepal. There are frequent flights (taking 20 minutes) from Kathmandu to Chitwan (Bharatpur). By road, it takes about 4 hours' drive from Kathmandu to Bharatpur. The distance between Bharatpur airport and symposium venue Sauraha is about 15 km.

Sauraha is a small village in the Chitwan Valley that has emerged from a Tharu village of mud and daub huts and houses, with a half dozen mud and daub hotels, into a small quiet town full of western style hotels and resorts, restaurants, internet cafes, and gift shops. The village is the eastern gateway to the nearby and large Chitwan National Park, famous for Jungle/Wildlife safari. Sauraha is also considered as Buffalo Hub in Chitwan. The distance between Sauraha and Agriculture and Forestry University, Rampur is 31 km.

Schedule of Events:

The on-site registration, inaugural session and reception dinner will be on Wednesday, November 15, plenary sessions in different thematic areas will be held on November 16 and 17 followed by closing session on November 17 evening. Post symposium tour is scheduled for November 18 Morning.

Registration Hours

On-site registration desk will be located in the Hotel Jungle Crown, Sauraha, Chitwan that opens from 10.00 am to 6.00 pm on November 15.

Important Contacts:

Overall: Bhuminand Devkota (9855054487)

Domestic flights and accommodation: Rebanta Bhattarai (9855081572), Hotel Jungle Crown (9855065023)

Technical matters: Manoj Kumar Sah (9845053569), Nirajan Bhattarai (9855060704), Gokarna Gautam (9855064941), Himal Luitel (9855051156)

Local transportation: Anil Kumar Tiwari (9855057430), Dawa Tshiring Tamang (9855059484)

Media Room

A media room will be available throughout the meeting to provide a space for media representatives to work. Meeting press releases will be available there. Krishi TV channel will live broadcast the major part of the event.

Hospitality Lounge

The hospitality lounge will be located in the Hotel Jungle Crown. Lounge will offer attendees an area to relax, network, and catch up with friends.

Side-line Meeting

Side-line meetings will be organized in the meeting room of the Bodega Wine Bar of Hotel Jungle Crown between the Agriculture and Forestry University Authorities, Michigan State University Authorities, other University Authorities, Head of the Professional Bodies, Visiting Scientists, Guests and Contributors.

Camera, Video Camera, and Cell Phone Policy

Use of cameras, video cameras, tablets, or smartphones (For calls or audio/video recording) is prohibited during oral and poster presentations to minimize disruption and unauthorized dissemination of data. The symposium sessions will be recorded and uploaded in the symposium website by the Organizing Committee.



B. Program Schedule

Day 1: November 15, 2017, Wednesday

Registration, Inaugural Session and Reception Dinner

10.00-18.00	Registration
15.00-18.00	Inaugural Session
Master of Ceremony : Bhuminand Devkota, Organizing Secretary	
15.00 – 15.20	Session chairing by Prof Dr Sharada Thapaliya, Organizing Chair Formal reception to the Chief Guest Hon'ble Vice Chancellor of the University Prof Dr IP Dhakal and Special Guests National anthem Welcome by the Chair Inauguration by the Chief Guest
15.20-16.00	Special papers IBS2017: Objectives and highlights by Bhuminand Devkota, organizing secretary and coordinator, Post Graduate Studies Center, Agriculture and Forestry University IBS2017: Partnerships and way forward by Nanda P Joshi, advisor of the IBS2017 and director of International Programs, Department of Animal Science, Michigan State University Strategy to enhance the production and productivity of buffaloes for the growth of livestock gross domestic production (LGDP) of Nepal by Bimal K Nirmal, advisor of the IBS2017 and Director General of Directorate of Livestock Services, MoLD Value chain analysis and dairy development in Nepal by Satish Joshi, Professor of Michigan State University Livestock ownership, animal source foods consumption, and maternal and child nutrition outcomes in Nepal: findings from the 2014 PoSHAN community survey by Robin Shrestha, Feed the Future Innovation Lab for Nutrition, Tufts University
16.00-17.30	Address to the symposium Dr Inderjeet Singh, Organizing Secretary of 9 th ABC and President of Asian Buffalo Association Prof Dr Antonio Borghese, General Secretary of International Buffalo Federation V Padmakumar, Regional Coordinator (Asia), Feed the Future Innovation Lab for Livestock Systems Prof Dr Naba Raj Devkota, Advisor of the IBS2017 and Director, DOREX of the University Dr Purna Bahadur Chhetri, Senior Agriculture Specialist, World Bank Nepal Prof Dr Nasim Ahmad, Pro-Vice Chancellor of UVAS, Lahore, Pakistan Dr Baidhya Nath Mahato, Advisor of the IBS2017 and Executive Director, Nepal Agricultural Research Council Prakash Mathema, Secretary, Ministry of Livestock Development, Nepal Dr Yubak Dhoj GC, Secretary, Ministry of Forest, Nepal Dr Suroj Pokharel, Secretary, Ministry of Agricultural Development, Nepal Honoring the Guests and Speakers by the Chief Guest
17.30-18.00	Address by the Chief Guest Honoring the Chief Guest by the Chair Vote of thanks and closing by the Advisor of the IBS2017 and Registrar of the University Prof Dr Mana Raj Kolachhapati
18.00-20.00	Reception Dinner

Day 2: November 16, 2017, Thursday

Plenary Session I: Policies and Socio-economics of Buffalo Production

Chair: John Medendorp Moderator: Lok N Paudel

KN1	8.00-8.25	Current status and economics of buffalo production worldwide	Antonio Borghese, Italy
KN2	8.25-8.50	Animal welfare issues in dairy farming	Janice Swanson, USA
OP1	8.50-9.05	Mind the gap: awareness and practices among Nepali buffalo farmers related to zoonoses	Durga Devkota, Nepal
KN3	9.05-9.30	Buffalo production for household food security in Nepal	Drona Rasali, Canada
OP2	9.30-9.45	Role of buffalo for diversified enterprises development in Nepal	Raj K Adhikari, Nepal
OP3	9.45-10.00	The economic losses and burden of endoparasites in buffalo and cattles of rural punjab "an underestimated emerging threat"	Muhammad Hassan Mushtaq, Pakistan

10.00-10.10 Coffee Break

Plenary Session II: Genomics and Biotechnology for Buffalo Production

Chair: Bianca Moiola Moderator: Madhav P acharya

KN4	10.10-10.35	Developments and prospects of advance reproductive biotechnologies in buffalo production	Danilda Hufana-Duran, Philippines
KN5	10.35-11.00	Buffalo reproductive biotechnologies: the current status in China	Yangqing Lu, China
KN6	11.00-11.25	Genetic studies in water buffalo	Shujun Zhang, China



KN7	11.25-11.50	Understanding the fertilising ability of buffalo oocytes & spermatozoa in functional genomics prospective	Tirtha Kumar Datta, India
OP4	11.50-12.05	Assessment of X chromosome genetic variation for population genetic analyses of domesticated water buffaloes in Nepal	S. Pérez-Espona/D. Chhetri, UK/Nepal

12.05-13.05 Poster Session (PP1 to PP16)

13.05-14.00 Lunch Break

Plenary Session III: Genomics and Biotechnology for Buffalo Production

Chair: Shujun Zhang Moderator: Doj R Khanal/Saroj Sapkota

KN8	14.00-14.25	Genetic improvement of dairy buffalo, constraints and perspectives. The Italian example	Bianca Moioli, Italy
OP5	14.25-14.40	Function and mechanism of MAP4K4 gene on milk traits and scs between dairy cow and buffalo	Dinesh Bhattarai, China
OP6	14.40-14.55	Authentication of buffalo meat using mitochondrial cytochrome-b gene	Neena Amatya Gorkhali, Nepal
OP7	14.55-15.10	Inhibin-alpha gene silencing on granulosa cells led oocyte development in transgenic mice model	IP Kadariya, Nepal

15:10-15.20 Coffee Break

Plenary Session IV: Buffalo Nutrition and Production

Chair: Nanda P Joshi Moderator: Netra P Osti

KN9	15.20-15.45	Enhancing buffalo production based on feed resources development and innovations	Metha Wanapat, Thailand
OP8	15.45-16.00	Effect of different silage on milk production of indigenous buffalo in western hills of Nepal	MK Shah, Nepal
OP9	16.00-16.15	Effect of challenge feeding during pregnancy on the production and reproduction	SS Hiremath, India
OP10	16.15-16.30	"Weaning induced stress alters immune and endocrine functions in buffalo-calves"	Anas Sarwar Qureshi, Pakistan
OP11	16.30-16.45	Buffalo production and management at National Buffalo Research Program, Nepal	Mohammad Shah Navaz Ali, Nepal
OP12	16.45-17.00	Comparative hoof volume assessment of Nili-Ravi buffalo being reared on different flooring systems	Hafsa Zaneb, Pakistan
OP13	17.00-17.15	Morphological and productive traits of terai buffaloes of eastern Nepal	Saroj Sapkota, Nepal
OP14	17.15-17.30	Effect of Non-genetic Factors on Milk Production Traits of Indigenous Buffaloes (Bubalus bubalis L.) in the Western Hills of Nepal	Nirajan Bhattarai, Nepal
OP15	17.30-17.45	Risk factors, assessment and outcome of failure of passive transfer of immunity in buffalo calves	ST Singh, India

Day 3 (November 17, 2017) (Mangsir 1)

Plenary Session V: Buffalo Reproduction and Breeding

Chair: Inderjeet Singh Moderator: Bhola Shankar Shrestha

KN10	8.00-8.25	Improvement in reproduction through synchronization and resynchronization studies in dairy buffaloes	Nasim Ahmad, Pakistan
OP16	8.25-8.40	Characterization and treatment of infertility in buffaloes of hills and terai region of mid Nepal	Gokarna Gautama, Nepal
OP17	8.40-8.55	Reproductive performances of water buffaloes in selective areas of bangladesh	Nasrin Sultana Juyena, Bangladesh
KN11	8.55-9.20	Update on attempts of developing breeding strategy of Nepalese buffaloes (Bubalus bubalis) during low breeding season	Bhuminand Devkota, Nepal
OP18	9.20-9.35	Timed artificial insemination (TAI) in bangladeshi buffaloes during the off breeding season	Mohammad Harun-Or-Rashid, Bangladesh

9.35-9.45 Coffee Break

Plenary Session VI: Buffalo Reproduction and Breeding

Chair: Nasim Ahmad Moderator: Shambhu Raj Pandey

KN12	9.45-10.10	Fourier harmonic analysis as tool in predicting bull fertility for improved buffalo breeding	Peregrino G Duran, Philippines
OP19	10.10-10.25	Effects of dietary supplementation of feed with omega-3 fatty acids on the freezeability of Nili-Ravi buffalo (Bubalus bubalis) bull semen	Muhammad Zubair, Pakistan
OP20	10.25-10.40	Age related changes in body weight, orchidometry and testicular dynamics in azikheli buffalo bulls raised in swat khyberpukhtunkhwa, Pakistan	Saima Masood, Pakistan
OP21	10.40-10.55	Evaluation of soya lecithin based extender as a substitute of tris-egg yolk extender for the cryopreservation of Nili-Ravi buffalo bull semen	Ijaz Ahmad, Pakistan
OP22	10.55-11.10	Clinical outcome of correction of uterine torsion in pluriparous buffaloes at field level initially treated by non-veterinarian	DK Singh, Nepal



- OP23 11.10-11.25 Breed improvement through intensive local selection of indigenous buffaloes for food & nutritional security and livelihood improvement in mid-hills of Nepal Lok Nath Paudel, Nepal
- 11.25-12.50 Poster Session (PP17 to PP36)**
12.50-13.40 Lunch Break

Plenary Session VII: Buffalo Health

Chair: Daniel Grooms Moderator: Krishna Kafle

- KN13 13.40-14.05 Buffalo mastitis in dairy production: technological advances in understanding of mastitis Neelesh Sharma, India
- OP24 14.05-14.20 Evaluation of mastitis related measures and their application to classify buffalo milk in Chitwan, Nepal Ishwari Prasad Dhakal, Nepal
- OP25 14.20-14.35 Morphometry of udder, teat, and milk vein in Nili-Ravi buffalo (*Bubalis bubalis*) and it's relationship with milk yield Mirza Muhammad Usman, Pakistan
- OP26 14.35-14.50 Udder morphometric observations in murrhah and Nili-Ravi buffalo breeds R.S. Singh, India
- OP27 14.50-15.05 Latent bacterial infections in buffaloes: earlier diagnosis through acute phase proteins (APPs) Shafia Tehseen Gul, Pakistan

15.05-15.15 Coffee Break

Plenary Session VIII: Buffalo Health

Chair: Melinda J Wilkins Moderator: Swoyam P Shrestha

- KN14 15.15-15.40 Adapting technologies to improve buffalo health and productivity Daniel Grooms, USA
- OP28 15.40-15.55 Serological and molecular studies of Brucellosis in buffaloes of Punjab, Pakistan Ahrar Khan, Pakistan
- OP29 15.55-16.10 Hemato-biochemical alterations in tuberculin reactor water buffaloes in Pakistan Imtiaz Ahmad Khan, Pakistan
- OP30 16.10-16.25 Pathological investigation of foot and mouth disease in buffaloes (*Bubalus bubalis*) in southern areas of Punjab province, Pakistan Riaz Hussain, Pakistan
- OP31 16.25-16.40 Clinico-hematological and oxidative status of Nilli-Ravi Buffaloes infected with Trypanosomiasis Riaz Hussain, Pakistan
- OP32 16.40-16.55 In vitro and in vivo evaluation of traditional medicinal plants against *Rhipicephalus microplus* (Asian cattle tick) Muhammad Arfan Zaman, Pakistan
- OP33 16.55-17.10 Tick borne diseases exploiting the buffaloes (*Bubalus bubalis*) of Khyber Pakhtunkhwa, Pakistan Said Sajjad Ali Shah, Pakistan
- OP34 17.10-17.25 Impact of climate change on epidemiology of tick-borne haemoparasites in Azakheli buffalo in district Swat, Pakistan Amjad Khan, Pakistan

17.25-17.40 Coffee Break

Closing Session: 17.40 - 18.40

Day 4 (November 18, 2017) (Mangsir 2)

Post-symposium Tour (8.00-12.00): Visit Agriculture and Forestry University, Rampur, Chitwan

Poster Sessions

Evaluation Chair: Janice Swanson, Members: Metha Wanapat, Nanda P Joshi, Drona P Rasali, Tirtha K Datta, Ahrar Khan, Danilda Hufana-Duran

Poster Session I (November 16, 2017)

- PP1 Milk yield response of bypass protein during dry season on smallholder dairy animals in the hills of Nepal Netra P Osti, Nepal
- PP2 Effect of medicated and non-medicated urea molasses multi-nutrient block (UMMB) on milk production, milk composition and gastro-intestinal parasites in buffalo Bijay Khanal, Nepal
- PP3 Use of feeding support tool for enhancing dairy animal productivity Bholu S Shrestha, Nepal
- PP4 Husbandry practice and reproductive performance of buffaloes in Chitwan and kaski districts of Nepal Prakash Adhikari, AFU, Nepal
- PP5 Can music influence milking behavior and yield in buffalo cows? : a review Sanjay Dhungana, Nepal
- PP6 Effect of parity on lactational efficiency of murrhah buffaloes (*Bubalus bubalis*) in central Nepal Dikshit Poudel, Nepal
- PP7 Effect of non-genetic factors on lactational efficiency of indigenous buffaloes at different stage of parity in western hills of Nepal Surya P Sharma, Nepal
- PP8 Status of buffalo (*Bubalus bubalis*) production in nepal: a review Ganesh Gupta, Nepal
- PP9 Performance of murrhah buffalo, a case of Kaski district of Nepal Uddhav Paneru, Nepal
- PP10 Seasonal variation in fat and SNF contents in the milk of murrhah buffaloes in mid-western region of Nepal Jib Raj Poudel, Nepal
- PP11 *Leucaena leucocephala*: a promising forage for improving swamp buffaloes production in Thailand Thongsuk Jetana, Thailand
- PP12 Utilization of rain tree pods (*samanea saman*) as a swamp buffalo feed in Thailand Thongsuk Jetana, Thailand



PP13	Knowledge attitude and practice (KAP) analysis on nutrition, breeding and health management of small holders buffalo farmers around gunjanagar dairy plant, Chitwan Nepal	Sweta Ghimire, Nepal
PP14	Shortening First Calving Age in Buffalo Through Feeding Management	Suman Karki, Nepal
PP15	Arna cross breed: potentiality and scope in Koshitappu region Nepal	Bhojan Dhakal, Nepal
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PP21	Re-assumption of Ovarian Cyclicity to Induce Pregnancy in Anestrous Buffaloes Using CIDR Synchronization Protocol During Poor Breeding Season	Deepak Lamsal, Nepal
PP22	Evaluation of reproductive traits of Lime and Parkote (<i>Bubalus bubalis</i>) buffaloes in the western hills of Nepal	Pratima Bhandari, Nepal
PP23	Study on factors affecting novel hormonal protocols in anestrus buffaloes during active breeding season	Yagya Raj Pandeya, Nepal
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Note: We have a dedicated time block on November 16 and 17 to poster presentations. All the posters should be placed on the designated poster stand by 11.00 am on November 16 and removed by 5.00 pm on November 17. Each poster will be available for poster viewing for the entire period, with the presenting author in attendance during the poster time. Posters will be evaluated for "Best Poster Awards".



LIST OF ABBREVIATIONS

AFR	Adjusted Farrowing Rate
AFU	Agriculture and Forestry University
AGDP	Agriculture Gross Domestic Products
AHRD	Animal Health Research Division
AI	Artificial Insemination
AMY	Average Daily Milk Yield
ANASB	Italian Buffalo Breeders Association
ANOVA	Analysis of variance
ATP	Adenosine Triphosphate
AV	Artificial Vagina
BC	Bacterial Count
BCS	Body Condition Score
BQ	Black Quarter
C2	Acetate
C3	Propionate
C4	Butyrate
C5	Valerate
C6	Caproate
CH4	Methane
CI	Calving Interval
CIDR	Controlled Internal Drug Release
CL	Corpus Luteum
CMT	California Mastitis Test
CNS	Coagulase Negative Staphylococcus
DAM	Dairy Animal Management
DCP	Digestible Crude Protein
DF	Dominant Follicle
DHP	Di hydroxypyridine
DM	Dry Matter
DP	Dry Period
EC	Electrical Conductivity
ECG	Equine Chorionic Gonadotropin
EO	Essential Oils
ERCR	Estimated Relative Conception Rate
ET	Embryo Transfer
FHA	Fourier Harmonic Analysis
FISH	Fluorescence In Situ Hybridisation
FMD	Foot And Mouth Disease
FSH	Follicle Stimulating Hormone
FTAI	Fixed Timed Artificial Insemination
GDP	Gross Domestic Product
Ghgs	Greenhouse Gases
GnRH	Gonadotropin Releasing Hormone
HA	Harmonic Amplitude
HCG	Human Chorionic Gonadotropin
HS	HaemorrhagicSepticaemia
ICAR	International Committee For Animal Recording
ICAR	International Committee For Animal Recording
IU	International Unit
IVF	Invitro Fertilization
LAMP	Loop Mediated Isothermal Amplification
LH	Luteinizing Hormone
LL	Lactation Length
LN2	Liquid Nitrogen
LSM	Least Square Mean
MAP4K4	Mitogen Activated Protein Kinase KinaseKinaseKinase 4
MD	Microdrops
MOET	Multiple Ovulation And Embryo Transfer
N2O	Nitrous Oxide
NaOH	Sodium hydroxide
NARC	Nepal Agricultural Research Council
NIK	Nck interacting kinase
NPN	Non Protein Nitrogen (Urea)
OD	Optical density
OPU	Ovum Pick Up
P4	Progesterone
PCC	Philippine Carabao Center
PCR	Polymerase Chain Reaction
PCR	Polymerase chain reaction
PDs	Purine derivatives
PEG	Polyethylene glycol
PGD	Preimplantation Genetic Diagnosis
PGF2 α	Prostaglandin
PMSG	Pregnant Mare's Serum Gonadotropin
PMY	Peak Milk Yield
PPE	Post partum estrus
PR	Pregnancy Rates
PRID	Progesterone Releasing Intra Vaginal Devices
PSPB	Pregnancy Specific Protein B
RDP	Rumen degradable protein
ROP	Retention of placenta
RTP	Rain tree pods
SCNT	Somatic Cell Nuclear Transfer
SCS	Somatic Cell Score
SPSS	Statistical Package for Social Sciences
TAI	Timed Artificial Insemination
TB	Total Born
TDN	Total Digestible Nutrient
TROFREC	Tropical Feed Resources Research And Development Center
UD	Udder depth
UL	Udder length
UW	Udder width
VER	Vaginal Electrical Resistance
VFA	Volatile Fatty Acid
VTH	Veterinary Teaching Hospital
WOW	Well of the Wells
WTO	World Trade Organization



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INTERNATIONAL BUFFALO SYMPOSIUM 2017: OBJECTIVES AND HIGHLIGHTS

B. Devkota, Organizing Secretary

BACKGROUND AND OBJECTIVES

Buffalo is one of the important livestock species in several countries of South Asia, South-East Asia and Arabian, Mediterranean and Central and South American countries. Buffaloes are considered as the “black gold” that reflect their immense economic value whereas it is considered as one of the top most valuable livestock species in Nepal. There are about 5.2 million heads of buffaloes in Nepal that contributes greatly in national economy and animal source food supply system. Buffalo alone contributes about 67.7% and 57.4% to the total milk, and meat production in the country, respectively. There are 3 indigenous breeds of buffaloes in Nepal such as Lime, Parkote and Gaddi widely spread in the hills and valleys whereas, Terai and Inner Terai region is dominated by exotic breed Murrah and their crossbred population. Buffaloes being a major source of milk in the country, they are widely used for meat as well. However due to unplanned slaughtering and rampant use for meat, the emerging scenario of need is to conserve this species.

Conventionally, buffaloes were part of the subsistence farming system and little attention was given to buffalo research and development. However, realizing the huge potentiality of the buffalo to contribute in food and economy, particularly of the rural communities, last 3 decades has seen a dramatic focus on buffalo research and development of technology to enhance their productivity, conserve the genetic potentiality and address the welfare issues. In Nepal, this is well reflected in the government plans and policies as well as development projects related to the livestock. In research, cytogenetic study of indigenous buffaloes was started in Nepal about 20 years back aiming to conserve the genetic pool, and the universities and other research stations had started focusing on buffalo research to understand the genetic variation, enhance the production capacity and implement techniques to obtain economic production. However, there exists a huge gap between the food and economic value of this animal and the priority based research efforts. Therefore, establishment of a “Center of Excellence in Buffalo Research” and developing buffalo research hub in the country is of prime importance.

Agriculture and Forestry University (AFU) was established in 2010 as the first government technical university in the country. It envisions of being a pre-eminent university for building Nepal into a food secure, economically vibrant, environmentally sustainable and socially equitable nation. The university is committed to improving the quality of life of the people of Nepal through agriculture including livestock education, research and extension. AFU is playing a lead role in buffalo research. In the short span of time, AFU has established a wide network among national and international institutions to facilitate academic and research activities. Recently, we have accomplished an USAID-funded multi-institutional collaborative research project on- “Improving Nutrition and Productivity of Buffalo to Adapt to the Impacts of Climate Change in Nepal (2012-2015) in collaboration with Nepal Agricultural Research Council (NARC) and Directorate of Livestock Services (DLS), under the Ministry of Livestock Development (MoLD) that was led by Michigan State University (MSU) in the US. Similarly, its faculty members are actively involved in several other research projects on different facets of buffalo production as a lead researcher or a collaborative partner that are supported by national and international institutions. As research and extension, besides teaching are mandatory functions at AFU, Directorate of Research and Extension (DOREX) has been established in the university that provides fund as well as implements the research projects in the university. In livestock sector, DOREX has allocated significant number of projects including buffalo reproduction, nutrition, and health and genetics improvement. We have already achieved encouraging results in developing breeding strategy of Nepalese buffaloes during low breeding season. The impact of such kind of works would be important on addressing the problem of acute shortage of milk in the country during spring and early summer months. Thus, AFU in its research priority has considered buffalo as a lead livestock species realizing its economic value and the pool of researchers in the university working on buffalo. The university envisages itself as a buffalo research hub in the future.

Michigan State University is a strategic partner university of AFU. Since the establishment of AFU, MSU is contributing greatly in developing strategic planning document and strengthening research capacity in the university. In livestock sector, AFU and MSU have jointly identified buffalo as a major commodity to work together on its promotion by research for technology development, conservation of a diverse genetic pool and several other prospective for enhancing its productivity. A series of bilateral visits, meetings and discussions were held between the authorities of the two universities in recent years, as well as multi-lateral meetings were held between AFU, MoLD and NARC. This helped on further strengthening the relationship and partnership between the universities, government bodies and research bodies locally and globally to develop potential collaboration for buffalo research and development. Further considering its strength of a good pool of human and other resources, as well as its location in Inner Terai region where buffalo is the predominant livestock, the wider consideration



is that AFU is a unique institution that can lead in the different aspects of buffalo research. Further, considering geological and political scenario, Nepal can lead in developing buffalo production function in the days to come as its neighbors in the Asia and collaborators/partners in the US and Pacific region can well support to this paradigm of promoting buffalo production in overall. In this sense, AFU can take a lead role in creating buffalo research hub in the region that this is high time to realize and move forward upon this fact. Considering this fact, a joint meeting of AFU and MSU authorities in March, 2017 decided to organize an International Buffalo Symposium (IBS) in November 2017 in Chitwan. Thus, IBS2017 has been organized during November 15 to 18, 2017 with the lead role of AFU, in collaboration with MoLD, NARC and MSU. The symposium is the first of its kind, organized in Nepal. In the meanwhile, Feed the Future Livestock Systems Innovation Lab, University of Florida, USA, Heifer International Nepal and Nepal Feed Industry Association also joined hand in supporting the symposium. The theme of the symposium is “*Enhancing Buffalo Production for Food and Economy*” and the overall objective is sharing and generating knowledge on buffalo related research among national and international scientists and concerned stakeholders working in this field. The mission of the symposium is to develop AFU as a “Center of Excellence” for multi-dimensional buffalo research activities.

HIGHLIGHTS OF THE IBS2017

A. Program

The symposium is scheduled from November 15 to 18, 2017 and the venue is Hotel Jungle Crown, Sauraha, Chitwan. The outline of the program is as follows.

November 15: Registration, inaugural session and reception dinner

November 16 and 17: Plenary sessions (Total sessions 8 in 6 different thematic areas) followed by a brief closing session.

November 18: Post-symposium tour (AFU tour)

B. Thematic areas:

1. Buffalo Nutrition and Production
2. Buffalo Health
3. Buffalo Reproduction and Breeding
4. Genomics and Biotechnology for Buffalo Production
5. Policies and Socio-economics of Buffalo Production
6. Buffalo as a Source of Food and Nutrition

C. Number of papers

1. Special papers: 5
2. Keynotes: 14
3. Orals: 35
4. Posters: 36

D. Countries of the speakers/participants/guests

Nepal, India, Pakistan, China, Bangladesh, Thailand, Philippines, Italy, UK, USA, Canada

E. Foreign dignitary scientists

In the symposium, more than 50 foreign scientists are participating with papers or as a special guest participant. We have the Department Chairs with a team of 9 Professors from MSU. Similarly, we have the President of Asian Buffalo Association (Dr Inderjeet Singh, India), University Pro-Vice Chancellor (Prof Nasim Ahmad, Pakistan), Secretary of International Buffalo Federation (Dr Antonio Borghese, Italy), well recognized professors, scientists and researchers making an elite group of foreign scientists in the IBS2017.



F. Keynote Speakers

Name: Prof. Dr. Nasim Ahmad
 Country: Pakistan
 Position: Pro-Vice Chancellor, Ex Dean,
 UVAS, Lahore
 Speaker Category: Keynote
 (Reproduction)
 Topic: Improvement in reproduction
 through synchronization and
 resynchronization studies in dairy
 buffaloes



Name: Dr. Drona Prakash Rasali
 Country: Canada
 Position: Director, Population Health
 Surveillance & Epidemiology
 Population and Public Health
 Speaker Category: Keynote (Policies
 and Socio-economics)
 Topic: Buffalo production for household
 food security in Nepal



Name: Prof. Tirtha K. Datta
 Country: India
 Position: Principal Scientist,
 Genomics and Biotech, NDRI,
 Karnal
 Speaker Category: Keynote
 (Genomics and Biotechnology)
 Topic : Understanding the fertilizing
 ability of buffalo oocytes &
 spermatozoa in functional genomics
 perspective



Name: Prof. Daniel Grooms
 Country: USA
 Position: Chair, Large Animal
 Clinical Science, Michigan State
 University
 Speaker Category: Keynote (Buffalo
 health)
 Topic: Adapting technologies
 to improve buffalo health and
 productivity



Name: Dr. Neelesh Sharma
 Country: India
 Position: ICAR Int. fellow, Sher-e-
 Kashmir University of Agriculture
 Science & Technology
 Speaker Category: Keynote (Health)
 Topic: Buffalo mastitis in dairy
 production: Technological advances
 in understanding of mastitis



Name: Prof. Janice Swanson
 Country: USA
 Position: Chair, Department of Animal
 Science; Professor and Director,
 Animal Behavior and Welfare
 Speaker Category: Keynote (Policies
 and socio economics of buffalo
 production)
 Topic: Animal welfare issues in dairy
 farming



Name: Prof. Dr. Shujun Zhang
 Country: China
 Position: Huazhong Agriculture
 University, Wuhan, China
 Speaker Category: Keynote
 (Genomics and Biotechnology)
 Topic: Genetic Studies in Water
 buffaloes in China



Name: Dr. Danilda Hufana-Duran
 Country: Philippines
 Position: Scientist I & Senior
 Agriculturist Reproductive
 Biotechnology Laboratory Philippine
 Carabao Center
 Speaker Category: Keynote (Genomics
 and biotechnology)
 Topic: Developments and Prospects
 of Advance Reproductive
 Biotechnologies in Buffalo
 Production





Name: Prof. Metha Wanapat
Country: Thailand
Position: Department of Animal Science,
Khon Kaen University
Speaker Category: Keynote (Buffalo
Nutrition)
Topic: Enhancing buffalo production
based on feed resources development
and innovations



Name: Dr. Bianca Moioli
Country: Italy
Position: CREA, Research Center for
Animal Production and Aquaculture,
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Italy
Speaker Category: Keynote (Biotech)
Topic: Genetic improvement of dairy
buffalo, constraints and perspectives.
The Italian example



Name: Prof. Dr. Yangqing LU
Country: China
Position: Animal Reproduction
Institute, State Key Laboratory for
Conservation and Utilization of
Subtropical Agro-bio resources,
Guangxi University, Nanning,
Guangxi 530004, China
Speaker Category: Keynote (Genomics
and biotechnology)
Topic: Buffalo Reproductive
Biotechnologies: the Current Status
in China



Name: Dr. Bhuminand Devkota
Country: Nepal
Position: Coordinator (Post Graduate
Studies Center), Associate Professor
(Department of Theriogenology),
Agriculture and Forestry University,
Chitwan.
Speaker Category: Keynote
(Reproduction)
Topic: Update on attempts of developing
breeding strategy of Nepalese
buffaloes (*Bubalus bubalis*) during
low breeding season



Name: Prof. Antonio Borghese
Country: Italy
Position: General Secretary,
International Buffalo Federation
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Monterotondo, Roma, Italy
Speaker Category: Keynote (Policies
and socio economic)
Topic: Current status and economics of
buffalo production worldwide



Name: Dr. Inderjeet Singh
Country: India
Position: Organizing Secretary, IX Asian
Buffalo Congress; President, Asian
Buffalo Association; Director ICAR-
Central Institute for Research on
Buffaloes, Hisar 125001, Haryana
Category: Special guest



Name: Dr. Peregrino Ginaldo Duran
Country: Philippines
Position: Philippine Carabao Center
National Headquarters and Gene
Pool Science City of Muñoz, Nueva
Ecija 3120, Philippines
Speaker Category: Keynote (Reprod)
Topic: Fourier Harmonic Analysis as
Efficient Tool in Predicting Bull
Fertility for Improved Buffalo
Breeding



CONCLUSION

We look forward that the IBS2017 would be a great success to meet the envisaged objectives, further strengthen the multi-lateral partnership, locally and globally and make a strong foundation to move forward together to meet the the mission of developing AFU as a multi-dimensional buffalo research hub. We hope that we will come forward with the symposium recommendation and resolutions that will be a milestone to move ahead in this important endeavor. As an organizing secretary, I would like to thank everybody and every institution contributing in this great event.



STRATEGY TO ENHANCE THE PRODUCTION AND PRODUCTIVITY OF BUFFALOES FOR THE GROWTH OF LIVESTOCK GROSS DOMESTIC PRODUCTION (LGDP) OF NEPAL

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ABSTRACT

Livestock sector plays a pivotal role in the Agriculture based economy of Nepal. Nepalese statistics shows the agriculture contribution of about 26.8% to the National GDP of which around 13% of the contribution comes from the livestock sector and 6% comes from the buffaloes (MOAD, 2016). Livestock statistics of FY 2072/73 estimates a total of 5.16 million buffaloes in Nepal contributing to the annual milk production of 1.21 million MT (65% of national milk production) and meat production 0.17 million MT (54% of national meat production). Buffaloes are considered black gold in Nepal. They are supposed to be reared in plain, mid hills and high hills with minimum cost of investment. It is not only the source of family income but also the best source of nutrition in all parts of the country. Nepal buffalo population consist of the three native indigenous buffaloes (Lime, Parkote and Gaddi) ranging from terai to hills along with other exotic and cross-breed buffaloes mainly of the Murrah breed. The production of native buffalo breeds is comparatively low (about 900 liter/lactation) as compared to the Murrah and their cross breed (1500 liter/lactation), but still the improved breed performance has been far behind the actual productivity of 2300 liter (NARC, 2015). Nepal government has given priority in the improvement of production and productivity of buffaloes. Through its structure of Department of Livestock Services and collaborating with other organization and institutions, the government has implemented various programs to improve the production and productivity of Nepalese buffaloes. Establishment of a separate dedicated office namely Central Cow Buffalo Promotion Office under Directorate of Livesin Department of Livestock services (DLS) and implementing activities for buffalo production and productivity shows the priority of Nepal government in this sector. The government has also given priority to the conservation of native indigenous buffaloes by endorsing and supporting implementation of the Long Term Action Plan of Animal Genetic Resources in Nepal (2011-2021). Government, through its formal structure of DLS, has implemented some of the important programs to enhance the production and productivity of buffaloes. Various programs like Buffalo Genetic Improvement Program (BGIP), community buffalo bull distribution program, Artificial insemination and Forage missions, buffalo conservation, nutrition program of newly parturition buffaloes and male buffalo fattening for meat production are some of the programs that have been implemented in many districts. Though the public private partnership, establishment of buffalo pocket area, resource centers and intensive resource centers has been implemented. However, genetic interventions, nutritional supplementation, commercialization, studying effects of climate change and its minimization, product diversification of buffalo milk and meat as niche products could be some of the important way forward to increase the productivity and utilization of buffaloes in the country. The need of technical intervention to shorten the calving interval and anestrus state is important tool to enhance the productivity of buffaloes. This paper will elaborates the aspect of policy and strategy for overall development of buffaloes in the country.

Keywords: Buffalo, Nepal, buffalo genetic improvement program (BGIP), production and productivity, GDP

VALUE CHAIN ANALYSIS AND DAIRY DEVELOPMENT IN NEPAL

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ABSTRACT

We present an overview of the value chain approach in industry development covering both the scope of value chain analysis and considerations in designing and implementing appropriate interventions. Successful value chain intervention considers the entire market system with clear focus on end markets, understands the role of various players, their interrelationships and governance structures, and targets key leverage points for changing participant behavior and transforming relationships. The current state of dairy value chain in Nepal is briefly described, identifying the key opportunities, major strengths and weaknesses. Key strategic options are identified along with potential intervention pathways.

Keywords: Value chain approach, pathways, interventions



INTERNATIONAL BUFFALO SYMPOSIUM - 2017: PARTNERSHIPS AND WAY FORWARD

N. P. Joshi
Michigan State University

It gives me tremendous pleasure to be amongst the august body of eminent scientists and researchers gathered in the symposium in this very beautiful Chitwan where I spent my adulthood as a young lecturer at the then Institute of Agriculture and Animal Science (IAAS). I am happy to be back. . On behalf of Michigan State University, I congratulate the conference organizing committee for putting together such an important Symposium in very short time. We are proud to join hands in this important initiative.

Michigan State University (MSU) has a long history of engagement in Nepal. In mid-seventies, MSU collaboration with the then Institute of Agriculture and Animal Science (IAAS) in its institutional capacity building with USAID assistance. Since the establishment of Agriculture and Forestry University (AFU) in 2010 MSU has been collaborating in various areas of capacity building. In 2012, MSU jointly with AFU, Nepal Agricultural Research Council (NARC) and Department of Livestock Services (DLS) received funding for a project entitled “**Enhancing Nutrition and Productivity of Buffaloes to Mitigate Potential Impact of Climate Change in Nepal**” through USAID LCC CRSP Program managed by Colorado State university that was completed in 2015. This collaboration brought the scientists working in various aspects of improving production and productivity of buffaloes through series of meetings and review and training workshops. The idea of convening a conference to bring together the Nepalese scientists and researchers working in buffaloes was discussed in East Lansing during the visit of honorable VC Dr. Dhakal and Dr. Nab Raj Devkota Director of research, AFU (Co-PI of the project) in April 2016. Rest is history. The symposium organizing committee has made great arrangements for this important international gathering of scientists from across the globe. Congratulations!!!

Dean Dr. Thapaliya, has highlighted the importance and timeliness of this symposium. Dr. Bhuminand Devkota, IBS2017 Organizing Secretary and Coordinator, PG Program has given the details of the proceedings for this important 3 days of deliberations. Importance of buffaloes in the economies of South Asian countries is well recognized, especially for the smallholder livestock farmers. The theme of the conference “**Enhancing Buffalo Production for Food and Economy**” is cohesive with the objectives outlined in the proceedings and the needs of smallholder buffalo farmers.

Many papers will be presented highlighting the experiences in efforts to address the problems faced by buffalo farmers and opportunities to mitigate those problems. Nepal has tremendous resources in buffalo germplasm. There are three important indigenous breeds of buffaloes in Nepal namely; Lime, Parkote and Gaddhi. These breeds are well adaptive in the region with disease resistant capacity and they poses extraordinary ability to convert less nutritious diet into most important source of meat and milk. Their genetic improvement work may provide ample opportunity to strengthen the productive performance and thus AFU, in collaboration with NARC, DLS and international organizations in the region could help improve its production and productivity.

Buffalo in the region is under threat due to over exploitation; from both milk and meat consumption. Due to its nature of slow growth, and fluctuation in population, serious attention is needed to assure availability of replacement stocks in timely manner. In this regard, Nepal has three major buffalo research and development centers (Lampatan – DLS, Tarahara – NARC and Rampur – AFU). To address these issues and concerted effort to improve production and productivity of buffalos we need a strong research networking that could help develop technologies appropriate for the region. In this regard, AFU has developed strong linkages in terms of research network inside the country and also internationally. Considering large pool of genetically pure Murrah breed herd at AFU, research collaboration to develop research projects would be meaningful for research focused on issues related to buffalo production.

Chitwan has become a very important hub for dairy production in the country and AFU graduates, faculty are engaged in many ways with the dairy farmers and producers. I am hoping that AFU plays a role as a hub for research on buffaloes in close coordination with NARC and DLS. This hub could host national and international scientists to conduct research and training of new generation of scientists and researchers. It could be a **Center of Excellence in “Buffalo Research, Training and Outreach”**. This could benefit of smallholder buffalo farmers of the region and across the region. This gathering is very important and special as the conference recommendations and guidance will be a road map to establish such a research hub in Nepal.

Thank you.



CURRENT STATUS AND ECONOMICS OF BUFFALO PRODUCTION WORLDWIDE

A. Borghese*

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ABSTRACT

The dairy buffalo holds a strategic role in the world economy and society, as human requirements of proteins of high nutritional value could be satisfied by milk and meat availability coming from buffalo species, represented in the world by more than 200 million head. The milk production is depending particularly from genetic capacity of females that can be increased by animal recording and selection. The genetic improvement can be realized easily by the spreading of artificial insemination of the best selected bulls. Correct management and nutrition of the herd, as health and welfare of the animals are basic conditions for the expression of the capacity to produce meat and milk of high quality. Meat and milk quality is today a pivotal factor in the progressing and in the diffusion of the meat and milk processing industry. The buffalo market in Italy is given particularly by mozzarella cheese, vary famous example of Italian food style exported in all the countries, in America the buffalo is used more as meat producer even if many farmers understood the better economy coming by dairy buffalo for cheese industry, in Asia both the meat and milk lines have a priority role for feeding people in the villages as in the big cities. Buffalo products coming from all the countries in the world are reported.

Keywords: buffalo meat and milk, genetic improvement, food quality, buffalo market.

BUFFALO POPULATION TREND LINKED TO DAIRY BUFFALOES

The buffalo is a priority animal in the world as a source of protein food for human survival and sustainability, as it is evidenced in each country, where buffalo is bred and where it has a pivotal role in human food sustainability.

In the World, according FAO data (FAOSTAT, 2010), there were 180,702,923 buffalo head on 2010, about 182 million according Borghese on 2013 (Borghese, 2013), with a positive trend (+8.3%) in comparison with the value of 168 million reported in a previous book "Buffalo Production and Research" (Borghese, 2005). And, as now the total population is more than 200 million, the positive trend is confirmed. This positive trend is due to the increasing population in India and Pakistan, where milk purpose breeds were selected and where the buffalo milk market is very strong, balanced by the decreasing in other countries where draught animals are less required than in the past.

In Africa, where the buffalo is found only in Egypt, there are 5,231,162 head (FAOSTAT, 2010), the 2.9% of the total population in the world, as buffalo in Egypt is a basic draught animal in rice fields and irreplaceable for milk production for direct consumption.

In Europe the most of population is in Italy with about 403,000 buffaloes (ISTAT, 2014), while the European buffalo population is 459,000 (0.25% on world population), less than the found one 7 years ago (500,000; 0.30%) (Borghese, 2005) for the decreasing trend in many Balkan countries as Romania, Bulgaria, Macedonia, Greece, Albania, Serbia, partially balanced by the increasing trend in Italy, link to the expansion of mozzarella market of mozzarella and other cheeses and milk byproducts .

In America we can consider this year 4,227,000 buffalo head, 2.32% of total population, while 11 years ago there were only 3,345,000 (Rocha Loures, 2001), about 2% of the global one, with a very positive trend (+26.4%) due to the enormous availability of land and free pasture in South America, the unique adaptability of buffalo to lagoons and marshy land, the changing from a meat purpose breeding to a double purpose (milk and meat) livestock.

The increasing trend in Asia, Italy and in America is essentially due to the increasing number of dairy purpose buffalo breeds, linked to the high demand of milk, cheese and processed products in all the market of the world. Really now the Governments and the people, also in some Asian and American countries where milk and cheese were not traditional food at high level as in European culture was for centuries, understood that milk is very important for human need of food and particularly for children request of protein of high biological value.

Cheese and products of milk processing in general are going to be introduced more and more in Asian and American countries too, as the consumers are being more exacting and requiring food of nutritional and taste quality.

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ECONOMY FROM DAIRY BUFFALO IN ITALY

Genetic Improvement

In Italy the Buffalo Genealogical Book was instituted by Italian Ministerial Decree on June, 23, 1980 and was held by A.I.A. (Italian Association of Breeders). The A.N.A.S.B. (National Association of Buffalo Species Breeders) had been instituted on 1979 and recognized on the 1994 by Agricultural Ministry. The Ministry, with Ministerial Decree n° 20154 has entrusted the management of the Book to A.N.A.S.B. on year 2000. In the same year other decree (D.M. 201992 on July, 5, 2000) recognized buffalo enrolled in Genealogical Book pertaining to the only own race: “Mediterranean Italian”. The animals rose in Campania and in Lazio Regions of Italy give the milk used for the production of the famous “Mozzarella di Bufala Campana D.O.P. ”. The D.O.P. (Denomination Origin Protected) Mozzarella di Bufala Campana was recognized with the Ministerial Decree on May 10, 1993, published on the G.U. n.219 on 17/9/1993, and after from European Union; that means that this cheese mozzarella has to be produced in defined areas, coming only from fresh milk of buffalo cows of Mediterranean Italian breed, registered in the Buffalo Genealogical Book. The Decree establishes the milk characteristics (fresh within 16 hours from milking, raw, minimum fat 7%), processing techniques (acidification, coagulation, stretching, moulding) and mozzarella characteristics. The control and guardianship is effected by “Consorzio per la tutela del formaggio Mozzarella di Bufala Campana”, so the European consumer is guarantee that the logo means a quality product of the made in Italy, according the best standard of animal management, welfare and health, according also the best characteristics of mozzarella, as sanity, freshness, flavour and juiciness. The main factors that have contributed to the development of buffalo in Italy in the past few years have been the following: increase of the consumption of mozzarella in Italy and export in many countries in the world; the high price of buffalo milk (1.20–1.50 euro/ kg) in comparison with cattle milk (0.38 euro/kg), the high technology of farmers, high level of management and breeding, high genetic value of the herd, obtained by performance and progeny testing, animal recording and selection, artificial insemination applying, starting with an organization born more than 50 years ago.

For Mediterranean Italian Buffalo, the productive controls regard: the quantity of milk in kg, the determination of the percentage of fat and of proteins (kg and %) and the somatic cells.

The beginning of the official lactation starts at calving, the first control cannot be carried before the five days from calving and not beyond 75 days. The duration of the reference lactation is 270 days, in any case the duration of the effective lactation must be indicated.

For every subject are reported the following data: number of current lactation, daily production expressed in kg milk, % fat, % proteins and number of somatic cells, the effective production from the calving for: kg milk, kg fat and proteins and the daily medium production, the milk production in comparison to the reference lactation of 270 days, “equivalentebufalamatura” (E.B.M.) expressed in kg of milk, fat and proteins (Coletta and Caso, 2008).

In Italy there are 402 000 buffaloes and the mean milk production is over 2200 kg for lactation, in confront of other Mediterranean countries where the maximum production is less than 1900 kg. In 2013, the number of recorded buffaloes increased until 56, 812 (ANASB, 2014, Table 1) as too the number of head per farm and the milk production increased.

Table 1. Italian Buffaloes (ANASB 2014)

N° Head	402,000
N° Dairy buffaloes L.G.	194,398
N° Recorded buffaloes	56,812
% Recorded Buffaloes	29.2
N° Recorded farms	318
N° Head/farm	178.6
kg milk production (in 270 d)	2,222
% Fat	8.16
% Protein	4.67

In the last few years, in Italy, milk composition has been improved too: the average protein content has raised from 4.4% in 2002 to 4.6% in 2010 while the average fat content raised from 7.3% in 2002, to 8.5% in 2010, without operating any selection for the character of protein and fat content. Moreover the possibilities for genetic improvement for milk quantity and quality will be higher, if the selection pressure will be increased reducing the number of bred females, discarding the low productive females. At the present time there are many females in Italy producing more than 5,000 kg milk/270 days of lactation) until the maximum production of 5600 kg, 8.32% fat, 4.63% protein.



There are in Italy two Bull Buffalo Centres for semen production: the COFA (Cooperativa Fecondazione Artificiale) in Cremona Province, Lombardia Region, in North of Italy, where there are many bulls with genetic potential to produce more than 4000 kg milk/lactation as Malandrino Bull and O-B-One Bull lines. The Chiacchierini Bull Centre in Perugia Province, Umbria Region, in Middle Italy: this one started a genetic selection programme with CIPAB consortium and actually produces semen from 16 tested bulls from different bloodlines, coming from mothers over 3100 kg milk yield per lactation with more than 4.5% protein: there are Ciripicchio Bull with 4494 kg milk/lactation and Jesce Sole Bull with 4157 kg milk/lactation, as recorded in the best daughters, and Brillante, the best bull for pedigree index of 2010-2011 progeny test. This stud is one of the few European A.I. studs authorized as insect-proof quarantine barn, located away from the semen production zone. It is authorized for worldwide export for the excellent sanitary level. Chiacchierini Bull Centre produces sexed semen too, available from a lot of bulls.

Hereafter the selection will be directed at the improvement of the yield of mozzarella cheese, not simply for milk production, since the farm income is based firstly on mozzarella cheese, secondly on the sale of pregnant heifers, lastly on beef sales and finally on the sale of semen and embryos of high genetic value.

Management and Nutrition

Italian buffalo management is today exclusively intensive, as in the past it was extensive too with calves milking from cows in the open air, separately or together with milking men by hands. This figure is disappeared, now existing only intensive system: dairy buffaloes are kept loose in paddocks close to the milking room, where the cows are submitted to udder control and mechanically milked twice a day. The females are normally artificially inseminated in the paddock, using high genealogy semen, preferably in February-March after oestrus induction, to obtain calving before spring (about 50% fertility), as the milk is paid more in spring and summer according the consumer demand. After one month from artificial insemination the empty females are naturally mated, obtaining 30% fertility with a total mean fertility rate of 80%.

Milk production is sustained by diets with a high energy (from 0.85 to 0.95 MFU/kg dry matter (DM) and a high protein concentration (14-16 % crude protein on DM), based on maize and other silages, cereal grains, soya, alfalfa or "graminaceae" hay and by-products. The feeding stuffs movement and distribution is effected by mixing trucks along the feeding line in paddock or in feed-lots; the movement and stocking of dung is also mechanized; therefore there are no smallholders in Italy, but only farmers with an average herd size of 161.3 head per herd. Heifers are also fed intensively in order to achieve puberty before 20 months (Borghese et al., 1997, Borghese, 2005). The heifers are housed loose in paddocks all year long, utilizing the same modern systems used for dairy cows.

In intensive systems the buffalo cows normally receive unifeed composed of maize silage, concentrates, hay, straw and sometimes by-products. For example, a 600 kg live weight buffalo cow producing 10 kg milk, would be fed 15.3 kg DM (33 % maize silage, 42 % alfalfa hay, 8 % maize grain and 17 % concentrate with 38 % proteins,) with 12.7 Milk Feed Units (FU), 2.1 kg crude proteins and 3.5 kg crude fibre. Maize silage can be highly increased: some rations foresee until 60% maize silage, 26% concentrates, 14% hay and straw, sometimes by products (tomato peel, brewer grain residuals, sugar beet pulp) (Borghese, 2010).

Buffalo Food and Market

The market is mainly based on mozzarella cheese, very famous one, not only for the local consumption according the traditional Italian cooking style, but also in many foreign countries. There are different types of mozzarella, the best one is produced in D.O.P. area, according the regulations: it is hand made by raw buffalo milk, soft, juicy and tasty, rich of live ferments, natural yeasts and microbes, it is coming from a difficult processing schedule, particularly for stretching phase, it changes taste during time, not preserving in fridge but in mozzarella water and the shelf life is about 5 days. The industrial mozzarella, even if produced in D.O.P. area according the regulations, is made by machines and microbes die during pasteurization, with the advantage of a longer shelf life, preserving in fridge (until more than 2 weeks) but the material is too compact and the taste is hard and anonymous; this product is distributed in supermarket and for export. The basic price of mozzarella at cheese industry is 10 euro/kg, with a good profit, utilizing 4 litres of milk/kg mozzarella and starting from the milk price as 1.20 €/litre that is more than 3 times the price of cattle milk. The price in the shop increases as more as the quality of mozzarella and the distance from the site of production until 20-30 €/kg. The mozzarella D.O.P. consumption is about 82% for the Italian market, 18% for the export, particularly for Germany (20% of the export), France (20%), USA (18%), U.K. (12%) (Borghese, 2005, 2010).

In the year 2010, 36000 tons of mozzarella were produced, with an increase of 12.5% respect to the 2009, with a sales volume of € 300 million at the production, € 500 million at the consumption (Borghese, 2011). In the year 2011, 37000 tons of mozzarella were produced (320 million €), in the first 6 months of 2012, 18203 tons were



produced.

Another very appreciated product is the ricotta, that is not really cheese because it is produced boiling the serum proteins remaining after the produced curd.

After mozzarella market, now meat market is rapidly increasing: now there are some fattening centres for the production of excellent buffalo carcasses. Calve carcasses are appreciated for clear and tender meat but normally the live weight at slaughter is 400-440 kg obtained at 15-16 months of age with 800-1000 g/d of daily gain, managed on slatted floor to avoid bad smell of urine and faeces: young bulls without defect or pathologies, beautiful carcasses with conformation R (good), medium fattening according Italian market requirements are obtained, 52% dressing percentage, 57% net dressing percentage, 62% meat on carcass, meat with low fat (less than 3%), very clear, tender and juicy, with good dietetic qualities: <50 mg cholesterol /100 g, unsaturated fatty acid/saturated fatty acid >1, iron >1.5 mg/100g. The first quality cuts are well represented with good muscular growth and are sold at 14-25 €/kg for typical restaurants and many products, as bresaola, salami, cacciatorini are sold in typical shop together with buffalo cheese, as meat also obtained I.G.P. (Indication Geographic Protected) "Carne di Bufalo Campana".

Very appreciated and common products are: mozzarella, treccia, scamorza, crescenza, robiola, caciocavallo and other cheeses, ricotta, yogurt; meat and meat industry products: bresaola, salami, sausages, caciiorollo, cacciatorini (little salami).

Finally Italy is a reference point as buffalo importance in human food sustainability for high quality.

ECONOMY FROM DAIRY BUFFALO IN INDIA

Buffalo livestock in India plays a crucial role to supply the requirement of animal protein for millions of people: buffalo supplies milk, meat, leather, bones, pharmaceuticals, dung and manure besides draft energy power. Buffalo population is almost 66% of the cattle population in India: 97 million compare to 146, respectively, but the milk produced by buffalo is 58 million tons, as only 47 million tons by cattle. These figures show the great superiority of buffalo in production ability (Madan, 2010) as buffaloes contribute 55% of total milk produced in the country, though their number is only 40% of the total bovine bubaline population. India buffalo population with 97 million animals represents 53.3 percent of the world buffalo population. India is the first country in the world for number of buffaloes and milk production (about 105 million tons, buffalo plus bovine, that is about the 15% of the world's milk production).

India is also the first country in Asia for scientific and technological development in buffalo nutrition, production, reproduction, biotechnologies and genetic improvement. India possesses the best River milk breeds of Asia as Nili-Ravi, Surti, Jaffarabadi and particularly Murrah that is the most diffuse breed in the world, from South America to Asia, all are originated from the north-western states and have high potential for milk and fat production, besides being used for work and surplus stock used for meat production (Sethi, 2003). Indigenous animals have high biodiversity and are considered to have higher disease resistance, better tolerance to high hot and humid conditions. These animals are also more efficient in feed conversion efficiency of crop residues and naturally available low quality roughages.

The lactation milk yield in 305 days of lactation is reported by Sethi (2009), as observed in various centers coordinated by the Buffalo Research Institute in Hisar, in last 5 years: Murrah 2075+54 kg, Nili-Ravi 1929+68 kg, Surti 1566+ 46 kg, Bhadawari 1434 kg, Pandharpuri 1841 kg, Swamp 412 kg.

Buffalo meat production is comparatively new area of buffalo production, as social taboos are not attached to the buffalo slaughter unlike bovine and swine. India produces 1.5 million tons of buffalo meat (FAO,2008) which is the 36% of the total meat produced in the country, India mainly being a dairy country, a tremendous export potential lies with buffalo meat. Annual per capita consumption of both beef and broiler meat is 1.5 kg, compared with 745 grams for lamb meat. The retail price of beef averages USD 1.4 per kg, compared with 1.8 for poultry meat and 2.9 for lamb (Kumar and Singh, 2010).

Buffalo males of Murrah breed have efficient work capacity especially for load pulling and ploughing in rice cultivation. The buffaloes can work for moderate work for 1-2 hours continuously without affecting their milk yield (Bhoite and Deokar, 2004).

Recent statistics concerning buffalo demography show that the buffalo population in some countries like India, Pakistan, China, Vietnam is increasing at a rate of 1.5 % per year. Buffaloes are well adapted to a hot and humid climate and play a distinct role in the economy of farmers, which is primarily based on agricultural production systems. Many important projects of buffalo development are carried out by the Buffalo Research Institute in Hisar, directed by Dr. Inderjeet Singh, while studies on milk processing are carried out by the National Dairy Research Institute, Karnal.

The most quantity of milk is used for direct consumption after skimming, fat is used to produce butter, ghee and the cream. Dry milk, condensed milk, milk replacers are very used in national market and for export, as



different industrial utilizations. Paneer is a cottage cheese, used in several vegetarian curry dishes in India and in other countries.

ECONOMY FROM DAIRY BUFFALO IN PAKISTAN

According to FAO (2009) data, buffalo population in Pakistan is about 30 million head, the second in the world, after India. Buffalo breeds are River types Kundi and Nili-Ravi and among the best milk producers of the world. The population increase was very fast, comparing with 22 million head reported by Borghese (2005) and comparing too with many Asian countries where the population has decreasing pictures because of low milk production due to Swamp breeds predominance.

Total milk production is 43.6 million tons of which 62% derives from buffalo with 27 million tons, therefore buffalo role in milk production system and in food availability in Pakistan is very important. The demand for milk and milk products are constantly rising, mainly because of increasing urbanization. The milk is used priority for direct consumption after skimmed. The fat is a richness as it is used to produce butter, ghee for cooking, cream, sweet. The skin is used in leather industry and the manure is the primary source of fuel in the villages and it is used too as bio-fertilizer.

The annual beef production in Pakistan in the year 2008-09 was 1.6 million tons (Pasha, 2010), in which buffalo contribution was 51%. Current buffalo meat production systems are traditional and inefficient. Most of the meat comes from cull cows and it is of bad quality.

The buffalo research and development is well organized: there is the Buffalo Research Institute in Pattoki, Punjab, where research areas are developed on delayed puberty, reproduction pathologies, reproductive technologies, productive parameters. Several researches are supported by UVAS (University of Veterinary and Animal Sciences) in Lahore, by the Livestock Production Research Institute in Bahadurnagar, Okara and finally by the Semen Production Unit in Qadirabad, Sahiwal, that has the role of progeny testing, semen collection and artificial insemination spread out.

ECONOMY FROM DAIRY BUFFALO IN CHINA

According to statistical data (FAO, 2008), the total number of buffalo in China is 23.27 million, the third largest population in the world, representing the 28.17% of the total bovine population in China. Chinese buffalo is Swamp type, as a total of 18 local breeds. Swamp breeds are mainly used for draught since their milk production is very low (500-700 kg milk yield for lactation) as they are very resistant working in the marshlands, particularly in rice fields. China imported Murrah buffalo from India in the late 1950s and Nili-Ravi from Pakistan in the late 1970s.: milk performance has been markedly improved in crossbreds through the crossbreeding system applied in upgrading the two breeds such as crossbred Murrah F1, F2 and crossbred Nili-Ravi F1, F2 with average milk yield per lactation respectively: 1240, 1423, 2041, 2325 (Bingzhuang et al., 2003). Murrah buffaloes are also used to provide milk and milk processed food to poor people in the villages. Recently crossbreeding was applied using Mediterranean Italian semen with better results. According to Cao et al. (2006), there are 30,000 milking buffalo in China, 61.5% of them are crossbreds, while 38.5% are local buffaloes. The buffalo milk production was 0.29 tons in 2008. There are few special buffalo milk processing factories distributed particularly in Guangxi Province, of small scale and poorly equipped. The products are pasteurized milk, yogurt, condensed milk, fancy milk drink, milk cake, milk bean curd, crème. Guangxi Buffalo Research Institute has successfully developed buffalo milk cheeses in 2009 and created food market (Yang Bingzhuang et al., 2010). The production of buffalo meat is 0.306 million tons (FAO, 2008). Most of the meat is directly sold to consumers, only little meat is processed such as dried beef, sausages, hams (Yang Bingzhuang et al., 2010). The production of buffalo hide is 92,000 tons (FAO, 2008), the hides were made into various products, which are sold well on the market. In conclusion the food availability from buffalo livestock in China is rapidly increasing by the widespread application of crossbreeding of River breeds with high genetic value (Mediterranean Italian, Nili-Ravi, Murrah) on local Swamp breeds.

ECONOMY FROM DAIRY BUFFALO IN THE NEAR EAST

Dairy buffalo is important also in the Near East Asian Countries, as Turkey, Iraq and Iran, where the buffalo population is represented by 122,000, 286,000 and 470,000 head, respectively.

The Anatolian Breed is present in Turkey and produces 962 kg as average milk yield for lactation of 224 days, as the Kuhzestani Breed is present in Iraq and in Iran and produces 2,100 kg milk per average lactation period of 210 days (Borghese, 2013). In Iran there are also Azeri and Mazandarani breeds, less milk producers.

In Iraq, buffaloes are bred in the marshes and in the Tigris and Euphrates rivers, managed by boys as docile animals or by farmers and swim far and wide for feeding on papyrus, reeds, common ash and other plants. They



like to stay in the mud, to maintain humid and protected skin. When the flood water is high their owners have to go out and collect these plants in order to feed the buffaloes on platforms. Buffaloes in towns rarely graze on natural pasture; they are fed mostly on concentrates, green forage, straw and agricultural by-products.

In Khuzestan, Iran, near Ahvaz there are many farms with Arabian origin people: the khuzestani buffalo cows are living free in courtyard and are milked together with calves. The cows are very docile and are managed by boys without risk. The feeding stuffs are straw, alfalfa and sugar cane pulp. The desert is irrigated and produces sugar cane and dates. In other farms the animals are tied all the night and on the morning they are free to go to the lagoons. There is a great pollution, no control on diseases. Some people live in the villages in the lagoons and buffalo are milked at 4,00 and 16,00 hours producing 8 kg milk/day, living in the water of lagoons and feeding water plants as Chulan.

Anyway the milk and the milk products, that are very similar in these countries, are very appreciated by the consumers and play a pivotal role in local economy. Many products are available from buffalo in Iraq: fat skimmed from milk and used to produce butter, ghee, sweet and cake; skimmed milk is used for direct consumption and to produce curd, fresh cheese and sweet. The meat is used for direct consumption or to produce salami and ham, as these products cannot come from swine, as pig meat is forbidden in Islamic countries.

The most appreciated products in Iran are: yoghurt, fresh cream, fresh cheese, butter, ice-cream, rice pudding, churned yoghurt, dried whey, ghee. In Iran, the price of buffalo milk is twice that of cows' milk. Buffalo skin is used in the leather industry. Buffalo manure is used for fuel in rural areas.

The milk production of the water buffalo in Turkey is renowned and favoured particularly for the production of the famous Turkish desserts. This was one of the highest motivations for farmers to keep and raise water buffaloes near big cities (Soysal and Kok, 2004). A semi-hard cheese called "peyazpeyneri" is made from buffalo milk. Ayran is a drink with water and buffalo yogurt.

Common strategies are going to be adopted in the three Asian countries to increase the milk production and products availability for the market: to apply crossbreeding, using semen from Mediterranean Italian breed. A development project started in Iran few years ago, introducing 4095 doses of Mediterranean Italian buffalo semen in order to improve genetic and milk production, with the goal to increase buffalo products and market.

In 2002 Italian semen was introduced in Turkey, in Ilikpinar village in Hatay province, for the local population of buffaloes in order to improve genetic and milk productivity (Sekerden et al., 2003). In F1 (Mediterranean Italian X Anatolian), milk yield average was 1386 ± 246 kg for lactation of 305 days; yield average of 81 Anatolian buffaloes are calculated as 987 ± 327 kg (Sekerden, 2009); daily milk yield was respectively 4.12 and 3.23 kg, fat% was 6.45 and 7.20 respectively, protein% 3.14 and 4.35, TDM% 15.64 and 17.00 respectively.

Buffalo improvement program for Anatolian buffalo, included in the Turkey Nation wide improvement program, started on 2011, involving progressively 16 provinces and 27,693 buffaloes, applying selection on pedigreed buffalo population, with the goal to achieve 1200 kg milk yield in 250 days of lactation length (Soysal et al., 2015).

ECONOMY FROM DAIRY BUFFALO IN THE FAR EAST

Buffalo population and economy is going to be more attended now in Bangladesh, Indonesia, Philippines and Thailand, where in the past years there was a decreasing trend in population, because of the population is Swamp buffalo or River buffalo used for draft. The policy neglected in the past the selection of dairy purpose breeds and the possibilities to create a market of dairy products.

In Bangladesh developing programs by Agricultural Ministry and by LAL TEER SEED LIMITED Company are going to increase the milk availability by improving the genetic potential in local buffalo population, applying crossbreeding projects utilizing high genetic value semen of Mediterranean Italian breed, according artificial insemination programs started on 2011. In this country the 45% of total population (1.26 million) is River type, Bangladeshi breed. They are used mainly for cultivation of land, transportation and crushing of sugar cane. Their coat color is usually black and horns are curly. Some animals have brown color or hanging horns. Average milk yield is about 620 kg in a lactation period of 270 days. Their management system is semi intensive; a new semi-intensive farm was founded by LAL TEER Livestock Limited in Jamuna to apply artificial insemination with Mediterranean Italian semen to increase milk production (Borghese, 2013). Total consumption of milk is about 1.7 million tons of which 27% came from powder milk import. The purpose of the LAL TEER Company is to create a new market with dairy products for the people.

Indonesia has a buffalo population of about 2 million head in 2010. There was a declining trend as in 1985 the total population of buffalo was 3,285 thousand (Borghese, 2005). There are varieties of Swamp breeds in the different isles of Indonesia with divergence in size, weight, color, marking and horn dimensions. Most of rural buffaloes maintained by families in the villages produce less than 1000 kg of milk per lactation. The Swamp buffalo



is generally considered to be a working animal, but it also has a considerable capacity for milk production. Swamp buffalo are used for draught power in most areas and for beef in the Java lowland areas and the Sumatra uplands. Spotted buffaloes are highly prized, particularly in Tana Toraja, Sulawesi, (and therefore they command high prices) to be sacrificed and consumed on special occasions such as marriage and obsequies ceremonies for their sacral value. Therefore in Sulawesi there is a special market with animals paid until 100,000 US dollars when parents died and a particular selection to obtain spotted animals and very high profits.

Generally milk production not yet been marketed widely but is only processed traditionally in the form of typical food as sagonpuan, milk mixed with eggs and sugar, very appreciated in Sumatra (Borghese and Sodikin, 2008). Another product by Sumatra is dadiah: after the hand made milking the milk is put in bamboo-cane, where it is naturally acidified and fermented for 2 days. It is rich of probiotics, similar as taste to kefir or to yogurt but it is really a fresh cheese cream, rich of fat and protein. It is possible to conserve dadiah for 8 days and to distribute and sell it in many lands of Sumatra at the price of 7.000 rupias/ piece. When people like to eat dadiah, they cut the bamboo-can and take it with a spoon to eat directly or with cereals, wheat, dried gluten rice or rice (Borghese and Sodikin, 2008). Other milk products in Sumatra market are buffalo oil, sugar puan, curdle, penjem .

Buffalo development in the future should be based on animal production improvement with population increase to achieve food of animal origin, actually very poor in the country: these goals could be achieved by increasing the production of buffalo milk and meat thought the improvement of the genetic capacity, producing F1 and backcross buffaloes from Swamp and Mediterranean Italian River buffalo (Borghese, 2005). Finally a new program started in Sumbawa to create a Buffalo research Centre, where the best local dairy buffaloes will be introduced and where the crossbreeding with Mediterranean Italian semen will be applied, with the purpose to create a Sumbawa buffalo breed with dairy characteristics. A similar project is starting also in Sumatra (Borghese, 2016).

In the Philippines there are 3.34 million buffaloes (FAO, 2010), 99 percent belong to small farmers that have limited resources, low income and little access to other economic opportunities. The buffalo, known as Carabao, is swamp type. Its history is basically of small-hold land-based agriculture, since for centuries, the Carabao has played a major role in draft animal-dependent farming, mainly in the production of major agricultural crops, such as rice, corn, sugar cane and coconut. The Carabao Development Programme is a massive programme started in 1993 to improve the native Swamp buffalo locally known as the Carabao to develop their meat, milk and draught potential. An elite herd of Riverine buffalo has been established at the Philippine Carabao Center, Science City of Muñoz by importing about 3 000 Murrah buffaloes with pedigree performance records from Bulgaria. The crossbreeding of Bulgarian Murrah (producing 1 800 kg per lactation) with a Swamp population (producing 400 kg per lactation, obtained F1 with 1 100 kg and F2 with 1 350 kg mean production respectively (Cruz, 2003). The program has established a distinct gene pool for the Philippines Swamp Carabao, apart from the gene pool of riverine buffaloes. This measure ensures that the outstanding indigenous animals are selected to form part of a continuing improvement program within the local animal breed. Along this line, there is also a gene bank where frozen semen, embryos and cells of outstanding animals are kept for future breeding usage (Cruz, 2010).

In the past Thailand had the second largest number of Swamp buffalo in the world. However this buffalo population drastically declined from 4.7 million in 1990 to 1.7 million (FAO, 2010). If the situation forces the buffalo population to decrease any further, the national buffalo population would risk disappearing completely (Suthikrai, 2002). Thai buffaloes are genetically of the Swamp type. There are very few farms that possess up to 50 head of buffalo and manage the herd as a commercial undertaking where animals are fed good quality feed and are well supervised. It is obvious that in general, there is no recording system approach at the farmer level as on the government farms. A project for the creation of a dairy buffalo breed in Thailand is going, to develop the milk market and satisfy the milk requirements by the people, finally to create an advanced Buffalo Institute in Thailand, by the Buriram Livestock Research and Testing Station of the Department of Livestock Development of Ministry for Agricultural and Cooperatives. In this project also the introduction of crossbreeding with dairy purpose breeds is foreseen.

ECONOMY FROM DAIRY BUFFALO IN THE AMERICAS

Today there is great enthusiasm about buffalo in America, particularly among buffalo breeders and livestock associations. Buffalo is considered to be the animal of the future, and there is justification for this. Buffalo numbers have significantly increased to about 5 million head and it is felt that breeding policies have led to an all-round improvement in quality. One of the characteristics that makes buffalo so widely used in these countries is their extraordinary ability to convert fiber into energy. Research trials indicate the superiority of the buffalo in food conversion and in the use of tropical forage and agricultural by-products. Therefore, it is emphasized that the buffalo does not compete with humans, for it does not necessarily use the main production from the crops. It is also an efficient tool in the recycling of nutrients in integrated production (Borghese, 2005). Other important characteristics



of the buffalo are their rusticity, their ability to adapt to different climates and their high fertility rates, always superior to those of bovines. Buffalo breeding is a synonym for low production costs and high levels of productivity (Rocha Loures, 2001).

Therefore the great availability of lands and the richness of pasture gave the opportunity to increase the number of buffaloes bred in extensive system for meat purposes as the meat market for export is a historical priority in South American countries.

But in the recent years the emerging request of cheese market produced a developing interesting for milk purposes, similarly to Italian feeding style. Therefore many American farmers developed the buffalo management for milking organization: this is the only role in the North American countries, but is coming more and more diffused also in Centre and South American countries.

The American country with the most of buffalo is Brazil with 3,500,000 head (Borghese, 2013). In some Brazilian States, buffaloes have become an economic option, mostly for their milk yield and, consequently, for the elaboration of mozzarella cheese, originally produced in Italy. This product is well accepted on the consumer market, and secures high prices due to the substantial demand. For this reason buffaloes have conquered a space in the national cattle husbandry sector and are no longer seen as marginal contributors to the meat and milk yield cycle.

Totally different is the management system in Amazonas lands, where the flood is the reality and the buffaloes swamp in lagoons during rain season, anyway the milk production is high for the use of Italian semen in Artificial Insemination by high genetic value bulls as Malandrino, Jafar, Napoli, obtaining several products as “La vera mozzarella” (Borghese, 2011). In the flood areas the used breed and their crossbreds are Jaffarabadi, Murrah and Mediterranean, the identification is on the horn and the health problems are brucellosis (30%) and TBC (16%) (Ribeiro, 2011).

To avoid the buffalo disappearing in Amazonia, one time the buffalo paradise, it is urgent a project for a new development of buffalo, taking in account the socioeconomic impact for the availability of draught animals, producing high value proteins for children as meat, milk and the effects on local market, where a lot of different products coming by meat and milk processing will be offered. To obtain these goals a new project was presented by Prof. Antonio Borghese at the XI Encontro Brasileiro de Bubalinocultores (Manaus 7-9 October 2015) for the socioeconomic development of Buffalo in the Amazon valley with the involving of the Pará State, UFOPA University of Amazonia, Amazonia State, Federal, FAO, International Buffalo Federation and Italian Institutes (Borghese, 2015). The first step is the creation of a Buffalo Centre as adequate space protected by flood, with controlled nutrition and reproduction, where the genetic improvement could be effected by artificial insemination using semen with high genetic value by Mediterranean Italian Breed. If the system of semi-intensive herd management will be adopted for regular, no-seasonal production, then modern husbandry techniques should be applied. An optimum feeding regime, utilizing Amazonian local green fodder will be applied throughout the year, and proper management would also be beneficial. The rate of conception could be raised to as high as 80 to 85 percent, compared to that achieved using traditional methods, less than 60 %.

After that there was a development of the milk production potential with the introduction of better genetic lines in Venezuela too. In the same way the technologies for milking cows, storing milk and for cheese production (together with technologies related to other milk products) are also developing. Although the promotion and expansion of buffalo production could solve the problem of the meat and milk deficit, there were many limiting factors such as government inertia regarding, the existing sanitary problems and the absence of national development programs (Reggeti, 2004). According Reggeti and Taracay (2006) there were 472 buffalos farms in Venezuela but no record were kept by the Venezuelan Buffalo Breeder Association, founded in 1986. Venezuela buffalo livestock, with 350 000 head on 700 000 hectares, produces 105 million kg of milk per year, 17 million kg of meat per year (Zava, 2011) as in Venezuela too buffalo is becoming a dual purpose animal.

In Colombia, with 250,000 buffaloes, the actual trend is to use buffalo for milk production too, increasing milk potential by importation of high quality semen from Brazil and from Italy, respectively Murrah and Mediterranean Italian, as milk is used for direct consumption, mixed with cow milk, or it is processed for producing cheese and sweets. The Colombian Buffalo Breeders Association is developing programs such as genetic improvement, genealogic registry programs, milk recording, ovulation synchronization, artificial insemination, embryo transfer, animal identification by ear and tail tattoos, skin and horns fire branding, skin cryogenic branding, microchips and inner stomach skittles (Roldan, 2005). This is an example of the great possibilities to increase milk production in Colombia, substituting original Buffalypso breed with dairy breeds as Murrah or Mediterranean Italian by crossbreeding. Today hand milking is the more used system for low cost of workers.



Figure 1. Mediterranean Italian Buffalo cow in intensive system. Tor Mancina farm, Rome (Photo: Borghese, 2006).



Figure 2. Kuhzestani buffalo cows. Ahvaz, milking in open air. (Photo: Borghese, 2005).



Figure 3. Murrah bull. Buffalo Research Institute, Hisar (Photo: Borghese, 2010).



Figure 4. Nili-Ravi Bull, Semen Production Unit, Qadirabad District, Sahiwal (Photo: Borghese, 2010)



Figure 5. Mediterranean Italian x Buffalypso in Bolson farm, Costa Rica.



Figure 6. Buffalypso herd with albino animal. Livestock Company “El Cangre”, Cuba (Photo: Borghese, 2011)



Figure 7. Bangladeshi buffalo in LAL TEER Livestock Breeding Farm, Jamuna, Bangladesh. (Photo: Borghese, 2012).



Figure 8. Indonesian Spotted Buffalo, Tana Toraja, Sulawesi (Photo: Borghese, 2008).



Figure 9. Carabao buffalo near Munoz, Philippines (Photo: Barile, 2004).



Figure 10. Fuel from manure in the villages (UVAS, Lahore, 2009)



Figure 11. Leather products from skin (UVAS, Lahore, 2009)



Figure 12. Italian mozzarella and ricotta.



In Argentina, with 100,000 buffalo head, the buffalo is used mostly for beef production, as meat export is very important for Argentina economy to introduce money from many countries of Europe, particularly Germany, USA and Canada. The milk production is effected in some farms in Formosa and Corrientes provinces utilizing selected groups of Murrah breed or Murrah x Mediterranean or Mediterranean, as Mediterranean Italian semen is continuously imported to increase rapidly the milk potential. The average milk production is 5-6 liters per day, the lactation length is about 240 days. The milk is used to produce mostly mozzarella cheese: 10 liters give 2.2 kg mozzarella (Zava, 2011). The mozzarella export is toward Chile.

In some, Center American countries as Costa Rica, Trinidad and Tobago and Cuba we find Buffalypso breed, a draft animal, mostly used for drawing carriages, for work in sugar cane fields and for meat purposes. The Buffalypso or Trinidadian Buffalo is the result of crossbreeding between the Carabao and other River breeds such as the Murrah, Nili-Ravi, Jaffarabadi, Surti, Nagpauri and Bhadawari, which was undertaken in the sugar cane factories of the Sugar Carone between 1920 and 1930.

The policy in Costa Rica, Cuba and in Trinidad and Tobago is to substitute the Buffalypso by crossing with dairy purpose breeds as Mediterranean Italian to increase milk production, according the requirements of the population and the new market. According Mitat, 2011, the buffalo population in Cuba is represented by 67 246 head, the breed is named Cuban Buffalypso, as the more evident characteristics are of this breed, even if the variability is very high, with albino animals too. As no selection was applied for dairy purpose, the milk production is very low: about 870 kg for lactation of 244 days. As no market was created for buffalo products, the consumer demand is very poor. In a trial of recording 826 lactations, the milk yield was 1011 kg in lactations of 244 days, 1232 kg in lactations of 305 days. In the 6th Symposium of Americas and Europe, held in Havana, Cuba, on November 21-30 2011, the Ministry of Agriculture declared the Government proposal to develop Cuban Buffalypso to increase the milk and meat production, as people need food rich of protein, as Buffalypso is able to utilize the pasture of internal land of the isle and the byproducts coming from sugar cane cultivations. For this purpose, artificial insemination has been applied with Mediterranean Italian semen, using too the semen of champion Millennium on Buffalypso herd.

Today in Trinidad and Tobago Buffalypso is considered as a dual purpose breed (milk and meat) and the population is 5,700 head (Rastogi, 2011). Different milk products are available in the market: yogurt, butter, ghee, mozzarella, quesoblanco, soft cheese, dahi and paneer (cottage cheese), very used in Indian vegetarian dishes.

In Costa Rica too, with 5000 head, the milk production from buffalo is going to be a priority, as in the farm of Mendoza family, where on rich and beautiful pasture of Brachiaria Mediterranean Italian x Murrah x Buffalypso crossbreeds are held, as the last one breed is the primitive genetic basis in Costa Rica too, as in all the countries of the Centre America, called Caribe. Mendoza farm products are: yogurt, caso (not stretched cheese), cacioricotta. The same goal is purposed by the Italacteos industry, of Italian family Puchino, with a splendid modern cheese industry, where mozzarella, treccia, ricotta and high quality cheeses are produced and after distributed in all the sites of Costa Rica and on Panama market. In the Puchino farm the crossbreeding with Murrah on the local Buffalypso was applied to increase the milk production and where a modern milking room is present. In the Bolsòn farm too, near Filadelfia and near one of the many National Parks of Costa Rica, the crossbreeding with dairy buffalo was applied: actually this farm can show herds of Mediterranean Italian buffaloes, obtained with substitution crossbreeding on local breed, with evident goal to create a dairy purpose breed and a milk and cheese industry.

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ANIMAL WELFARE ISSUES IN DAIRY FARMING

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ABSTRACT

The care and treatment of farmed livestock is a concern of stakeholders in the dairy foods system. Animal welfare is an issue no longer active only in western countries, it has become an important transnational trade issue for the global food supply chain. Therefore the World Organization for Animal Health has been implementing global guidelines for the care, transport and slaughter of terrestrial livestock. Animal welfare encompasses the social expectations for the care of dairy cattle [*Bos taurus*] and dairy buffalo [*Bubalus bubalis*] and the farmer's obligation to provide dairy livestock with a good quality of life. Animal welfare is composed of three primary and overlapping spheres of influence on an animal's wellbeing: biological wellbeing including good health, freedom from injury, proper nutrition, and provision of shelter; emotional wellbeing including freedom from fear and use of low stress methods for human handling and transport; and natural living which includes meeting the behavioral needs of dairy livestock by designing production environments that promote healthy adaptation. Any one or more of these spheres can become the focus of public scrutiny when questions about the state of dairy animal welfare arise. Issues requiring attention within the dairy industry include abusive handling, lameness, chronic confinement, poor health, inappropriate euthanasia, and transportation conditions. Taken together farmers are responsible for managing the production environment such that dairy cattle and buffalo are comfortable, healthy, productive and free of abuse. Scientists are engaged in research that assists with elucidating best practices for the care, handling, transport, slaughter, housing and management of farmed livestock. Translating scientific evidence into practice requires working with dairy farmers to understand the benefits of using best practices; and acknowledging and overcoming the practical aspects of implementing change.

Keywords: animal welfare, dairy, cattle [*Bos taurus*], buffalo [*Bubalus bubalis*]

INTRODUCTION

The world markets are becoming more interconnected on issues of social importance relative to agricultural food production practices including dairy farming. The issue of the care and treatment of farmed livestock has been entertained by national and international government bodies, non-governmental organizations, and private institutions. Multinational corporations that market food have added internal business policies on food production that have created a new environment for agricultural production (Schweikhardt and Browne 2001). Corporate social responsibility statements (Doh and Guay 2006) and a commitment to address consumer concerns include animal welfare (Thompson et al., 2007; Olynk and Ortega 2013), food safety, human health, environmental sustainability, and the treatment of labor. As a condition of doing business farmers are also expected to meet corporate policies on animal welfare.

Multinational corporate policies on animal welfare may impact a nation's export markets for dairy products and even influence public policy making within a country. However this is not the only reason to consider animal welfare in the routine care of dairy cattle and buffalo. Attention to animal welfare also results in improvements to animal health and productivity (von Keyserlingk et al., 2009). An additional benefit is the public assurance that comes with knowing food producing livestock like dairy cattle and buffaloes have lived an acceptable quality of life.

This paper will provide an overview of the topic of animal welfare issues in the dairy industry. Selected research reviews, studies and books on dairy cattle and buffalo are used to provide examples and support for the concepts and topics presented in this paper.

Defining Animal Welfare

Animal welfare poses both ethical, cultural, scientific and practical questions about what constitutes "best practices" to support good animal welfare in the farming of dairy cattle and buffalo. The first step is to develop a working definition of animal welfare and to identify its constituent domains. The definition of animal welfare is not only influenced by the biological constituents that promote the mental and physical wellbeing of an animal but the social perceptions and expectations for providing a good quality of life.

The World Organization for Animal Health, also known by its French acronym OIE, (<http://www.oie.int/>) has long been recognized by its work in animal disease and health. The World Trade Organization (WTO) has

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used the OIE health recommendations to address animal health issues in trade actions or policy. At the request of its member nations, OIE added animal welfare to its animal health mandate in the mid-1990s. By 2008 the OIE had assembled a steering committee of representatives from its member nations and scientific work groups for the different farmed species. OIE commenced the development of basic global standards of the care, transport and slaughter for terrestrial animals including farmed livestock, and released its definition of animal welfare:

“According to the OIE *Terrestrial Code*, “animal welfare means how an animal is coping with the conditions in which it lives”. The OIE guiding principles on animal welfare also mention the universally recognised “Five Freedoms”, published in 1965 to describe the right to welfare of animals under human control.” (World Organization for Animal Health, 2008).

The “Five Freedoms” referenced by the OIE are based upon a U.K. government appointed committee report known as The Brambell Report (Report of the Technical Committee 1965). The committee was led by a scientist (Professor Roger Brambell) appointed to examine the growing public concerns about the confinement raising of livestock. The original report issued statements addressing the restriction placed on animal movement under emerging confinement housing conditions. Based on its findings, the committee identified basic metrics of what an animal ought to be able to do including the ability to stretch their limbs, lie down, groom themselves and to turn around. Over time the freedoms have been revised under the Farm Animal Welfare Commission (Farm Animal Welfare Council 1979) to address other important aspects of animal welfare. They have solidified into the Five Freedoms listed below.

- Freedom from hunger, malnutrition and thirst;
- Freedom from fear and distress;
- Freedom from physical and thermal discomfort;
- Freedom from pain, injury and disease; and
- Freedom to express normal patterns of behaviour.

As a result of the OIE adoption of the Five Freedoms they have had a powerful impact on the development of animal care standards/guidelines, codes, directives and laws around the world. The original intent of this list of five basic animal welfare outcomes were to guide policy decisions, bodies addressing animal care practices, scientific research, public assurance, and to help the agricultural animal industry maintain its place in food production worldwide.

The Constituents of Animal Welfare

The study of animal welfare requires a working conceptual model (Carenzi and Verga 2009; Hemsworth et al., 2015). Using a Venn diagram, David Fraser (1997) first laid out the primary domains of animal welfare. In three centrally overlapping circles he identified the domains of animal welfare as biological functioning, affective state, and natural living. Each circle has space of its own and space that overlapped with the other two circles indicating interactions and shared indices of animal welfare between them. Fraser also linked these domains to public concerns about animal treatment (Fraser, 2003). Of special note is animal and veterinary scientists have historically concentrated most of their research on the biological functioning of an animal based on mandates of achieving high productivity, efficiency and good health. Overtime the public has gravitated to questions concerning the emotional state of the animal such as conditions that promote fear and suffering (negative affective state) or promote contentment (positive affective state). More recently questions are have migrated to asking whether farm animals are living a life consistent with its nature (natural living) which focuses on issues such as confinement housing, access to grazing, behavioral integrity, and the provision of more enriched environments. The following descriptions of each domain of the working conceptual model of animal welfare is based on Fraser’s work (1997; 2003).

Biological functioning: Biological functioning in general includes disease, injury, preventative health, productivity, animal efficiency, animal nutrition, genetics, stress physiology and other objective indicators of animal biological functions. Historically studies of biological function make up the majority of research in the veterinary and animal production sciences.

Affective State: The affective state of the animal is its emotional state which can be positive or negative. Questions encompass painful conditions (including chronic lameness) leading to suffering, conditions of treatment and handling practices that promote fear and distress, and conditions leading to animal boredom or frustration such a barren environments. Scientific research in this area often overlaps with biological functioning research and also engages the behavioral and neurosciences to understand what the animal experiences and the identification of how best to measure and assess these affective states. Research has also begun to make progress in measuring and identifying positive affective states such as animal contentment, the role and need to play, and other states where animals signal pleasure and adaptiveness to their environment, management practices, and interactions with humans



(Mellor, 2015a).

Natural Living: Most farm animals have retained hardwired behaviors that create a desire to be performed even in the absence of stimuli required to fulfill the behavioral need. In some instances abnormal behavior or stereotypies erupt including tongue rolling, teat sucking and other damaging or repetitive behaviors in dairy animals. The effects of lack of grazing, exercise, chronic tethering, use of tie stalls, standing on cement, and other types of practices and housing used in dairy farming has been the focus of study. Public perceptions and concerns have also driven a focus on the natural lives of animals (Fraser, 1997 and 2003; von Keyserlingk et al., 2009) and the provisioning of environments conducive to satisfying innate behavioral needs and providing a good quality of life for farmed animals (Mellor, 2016).

Von Keyserlingk et al. published an invited review of dairy cattle welfare and the key role science plays in addressing questions of animal welfare. The authors cite specific examples of dairy cattle research conducted within each domain of animal welfare that have advanced knowledge and shaped best practices in dairy farming. Although fewer in number, scientific reviews focused on the behavior and welfare problems of the dairy buffalo have been published (De Rosa et al., 2009; Napolitano et al., 2013). In general more research has been conducted on the welfare problems of dairy cattle than buffalo. However they share common overlapping issues of relevance to their welfare.

Common Dairy Welfare Issues

By its nature dairy farming has common elements worldwide including the milking of cattle and buffalo, and the care, nutrition and management practices that enable milk production. Variable levels of confinement housing and restraint are necessary to facilitate the ease of milking livestock. There is a dual nature of the work these animals perform in producing milk, meat and in some cases performing draught work. Listed below are common focal areas where animal welfare issues arise in dairy animal farming.

Animal Treatment and Handling: Dairy farmers maintain daily close human animal contact through the milking and movement of cattle and buffalo. The temperament of the animal for high tolerance of human contact (Haskell et al., 2014), and the attitude and expert handling skills of the farmer (Coleman and Hemsworth, 2014), play key roles in mitigating problems related to the poor treatment of animals. Numerous studies have shown how the stockmanship and handling skills of dairy farmers impact the quality of life and ultimately the productivity of dairy animals (Hemsworth et al., 2000; Hemsworth, 2003). Poor handling and treatment of milking livestock cause fear and distress that impacts milk production. Gaining an excellent understanding of the natural behavior of dairy cattle and buffalo, the use of low stress handling techniques for these species, well designed facilities, and genetic selection for docile temperaments greatly enhance animal welfare.

Lameness: Lameness is a painful condition and as such causes suffering of the lame animal. Lameness induced by housing flaws including flooring type and poorly designed stalls as well as lack of hoof care and unsanitary conditions has been a mounting problem in dairy animals (Adams et al., 2016; Bouffard et al., 2017). Animals with pasture access can develop lameness too. Research, focused primarily on high confinement dairy cattle farming, has been and will continue to be conducted to discover new ways to prevent, detect early stage, and treat lameness. In addition to improving the living conditions to prevent lameness, scheduled hoof care can be important to maintaining good foot and leg health. Regardless of the production system the monitoring of foot and leg health is important to dairy cattle and buffalo welfare (Guccionne et al., 2016).

Painful Procedures: Special procedures that produce animal pain and discomfort garner considerable attention from the public. Tail docking (in steep decline due to changes in policy or recommended practices; Sutherland and Tucker, 2011), disbudding and dehorning (Stafford and Mellor 2011), and the removal of supernumerary teats in dairy livestock have been the subject of animal welfare concern. The lack of provisioning anesthetic or analgesic relief to animals undergoing the procedure, the method used, and the necessity of the procedure (tail docking in particular) have come under intense scrutiny (von Keyserlingk et al., 2009). Recent advances in genetic technologies hold promise of producing polled dairy cattle breeds (Tan et al., 2013). And research into effective pain mitigation strategies (disbudding example; Winder et al., 2017) for dairy animals undergoing painful procedures has also advanced knowledge about how and when an animal experiences pain (Gleerup et al., 2015).

Culling and Timely Euthanasia: End of life or culling decisions for dairy cattle and buffalo are often hard for dairy farmers to make. In some cases religious restrictions forbid the farmer to euthanize the moribund animal. For many farmers, the first instinct is to attempt to recover a seriously ill or injured animal. Dairy animals are valuable and represent an economic loss when they become old, ill or injured. Moreover there is a human-animal bond that fortifies the desire to continue treatment well beyond the time an animal should be culled or euthanized. The issue of timely culling or euthanizing of animals has caused unintended suffering and animal welfare issues for the dairy industry (Langford and Stott, 2012). Research has been conducted to help identify reasons for dairy animal



mortality (Compton et al., 2017) to better decide when culling or euthanizing an animal should be implemented. Research has been coupled with outreach programming (American Bovine Practitioners Association, 2013) to teach farmers which methods are most effective and humane when conducting on-farm euthanasia especially for debilitated animals that cannot be transported to a slaughter facility (Mueller, 2015).

Cow Nutrition: Dairy cattle and buffalo nutrition is vitally important to the welfare of the animal. Animal nutrition impacts the maintenance of good health, fertility, and productivity (Robinson et al., 2006; Sabia et al., 2015). In the dairy industry it is well established that pregnancy and lactation exact high costs not only to the cow but also to the calf if not managed well. The nutrients required to maintain these high cost physiological activities deserve special attention. Cows that are marginally fed and consuming low quality forages do not maintain the body condition required to adequately support a healthy calf or profitable milk production. The quality of milk may also be affected by poor nutrition. Body condition scoring to obtain an estimate of fat cover (energy balance) has been one method used by farmers to guide herd nutrition. There is an abundance of literature on dairy cow and buffalo nutrition (Sawar et al., 2009). The provision of guidance to dairy farmers through extension or outreach programming helps farmers to develop sound nutritional programs for their dairy herd.

Calf Care: The care of female replacement dairy calves, and the care and disposition of male calves, is an animal welfare concern for the dairy industry (Mee 2013; Windeyer et al., 2014). In particular, the timing and feeding of adequate amounts (Vecchio et al., 2013) and the correct preparation and storage of colostrum (Cummins et al., 2017) and milk replacers; and appropriate nutritional regimens for the growing calf. The weaning protocols practiced by dairy farms makes the feeding of colostrum and milk replacers to calves important (Patil et al., 2016; Dunn et al., 2017). Research has found that calves can be undernourished and left with a passive immunity deficit (Yang et al., 2015) due to poor protocols for the handling and feeding of colostrum milk and follow-up milk replacers. Even if calves are nursing cows, as practiced in buffalo production, attention to cow nutrition, timing of nursing (if not free choice) and milk intake are important to calf nutritional health.

The care and disposition of male calves is a dairy welfare issue. The neonatal male calf may be underfed and poorly housed due to their low value to the dairy farmer (Webb et al., 2015; Renaud et al., 2017). Finding value-added markets for male calves that place emphasis on calves arriving in good health and nutritional state (El-Deeb and El-Bahr 2014) greatly encourage farmers to invest more time and resources in raising male calves. In general inattention to calf nutrition and health can carry production set-backs whether the calf serves as a replacement heifer for a retiring member of the milking herd or is marketed into the meat supply chain.

Milking Protocols: The protocols implemented for daily milking do have impact on udder health. Poor milking technique, sanitary procedure and dry-off protocols allow for the introduction of mammary disease and infection. This can impact milk safety and quality as well as animal welfare. Mammary diseases such as mastitis can become chronic problems in a dairy herd. It is a painful condition and causes set-back in milk production. Dry-off protocols have undergone study with respect to the effects of abrupt versus gradual dry-off practices on mammary health (Silanikove et al., 2013; Zobel et al., 2013 and 2015; Silanikove 2014; Gott et al., 2016). Research abounds on both topics and the development of sound milking protocols and procedures for daily milking are important to maintaining good animal welfare.

Transition Cows: The period of three weeks before calving and three weeks after calving is one in which cows are highly susceptible to the development of metabolic diseases like ketosis (Youssef et al., 2010; Raboisson et al., 2014) and post calving issues such as retained placenta. The nutritional management of cows experiencing this physiological transition (Fiore et al., 2017) is highly important to the cow's health, rebreeding (Zebeli et al., 2015; Kalasariya et al., 2017) and ability to reach peak milk production (Esposito et al., 2014). Cow management approaching, during and post calving should be carefully monitored to prevent issues that can lead to higher herd morbidity and mortality and lower milk production. The proper management of the transition cow is considered an important animal welfare concern for the dairy industry.

Transportation: The transportation of livestock has long been an issue connected to animal welfare. It combines the elements of animal fitness, human handling, method of transport, trailer or lorry design, time in transport, and post transport handling practices. Transportation poses one of the greatest stress challenges to dairy livestock. Animal distress is cumulative under conditions of transport. For example, a neonatal dairy calf undergoes weaning stress, handling, and typically feed withdrawal pre-transport (Fisher et al., 2014). If vehicular transport is not used to take cattle or buffalo to market or slaughter, then driving and handling methods, rest, the provision of water and feedstock during the journey, distance travelled, and containment facilities enroute and upon arrival are important considerations. Problems can arise during each phase of transportation causing animal distress, injury, fatigue and debilitation which can lead to morbidity/mortality during and post transport. There are guidelines and standards, and in some countries laws, concerning animal transportation. Research and books on the transportation of livestock including dairy animals are available (Chandra and Das, 2001; Grandin editor, 2014; El-Deeb and



El-Bahr 2014). The OIE has developed recommendations for the transportation of terrestrial livestock (see World Organization for Animal Health. Terrestrial Animal Health Codes). The OIE recommendations may be a good starting place when no guidance is available from other resources.

Thermoregulatory Environment: Heat and cold stress are a concern in dairy farming (Cox et al., 2016). It is well known that heat stress depresses milk production especially in breeds of livestock not selected for or adapted to hot environments. Even so, there are implications for heat sensitivity for dairy buffalo living in hot environments (Kumar et al., 2015; Dash et al., 2016; Sevegnani et al., 2016). With the introduction of *Bos taurus* breeding such as Holstein to hotter climates special attention to weather adaptation and the use of heat relief measures to mitigate the effects of heat stress are important (Ferrazza et al., 2017). Provision of shelter, shade, well-ventilated housing, sprinkler systems, and free access to potable fresh water during heat waves can prevent large reductions in milk production and in the most severe conditions animal death (Caja et al., 2016).

Cold stress is an issue for dairy farming conducted in temperate climates. In these regions confinement housing during the cold winter months is typical. Protective housing that can capture animal body heat to warm the building (or the use of safe auxiliary heating devices), adequate space and bedding for animal comfort, and good ventilation of fresh air to prevent respiratory disease and gas build up, become important factors to monitor. Animal nutrition during heat and cold stress is important. During cold weather there is higher energy expenditure (Litherland et al., 2014) to maintain core body temperature. During heat stress lower body energy requirement and decreased feed consumption occur. Diet formulation for dairy cows may need to be more closely monitored and fine-tuned to improve the precision of nutrient use and cow productivity according to seasonal conditions (White and Capper, 2014).

Within each animal welfare concern listed above other types of animal welfare issues can be identified. These major issues have produced social scrutiny and stimulated scientific research and extension/outreach programming to offer solutions to dairy cattle and buffalo farmers. To encourage continuous improvement in dairy cattle and buffalo welfare, the dairy farmer must have access to the best and most recent information on dairy animal care and management. Considerable work has been conducted to identify measures of animal welfare that dairy farmers can use to assess their handling practices, facilities, management, and care of their dairy herd. To this point efforts have been undertaken to bring tools to dairy farmers that help them to monitor their use of best dairy animal welfare practices.

Assessing Dairy Cattle and Buffalo Welfare

Guidelines and standards, both voluntary and in-voluntary (laws or mandated private policies), have been developed and used to assess the welfare of dairy cattle and buffalo. Three basic types of measures have been used to assess the on-farm welfare of dairy livestock: animal-based, resource-based and management-based (von Keyserlingk et al., 2009; Green and Mellor, 2011; DeVries et al., 2015; Mellor, 2015b).

Animal-based Measures: Animal-based measures are used to assess the physiological and mental welfare of the animal. Examples of measures include disease and mortality rates, incidence of lameness, body condition, incidence of abnormal behavior, and other measures that are gathered specific to the animal.

Resource-based Measures: Resource-based measures include the type and condition of facilities, housing design (Caja et al., 2016), ventilation, fencing, quality of pasture or dry-lot (Chen et al., 2017), feed and water supply, milking facilities and machines (if used) and other types of conditions and resources used to support dairy cattle and buffalo welfare.

Management-based Measures: Management-based measures focus on the human dimension of maintaining conditions conducive to good animal welfare (Cook et al., 2016). These include the training of employees or family members to properly handle and care for dairy cattle or buffalo, preventative health maintenance programs used to promote good health, biosecurity to prevent animal disease, the management of animal nutrition, provision of veterinary care, and protocols/methods used to perform standard dairy animal procedures such as milking, breeding, calving etc. These measures are indicators of how well farmers implement best practices in the management of the animals to create conditions that support good animal welfare.

The combined use of the types of measures listed above are coupled with written guidance documents that articulate best practices to the dairy farmer. Assessing on-farm practice helps farmers to determine where improvements need to be implemented. Most of the dairy farm animal welfare assessment programs to date have been developed for dairy cattle. However, there is a growing body of literature focusing on the on-farm welfare assessment of the dairy buffalo (Zucali et al., 2016; De Rosa et al., 2005 and 2015). There is ample opportunity for dairy scientists and extension/outreach professionals to further develop guidance documents and on-farm assessment tools to help farmers achieve a standard of high quality of life for their dairy herd. This also facilitates meeting the public expectation for providing a good quality of life to dairy livestock.



SUMMARY

Scientific progress has been made in the study of dairy animal welfare. In combination with the attention to the issue of animal welfare by multinational food corporations and globally important organizations such as the OIE and WTO, it is imperative that dairy farmers and cooperatives worldwide consider the quality of life of the dairy cattle and buffalo they farm. The state of dairy animal welfare can affect the ability to enter supply chains, marketability of the milk or meat product, and most of all the public image of dairy farming. Improvements to animal management practices that promote high biological functioning, reduce fear and suffering, increase animal contentment, and living environments that accommodate important behaviors creates higher economic and social payoffs for the dairy farmer too.

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MIND THE GAP: AWARENESS AND PRACTICES AMONG NEPALI BUFFALO FARMERS RELATED TO ZOOSES

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ABSTRACT

Increasing livestock production to meet growing demands has resulted in greater interactions at the livestock-wildlife-human interface and more opportunities for emergence and spread of zoonotic pathogens. Livestock-associated zoonoses can have substantial impacts on health and livelihoods, undermining effective use of livestock for food security and income. Several livestock-associated zoonoses have been reported in Nepal. However, little is known regarding Nepali farmers' awareness of zoonoses and on-farm disease prevention practices. We conducted a study to investigate the knowledge base of Nepali farmers regarding livestock zoonoses, assess current health challenges, and identify farm practices that could modify transmission risk. We found that farmers' awareness of zoonoses was limited. Moreover, informally educated farmers, mainly women, were less knowledgeable about zoonoses and sought recipient of fewer livestock services than educated farmers. Further, farmers' preventive herd health, food safety, and sanitation practices were unaffected by their awareness of zoonoses, suggesting disconnect between the farmers' knowledge and practices. Our study highlights the need for improving Nepali farmers' knowledge regarding zoonoses and practices to reduce risk. Closing these knowledge practice gaps will require an improved understanding of farm-level risks through investments in disease surveillance and working with farmers to design targeted gender-inclusive training and disease prevention programs.

Keywords: Buffalo, cattle, Nepal, zoonoses, smallholder farmer

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BUFFALO PRODUCTION FOR HOUSEHOLD FOOD SECURITY IN NEPAL

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ABSTRACT

Food security provides a household the ability to acquire or consume an adequate quality diet or a sufficient quantity of food in socially acceptable ways. Buffalo in Nepal, with a population of 5.2 million in 2015/16, continues to be economically the most important livestock. Especially, the animal holds a special place in the economy of smallholder farms, fulfilling their manifold household needs. Buffaloes provide nutritious food, agricultural inputs, agricultural sustainability and cashable resource, serving as a dependable household asset. Many of the outputs of buffalo production system have pathways leading to the household food security, especially so in rural settings, where majority of the people live and work. In 2015/16, the milk production from buffalo (1,210 thousand MT) constituted 65% of the total milk production, and the buffalo meat production (175 thousand MT) constituted 54% of the total meat produced in the country. However, the current level of buffalo production is far less than optimal that is attainable, for producing food adequate to feed the ever growing population and for fulfilling potential multiple roles the buffalo can play in the household and the national economy. While buffalo is the main source of milk and meat in Nepal, the country is still deficient in minimum annual per capita consumption by 30% milk and 14% meat. A potential strategy for the development of composite breed types by interbreeding exotic blood types with the indigenous mixed stock, supported by improved production management, improved reproductive performance, adequate veterinary care and marketing is proposed. This can lead the buffalo production to a future state surpassing self-sufficiency in domestic milk and meat consumption needs at the adequate level, contributing towards household food security. A strong political will, strategic policy directions and prioritization of resources for an integrated national plan is essential for its realization.

Keywords: Food security, buffalo production, smallholders' farms, composite blood types

INTRODUCTION

The households become food secure when they have physical, social and economic access at all times to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life (WHO, 2017). Food security provides a household the ability to acquire or consume an adequate quality diet or a sufficient quantity of food in socially acceptable ways (Li et al., 2016).

Food security is not merely providing food to feed the hungry to meet the calorie needs of a person. As household food insecurity is one of the three underlying causes of malnutrition (other two are: poor social and care environment, and poor access to health care and unhealthy environment), it is rather a public health issue and is foundational to healthy eating, and is related to a number of health and social challenges that may arise for food insecure households- birth outcomes and maternal health, child development, health status and chronic diseases, mental health and emotional wellbeing, health care costs (Li et al., 2016).

In the context of Nepal, many issues of food insecurity and related nutritional gaps and deficiencies exist across agro-ecological zones. For an example, a study across three agro-ecological zones showed that the household common diet lacked sufficient vitamin B12, riboflavin, and calcium in the mountains; B6, B12, calcium, and iron in the hills; and vitamin A, calcium, and iron in the Terai (Biehl et al., 2016).

Globally, the need for buffalo development has been identified as a driving force for food security, self-sufficiency and sustainable development (Chantalakhana, 2001; Soliman, 2008; Mahmood et. al., 2014; FAO, 2017) due to manifold purposes the buffalo serves and high potential it has for increased production for reducing hunger and food insecurity. Several deficiencies of nutrients- proteins, fats, minerals and vitamins can be filled by increasing the production, marketing and access of buffalo milk and meats. Buffalo serving as a living bank for small farming households serves the purpose of securing food in emergency by disposing the buffalo for cash. In the overall scheme of a small farming household, buffalo holds a special position by providing asset for household economy, agricultural inputs (manure, draft power), and capital growth.

This paper aims to discuss the current and future state of buffalo production in the context that the future potential for buffalo improvement can be realized for ensuring food security in Nepal.

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BUFFALO PRODUCTION OVERVIEW

Buffaloes in Nepal have the total population of 5.2 million as of 2015/16, with their major regional-agro-ecological concentrations in Western Hills (863 thousands), followed by Central Hills (716 thousands), Eastern Terai (587 thousands) and Central Terai (779 thousands) (MoAD, 2017). They continue to be economically the most important livestock. They hold a central place among all livestock in the economy of Nepalese farms, accounting for 53% of the livestock share in the country's gross domestic products (GDP), fulfilling the manifold household needs, by providing nutritious food, agricultural inputs, agricultural sustainability and cashable resource, serving as a dependable household asset (Rasali, 2000). Many of the outputs of buffalo production systems especially in the small farming systems in the country have production pathways leading to the household food security (Rasali, 2015), especially so in rural farm settings, where majority of the people live and work.

In 2015/16, the milk production from buffalo (1,210 thousand MT) constituted 65% of the total milk production, and the buffalo meat production (175 thousand MT) constituted 54% of the total meat produced in the country (MoAD, 2017). However, the current level of buffalo production is far less than optimal that is attainable, for producing food adequate to feed the ever growing population and for fulfilling potential multiple roles the buffalo can play in the household and the national economy. While buffalo is the main source of milk and meat in Nepal, the country plans to meet the deficits in minimum annual per capita consumption, about 30% milk and 15% meat, by 2018/19 (MoLD, 2017). Expansion of buffalo production through improvement in genetic resource base and management of buffalo would be required for meet the needs of households for food security, while improving access to remote and rural areas will be a huge challenge to meet as well.

Indigenous Breed Types

Nepal has a diversity of buffalo breed types (Shrestha, 1996) owing to geographic isolation of animal breeding systems from time immemorial across three major agro-ecological zones: high mountains, hills and Terai plains. So far the indigenous breed types, namely Lime and Parkote in the western hills and Gaddi in the far-western hills, Terai Native type with some of their intermediate variant types are identified and characterised. Rasali et. al. (1998) statistically grouped the western hill buffalo into four main canonical cluster types that are consistent with four phenotypic types: Lime, Parkote, Lime dominant mixed (intermediate) type, Parkote dominant mixed type (Figure 1). Gaddi buffaloes characterised from the far-western hills are reported to be morphologically larger in body size (Kumar and Raj, 2007). Terai Native buffaloes, though low in productivity, are well adapted to different climatic zones in Terai and low hills, and can be crossed with Murrah for breed improvement (Minematsu et. al., 1996).



Figure 1. Four phenotypic clusters of hill buffalo types- Parkoto (far left), Lime (middle left), Parkote dominant intermediate type (middle right) and Lime dominant intermediate type (far left) based on external phenotypic characteristics were found in the Western hills (Rasali et al., 1998).

Karyotype Issue

Due to some similarity of phenotypic appearance of Lime buffalo with swamp buffalo that have ($2n=48$) chromosomes in other South Asian countries, there was a long held notion that Lime were swamp type that do not successfully interbreed with riverine buffalo with ($2n=50$) chromosomes. Rasali et al. (1998) studied, for the first time, the karyotype of Lime and Parkote breed types, and confirmed both having ($2n=50$) chromosomes, resolving the issue that they were all riverine type. This confirmation has cleared up the confusion regarding crossbreeding of Lime with riverine breeds such as Murrah buffalo, which was already in practice since 1970s.



Cross-breeding and Milk Recording Studies

Since the 1980s, the Lumle Agricultural Research Centre implemented a field campaign type programme of crossbreeding of local buffaloes in the districts of western hills with Murrah buffalo using artificial insemination. This was considered complementary to the district livestock services program of distributions of breeding buffalo bulls to the rural villages throughout the country. As a result, a sizeable number of Murrah crossbred buffaloes were born and raised in various districts of western hills, where field milk recording of local and crossbred buffaloes became possible. In the late 1990s, on-farm milk recording study of local hill buffalo, buffalo with 25-49%, 50-74% and 75% Murrah blood in the western hills indicated that there was a gradient of increase in lactation yield to 1013, 1191, 1440 and 1872, litres respectively (Rasali et. al., 1997).

Nutrition, Health and Reproduction of Buffalo

Buffalo production system in Nepal requires adequate attention to improvement in nutrition, health and reproductive performance of buffaloes in order for them to produce optimally, playing a significant role in food security. Buffaloes utilize non-conventional feeds efficiently, but would need adequate forage development for higher productivity. Control of disease conditions such as the Foot and Mouth Disease (FMD), Haemorrhagic septicaemia caused by *Pasteurella multocida* (HS) and internal parasites (Rasali, 2015), and reproductive disorders such as repeat breeding and anoestrus (Sah et al., 2012) is prerequisite for improved efficiency in the production system.

Prospects of Composite Blood Types

Procedures employed by researchers to create varieties of composite breeds have been well documented. The genetic divergence of indigenous breed types, especially across various mixed breed types existing across buffalo populations provides us an opportunity to create a '*composite breed*' for higher production and adaptability as a new breed. Substantial progress in crossbreeding of indigenous stock with Murrah blood by natural service or artificial insemination achieved so far has created crossbred populations of Murrah and indigenous breed types in pockets of geographical areas. Intermediate mixed breed type clusters of especially, Lime and Parkote dominance, can be used as two-breed crosses for crossing with Murrah breed or even crossbreds. Finally, the population resulting from multiple crosses is closed after the second generation, followed by *inter se* mating for a number of generations until composite breed stabilization. This nearly parallels with a simple procedure of creating a composite breed of sheep as described by Rasali et al. (2005). Thus, a potential strategy for the development of *composite breed types* by interbreeding exotic blood types with the mixed indigenous stock and their variants with or without the exotic blood, supported by improved production management, improved reproductive performance, adequate veterinary care and marketing is proposed for further discussion and planning.

CONCLUSIONS AND POLICY IMPLICATIONS

Nepal is one of the four countries, including Pakistan, Egypt and India, in the world that have the numbers of dairy buffaloes exceeding the numbers of dairy cows (Moioli, 2005). Despite the central role of buffaloes among livestock in the national economy of Nepal being undisputable, they have not yet received adequate national and international attention they deserve. A strong political will, strategic policy directions and prioritization of resources for an integrated national plan is essential for successful realization of improved buffalo production for food security. As national breeding programme has focused thus far only crossbreeding by Murrah to upgrade indigenous buffalo, a more enhanced genetic improvement based on milk performance recording has now been warranted to boost the milk production to make the households food secure and self-sufficient. A simplistic open breeding scheme proposed by Rasali and Joshi (1996) should be revisited for further development along the line of forming composite breed types for implementation, where feasible, as suited to agro-ecological zones. The formation of composite breed types and improvement in their feeding, production management, reproductive performance and veterinary care can lead the buffalo production to a future state surpassing self-sufficiency in domestic needs of milk and meat consumption at the adequate level. Accordingly, the following components of policy implications can be drawn:

- 1) Eastern and Western hills can be selected for the first stage of composite breed formation, as there exist geographical pocket areas with a sizeable stock of Lime and Parkote dominant mixed type and Murrah crossbred populations. All breeding operations should be organized on-farm.
- 2) Buffalo research centers for Lime at Lumle Centre, Parkote in Rampur Campus and Lampatan Farm, Terai Native in Tarahara Farm, Murrah in Lampatan Farm and Tarahara Farm, and Gaddi in a suitable location in Far-Western hills should be established or reinforced, maintaining a reasonable nucleus breeding stock of a breed (about 50 heads) in each location.



- 3) A series of specific projects on following research areas should be prioritised: a) high altitude buffalo production research on Lime buffalo, which has potential for utilization of abundant green forage of the high hills ranges; and b) research on reproductive performance of buffalo cows, fattening research of buffalo calves, preventative veterinary care, nutrition, market based production strategies in all agro-ecological zones.
- 4) A satellite buffalo farm in each of the newly formed provinces is recommended for providing a resource base for farmers' level production.
- 5) Agriculture and Forestry University (AFU) and Nepal Agricultural Research Council (NARC) should initiate a national collaborative buffalo research program such that strength of academic research of (AFU) is well coordinated with the governmental research mandate of NARC in the sponsorship of the Ministry of Livestock Development.
- 6) Multi-lateral or bilateral cooperation for buffalo research and development should be sought through Food and Agricultural Organization (FAO) for implementing the aforementioned project components as priorities.

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ROLE OF BUFFALO FOR DIVERSIFIED ENTERPRISES DEVELOPMENT IN NEPAL

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ABSTRACT

Buffalo is one of the integral components of the Nepalese farming system, mainly for milk and meat but there are numerous MSMEs based on raw materials originating from buffalo. The country with Majority of the Hindus worship cattle as god but the existing social norms, values and legal forces are in favor of promotion of buffalos in Nepal but there are scanty records and evidences scientific world. This article highlights the findings of dairy value chain study done by Heifer International Nepal in 2015. In 2015, total of 12.4 million heads of cattle and buffalo were farmed, only 41.4% were buffalo where only 26.2% were productive and contributing 65.3% in total (1.9 mt) milk production. Data from 2001 to 2015 revealed that the AAGR on total buffalo population (2.5%) and 3.0% on buffalo milk production. Different N/GOs contributed to boost the Nepalese dairy mainly in innovation, technology and extension but majority of them are focused on cattle, with no or poor priority on buffalos. The average cost of milk production was Rs 31.5/lit, notably higher for buffalo milk and the farm gate price was also higher for buffalo milk. Majority farmers preferred buffalo enterprise over cattle due to their multiple utility, lower risk and hardy nature against stresses. Buffalo is consistently major contributor (60%) for meat in Nepal. Numerous enterprises are based on different body parts of buffalo but lagging behind due to poor access to technology and promotional support. In spite of multiple benefits of buffalo, as a commodity and as an enterprise, limited research and development efforts were found on an around it. The study found adequate space and great prospect of buffalo for building sustainable enterprises and reducing poverty in Nepal and strongly recommend concerned agencies and authorities to pay attention for its promotion.

Keywords: Buffalo, enterprise, value chain, dairy, poverty

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THE ECONOMIC LOSSES AND BURDEN OF ENDOPARASITES IN BUFFALO AND CATTLES OF RURAL PUNJAB “AN UNDERESTIMATED EMERGING THREAT”

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ABSTRACT

Parasitism causes serious clinical infections, welfare problems and enormous losses in terms of production in buffalo (*Bubalus bubalis*) and cattle of rural smallholder farmers. Therefore, a cross sectional population based survey in parallel to mass vaccination, the first of its nature in Pakistan was conducted in province of Punjab to estimate the point prevalence and economic losses due to endoparasites in large and small ruminants, by endeavors of directorate of animal disease reporting and surveillance from January to March 2015. Fresh fecal samples were collected randomly from (n = 242,106) large and small ruminants all over rural Punjab. Standard parasitological techniques were performed to spot parasite eggs and larval determination of specific parasites was performed by copro-culturing. The result showed a cumulative higher prevalence of 57.02%. Cattle (57.15%) were found affected the most followed by buffaloes (54.43%). Geographically in South Punjab (75.8%) samples were positive, significantly (P<0.05) higher than (41.3%) in North Punjab and (35.37%) in Central Punjab. Amongst the parasite families specifically nematodes were (26.94%) found highly prevalent than trematodes and cestodes. The economic losses due to reduced milk and meat production calculated were approximately PKR: 12701.06 million (122.12 Million USD) per month in the Punjab. It was concluded that endoparasitism is one of the major health problems prevailing in livestock population under rural management causing massive economic losses and stern clinical infections. The results of this mega survey will have vital implications in future for the endoparasitic infections control in small and large ruminants in Punjab province, Sub-Tropical Continental Lowlands of Pakistan.

Keywords: Parasitism; buffalo; cattle; copro-culturing and Pakistan.

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DEVELOPMENTS AND PROSPECTS OF ADVANCE REPRODUCTIVE BIOTECHNOLOGIES IN BUFFALO PRODUCTION

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ABSTRACT

The multiple ovulation and embryos transfer, *in vitro* embryo production, ovum pick up (OPU), cryopreservation, cloning by somatic cell nuclear transfer (SCNT), ovulation synchronization, and sperm sexing are among the advance reproductive biotechnologies that posted high potentials for application to address both the reproduction and production problems in livestock. In water buffalo, to address the need of producing genetically superior buffaloes, the use of *in vitro* embryo production-vitrification and transfer techniques have resulted to the birth of live calves after embryo transfer to a river buffalo and swamp buffalo recipients that post the possibility of propagating river buffaloes in swamp buffalo dominated countries. Birth of twin calves by these techniques demonstrated the possibility of enhancing twinning in water buffaloes. To produce embryos of pre-determined sex, *in vitro* fertilization (IVF) using sexed-sperm was employed. To optimize the female contribution to genetic progress, OPU was used to collect oocytes from superior donors and sexed sperm was used for IVF. Use of SCNT to clone buffaloes also resulted to birth of cloned calves. With the huge contribution of this animal species on the lives of the farmers and in supporting the food requirement of the human population, improving the reproduction performance and potential is inevitable that the use and application of the advance reproductive biotechnologies is important. However, various technical problems still hinder the application of these reproductive biotechnology tools. The developments and prospects on these reproductive biotechnologies will be discussed to address the concerns of buffalo production.

Keywords: *In vitro* embryo production, cryopreservation, vitrification, embryo transfer, buffalo

INTRODUCTION

Recently, the total carabao inventory in the Philippines is 2.88 million heads. It decreased by 0.18% compared with the stocks of the same period the previous year. Commercial stocks went down by 5.50%, while inventory in backyard farms decreased by 0.16% compared with the previous year's inventory. This situation is brought by the increasing human population that enhances high extraction rate, a situation that without technical intervention will result to huge problem in the future.

Reproduction is one of the important aspects of livestock production to increase population not only in water buffalo but also in other livestock species as it dictates profitability and food security. Production of calves would mean new generation of animals for breeding, milk and meat production and parturition or delivery of calf by a dam would mean production of milk as source of income and nutritious food for human population. However, reproduction especially in water buffalo is widely known as problematic because of delayed age of puberty, high incidence of silent estrus, long service and gestation period, and long calving interval. Addressing the issue of hard-breeder heifers and cows is a big challenge that continuously being studied to improve reproduction efficiency.

Reproductive biotechnologies are tools in maximizing reproductive efficiency in animal agriculture. With the need to increase population and the problematic reproductive potential of water buffalo exhibited by long seasonal breeding, small ovaries with very few number of oocytes and those inherent features mentioned above; the advance reproductive biotechnologies provide water buffalo producers the ability to overcome reproductive problems and enable propagation of superior buffaloes beyond what was previously thought to be the normal reproductive potential and efficiency of the animal. It intends to address genetic improvement, production of purebred genetically superior animals, improvement of reproduction potential, and even the preservation and propagation of endangered buffalo species. This paper intent to present and discuss the developments of advance reproductive biotechnologies done so far in water buffalo and present the prospects that can be considered as far as improving buffalo productivity is concerned.

DEVELOPMENTS

Compared with the cattle, developments and advancements in water buffalo research is slow because of the limited number of studies conducted in this animal species and perhaps due to limited laboratories that deals on water buffalo research. The advances in reproductive technologies in water buffaloes were based on the works in cattle

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with few modifications to suit the water buffalo requirements. Slow as it is, successes on the use and application of advance reproductive biotechnologies were reported and these have potential application in improving reproductive efficiencies especially to hard breeder cows and in the production of genetically superior animals.

Improving Artificial Insemination Efficiency

Artificial insemination (AI) remains the most useful and largely adopted biotechnology in livestock reproduction. This is because AI is easier to carry out than embryo transfer (ET), more technicians are already trained to carry the procedure and frozen semen is now readily available. In the Philippines, village-base AI technicians are dispersed throughout the country to carry out AI. They are being supported by the government in terms of provision of AI paraphernalia including semen and liquid nitrogen. Their income is generated from the payment made by the carabao owner in every calf to produce or AI service they rendered. Frozen semen is now produced from high genetics bulls that have undergone the needed selection and screening process.

Involvement of AI in the implementation of the advance technologies especially in genetic improvement cannot be underscored. It remains an integral part of Multiple Ovulation and Embryo Transfer (MOET) to fertilize the ovulated oocytes *in vivo*. AI technology requires the involvement of other techniques such as the collection, processing and storage of semen from genetically superior males, observation of natural estrus or synchronization of estrus of females to make them in heat, and thawing of semen.

Semen Collection, Processing and Storage

At present, semen collection, processing and storage is already a routine work. In the Philippines, the Philippine Carabao Center is regularly producing frozen semen in a twice a week basis and distribute throughout the country for nationwide AI program. This technology is an important requisite of AI and IVF activities that of the many years of implementing it.

Estrus Synchronization

This technology is a control on ovarian function to allow the animal come to estrus at pre-determined time. In order to synchronize a group of cows or heifers into heat and induce ovulation, hormones are administered at specific times following a standardized system. Failure to follow the details of these systems will cause poor results.

Synchronization of estrus has become a subject of major interest in buffaloes *vis-a-vis* wide geographical distribution of the animals in a given village setting. The ultimate goal is to synchronize estrus effectively at a predictable time so that all the females can be inseminated at a predetermined time. Estrus synchronization programs improve reproduction efficiency by reducing the length of breeding and calving seasons and increasing calf weaning weights. Artificial insemination (AI) and embryo transfer (ET) techniques can also be used more efficiently. Considerable advancement has been made to develop techniques to control and synchronize estrus. However, fertility after breeding at pre-determined time subsequent to treatment of synchronizing agent has not gain consistent results compared to cattle. One of the reasons is the "silent heat" characteristics which is a unique feature in this animal species. The typical and clearly defined estrus signs are not obvious, less overt than in cattle impeding detection of estrus and insemination as well as ET. However, controlling estrous cycle and the relative synchrony of ovulation after synchronization resorting to fixed time insemination and ET provide the means of circumventing problem of estrus detection in buffaloes.

The major hormones used in synchronization programs are prostaglandin (PG), gonadotropin releasing hormone (GnRH) and progesterone. The use of these hormones is based on the fact that prostaglandin caused regression of the corpus luteum (CL). GnRH, when injected to buffaloes, causes either ovulation of the dominant follicle or formation of luteal tissue within the follicle which will regress when prostaglandin is injected later. Progesterone when used in heat synchronization programs is released slowly from a device that was inserted into the vagina for seven days. This progesterone treatment before an injection of prostaglandin ensures regression of the CL in response to a prostaglandin injection because most buffalo will have a CL that has developed over the seven-day period. Progesterone will also delay heat in buffalo that undergo natural regression of the CL during the seven-day period before a prostaglandin injection. The following is a list of general synchronization programs and the hormones used. 1. Standard prostaglandin program = prostaglandin (PG) injections, 2. Ovsynch program = PG and GnRH, 3. Presynch program = PG prior to standard Ovsynch program, 4. Exogenous progesterone administration = vaginal insert of progesterone (CIDR or PRID) followed by PG injection.

Sperm Thawing and Artificial Insemination

For optimum reproductive efficiency, it is essential that the frozen semen be thawed correctly. Thawing of sperm in water buffaloes could be done at 37 to 40°C for 15 sec. depends on the status of estrus. Beyond this range



would affect the quality of the sperm cells. It is important to note that even if all these facets are carefully covered, reliability of frozen buffalo semen is still immensely variable from bull to bull. However, handling of the frozen semen is equally important to maintain the quality of the semen. A major error that affects the quality of the frozen semen is the incidence of lifting the canister to the neck of the LN₂ tank and expose the semen to 0°C for more than 4 sec. Damage will start to occur to the frozen semen if it is exposed to temperatures greater than minus 80 °C for longer than 4 sec. As a "rule of thumb", the semen straws must not be raised above the point where frost appears in the neck of the container, for longer than 4 sec., and once 4 sec. of exposure is reached, the canister must be plunged back into the LN₂ to the bottom of the tank for at least 30 seconds. This practice will result in minimum damage to sperm.

Success rate of AI is affected by the following: 1) quality of semen (frozen or fresh), 2) proper detection of heat and timing of AI, 3) narrow cervix of the buffalo, which makes AI more difficult, and 4) technical aspects. If all these are carefully considered, success rate is guaranteed and efficient reproduction is insured.

Successful Embryo Transfer Records

Multiple Ovulation and *In Vivo* Collection of Embryos

Multiple ovulation is the technique involving hormonal treatment to a female to induce its ovaries to develop more follicles and ovulate matured oocytes in order to collect more embryos *in vivo* after artificial insemination. The female, called embryo donor should be a genetically superior female in order to maximize the female contribution to genetic progress.

In Multiple Ovulation and Embryo Transfer (MOET), oocytes ovulated by the donor are fertilized *in vivo* through AI or by natural mating with a bull, resultant embryos are collected non-surgically by flushing technique, and viable embryos are selected and transferred into a surrogate dam for full-term development. This technique exploits the genetic potential of females to accelerate the multiplication of superior animals for milk, meat and as breeding stocks. In swamp buffalo dominated countries; this technique is an alternative to produce purebred dairy buffaloes in a shorter period of time.

Using the MOET techniques, a river buffalo calf was produced in USA (Drost et al., 1983), then in India (Misra et al., 1990) and later in the Philippines (Cruz et al., 1991). Earlier attempts were reported from Thailand (Parnpai et al., 1985), Bulgaria (Alexiev et al., 1988) with modest results due to peculiarities inherent of the buffalo. Aside from optimizing the female contribution to genetic progress, MOET also increases genetic gain by 63 to 70% per year from juvenile and adult compared to progeny testing (Gandhi and Singh, 1994).

In Vitro Embryo Production

In vitro embryo production is an advance reproductive biotechnology that offers a lot of opportunities to do *in vitro* manipulation in order to address a particular need or problem and produce the desired embryo. To rescue a female genetic material at slaughter situation, the ovaries can be collected and the oocytes can be retrieved for *in vitro* maturation, fertilization and *in vitro* culture to the development to pre-implantation stages. If a genetically superior female is desired to become donor of eggs for the production of embryos, ovum pick up techniques can be adopted in the collection of eggs (Liang et al., 2008; Duran et al., 2013). If a pre-determined offspring is desired, then a sex-sorted sperm can be used for *in vitro* fertilization of *in vitro* matured eggs to produce the embryo of desired sex (Lu et al., 2007). If a genetically superior bull has difficulties to reproduce due to semen having low post-thaw motilities, a density gradient sperm separation technique (Hufana-Duran et al., 2005b) can be adopted to separate the motile sperm for use in AI or IVF. Similarly, intra-cytoplasmic sperm injection can also be done to enable reproduction of the said bull. If complete copy of a certain buffalo is desired, the somatic cell nuclear transfer (SCNT) can be adopted to produce a clone of the desired animals (Shi et al., 2007; Yang et al., 2010; Saha et al., 2013; Taspiroo et al., 2014). All these are now possible with successful results.

Earlier attempts (Hufana-Duran et al., 2004) on assessing the viability and full-term development of *in vitro*-produced vitrified-warmed river buffalo embryos resulted to 16.36% pregnancy and 10.91% birth to live, healthy and normal calves after an average of 312.8±2.99 days of gestation. The resultant calves have grown to the mature ages with growth rate comparable to the river buffalo calves born out of AI. The females have reproduced their own calves demonstrating the potential use of the technology as an effective tool in the production of buffaloes of desired genetics and as possible option in rescuing endangered buffalo species like the tamaraw (*Bubalus bubali mindoronensis*). The male calves are being used for breeding and have resulted to progenies that are deployed to farmer cooperators for the propagation of improved buffalo breed in the rural areas. Figure 1 shows the resultant calves out of *in vitro* produced-vitrified warmed and transferred embryos with members of the Project Team and the funding agency. Figure 2 shows the first born calf, named Glory, with the Project Team and the Ambassador of India in the Philippines and Senator Letecia Ramos-Shahani who is an advocate of milk revolution in the Philippines.



Figure 1. The produced river buffalo calves out of *in vitro*-produced vitrified-warmed and transferred embryos to river buffaloes as surrogate mothers



Figure 2. The first calf named Glory, born out in vitro-produced vitrified-warmed and transferred embryo with the Ambassador of India to the Philippines (lady in black gown), officers of the Philippine Carabao Center, members of the Project Team, and guests from JICA.



Figure 3. A river buffalo calf (with the owner) out of in vitro-produced vitrified warmed and transferred embryo with his Philippine Carabao surrogate mother (at the back) and an older Philippine Carabao calf born 34 days earlier (middle).



Figure 4. Twin calves born out of in vitro produced-vitrified warmed and transferred embryos at the Philippine Carabao Center with the Executive Director Dr. Liberto C. Cruz, Dr. Danilda Hufana-Duran who produced the embryos, Dr. Prudencio B. Pedro who vitrified the embryos, and Mr. Apolinario Salazar Jr. who transferred the embryos

Assessing the efficiency of swamp buffaloes (2n=48) as recipient of river buffaloes (2n=50) *in vitro*-produced and vitrified-warmed embryos, a pregnancy rate of 19.7% (45/288) was achieved with fifteen calves delivered presenting a 6.6% (15/288) full-term development rate (Hufana-Duran et al., 2007). Of the calves delivered to term, thirteen (86.7%) were delivered alive and healthy, while two (13.3%) were a case of stillbirth caused by dystocia. Figure 3 shows a river buffalo calf named Irfan with his surrogate mother and a native calf, a Philippine carabao that was born 34 days ahead of him. Irfan is 11-days old in this photo. The results suggest a need to carefully select the swamp buffaloes as recipients of river buffalo embryos to avoid dystocia.

On attempts (Hufana-Duran et al., 2008) to enhance twinning in water buffaloes, 2 to 3 *in vitro* produced-vitrified warmed embryos were transferred to recipient buffaloes and resulted in 16.7% (1/6) twinning rate and 83.3% (5/6) single births (Table 1). Improvement on the success rate was accounted to the improvement in the culture system (use of sequential media system, Hufana-Duran et al., 2008b) and sperm treatment (Hufana-Duran et al., 2005b), the use of recipients undergoing spontaneous estrus, and the transfer of embryos at different embryonic stages that suits the synchrony of the *in vivo* environment. Figure 4 shows the twin calves born with the Project Members.

Table 1. Twin calf production out of non-surgical embryo transfer of vitrified water buffalo embryos produced *in vitro*

Parameters	Total (%)
Number of embryos transferred	58
Number of recipients	26
Number of calves born	7
Full-term development rate, n/number of embryos transferred	(12.1)
Calving rate (from total recipients)	6 (23.1)
Singlet, n/number of calving	5 (83.3)
Twins	1 (16.7)



Recipient animals in spontaneous or natural estrus have higher pregnancy (29.0%) and calving (22.6%) rates than those recipients with synchronized estrus; 19.0% pregnancies and 5.7% calving rate, respectively with overall calving rate of 14.2% (Table 2). The low success rate is attributed by the difficulty to determine the perfect time of ET brought about by the lack of overt signs of estrus in water buffalo species that resulted in asynchrony between embryo and the recipient animal.

Analyzing the data on the basis of breed of recipient animals (Table 3), significantly higher ($P<0.01$) pregnancy rate was achieved in swamp buffaloes than in river buffaloes both for natural and synchronized estrus; 40.0 and 20.2% vs. 28.1 and 8.3%, respectively. Calving rate among recipients transferred with embryos after natural estrus was also higher in swamp than in river buffaloes. Overall calving rate, however, was significantly ($P<0.01$) higher in river buffaloes than in swamp buffaloes. These differences, however, could not be attributed as breed effect because the river buffaloes are in confined system of management while the swamp buffaloes were under field condition where heat stress is a major factor causing early embryonic death. Similarly, the number of samples is limited. The 40.0% calving rate among swamp buffaloes transferred after spontaneous estrus with river buffalo embryo shows that the technology is a potential technique in enhancing the production and propagation of desired animals like river buffaloes in swamp buffalo dominated countries.

Overall, the results demonstrated a promising tool to facilitate genetic improvement in water buffalo and possibility to rescue and preserve the endangered buffalo species. With further refinement, success rate could be improved. The herein results confirmed earlier reports indicating that compared to cattle, application of reproductive biotechnologies in water buffaloes has a lower rate of success (Totey et al., 1992; 1993a,b; Neglia et al., 2003). However, by applying several modifications such as the separation and use of motile sperm for IVF (Hufana-Duran et al., 2005b), selection of good quality oocytes for IVM and improvement of culture medium suitable for buffalo oocytes and embryos (Hufana-Duran, 2008), success rates were enhanced to a similar level to that achieved in cattle. This indicates that refinement of the technology to suit the water buffalo requirement is necessary.

In the cryopreservation of embryos, the vitrification technique was considered practical considering the efficiencies achieved (Hufana-Duran et al., 2004) and the absence of any sophisticated equipment. The most important consideration for a successful vitrification was the type and concentration of the vitrification medium, exposure time, and the material where embryos are loaded. In these studies, an open-poled 0.25 mL French straw was used in loading embryos to allow direct contact of the liquid nitrogen and ultra-rapid vitrification.

Table 2. Pregnancy and calving rate after embryo transfer of *in vitro*-produced vitrified-warmed water buffalo embryos (2n=50) after transfer to natural and synchronized estrus recipients

Nature of Estrus	Number of Recipients	Pregnancy Rate, n (%)	Calving Rate, n (%)
Natural	62	18 (29.0) ^a	14 (22.6) ^a
Synchronized	247	47 (19.0) ^b	14 (5.7) ^b
Average±SE		24.0%±0.4	14.2%±0.7

Figures in the same column with different superscripts are different ($P<0.01$).

Table 3. Pregnancy and calving rate after embryo transfer of *in vitro*-produced vitrified-warmed water buffalo embryo (2n=50) to river (2n=50) and swamp (2n=48) buffalo recipients

Recipient	Pregnancy rate, %(n/n)			Calving rate, % (n/n)		
	Natural	Synchronized	Total	Natural	Synchronized	Total
River	28.1 ^a (16/57)	8.3 ^a (2/24)	22.2 (18/81)	21.1 ^a (12/57)	4.2 (1/24)	16.0 ^a (13/81)
Swamp	40.0 ^b (2/5)	20.2 ^b (45/223)	20.6 (47/228)	40.0 ^c (2/5)	5.8 (13/223)	6.6 ^b (15/228)

Figures in the same column with different superscripts are different ($P<0.01$).

Non-surgical transfer of embryo to recipients undergoing natural or spontaneous estrus were done both in river and swamp buffaloes. However, success rate in terms of pregnancy and calving rates were higher from animals that underwent natural than those that underwent synchronized estrus (Hufana-Duran et al., 2004; Hufana-Duran, 2008). Embryo-recipient asynchrony was suspected to be a major problem as shown by the delayed ovulation among animals manifesting estrus symptoms after estrus synchronization. Ovulation synchronization needs consideration and emphasis in order to improve the success rate of ET.

With the above developments and results, applications and a wider scope of reproductive biotechnologies is being explored. At the same time, ovum-pick up activities was initiated to use high genetic live females as source



of oocytes in order to optimize the female contribution on genetic progress. The use of sexed semen for IVF and AI is another concern and considered for application.

The use of advance reproductive biotechnology remains a challenge that needs to be addressed. Though successful pregnancies and calves were produced, the efficiency needs further improvement in order to minimize the cost and enable practical application. Improvement of the *in vitro* culture systems, the skills of the technicians, and development of the overall system and programs in order to address these concerns is of prime importance.

Somatic Cell Nuclear Transfer

Cloning is an advance technology on the production of embryos that is an exact genetic replica or copy of the animal that you want to reproduce. This is done by either SCNT or embryo splitting. This technology offers the possibility of reproducing copies of a super buffalo, buffalo that produces huge volume of milk or meat, that would maximize and enhance a profitable buffalo production system. This means less number of animals needed to produce the required volume of milk and meat, demanding less manpower, water and feed resources, requires less area and secretes less methane gas that benefits the environment. Through cloning, farmers can select the best animals with the best genetics and basically copy them for an equally strong and healthy animal.

The potential benefit offered by this technology in the livestock industry is huge but the efficiency of implementation in water buffalo is slow and challenging. Successful cloning in water buffalo evident by the production of cloned calves were reported by the use of fetal fibroblasts or granulosa cells as donor cells (Shi et al., 2007), ear fibroblast nucleus from river buffalo fused into swamp buffalo oocyte cytoplasm (Yang et al., 2010), and in hand-made zona-free cloned vitrified embryo derived from enucleated oocytes reconstructed using adult skin fibroblast cells as nucleus donor.

Production of Pre-Determined Sexed Offsprings

Embryo sexing and diagnosis offers the procedure of knowing the sex or genetic disease of the embryo prior to its implantation into a recipient. Preimplantation embryos can be sexed or screened for genetic abnormalities using techniques such as polymerase chain reaction (PCR), fluorescence *in situ* hybridisation (FISH) and karyotyping. Preimplantation genetic diagnosis (PGD) allows the screening embryos before transfer and excluding affected embryos.

Embryo sexing is a potential partner of embryo splitting technique as a representative of the sectioned blastomeres could be sexed and diagnosed and the others are cultured for further splitting and development of a sex-predetermined pre-implantation embryo. This would guarantee production of embryos of known sex and assurance of genetic quality.

PROSPECTS

Successful trials on the use of reproductive biotechnologies have been achieved and were reported. With the increasing human population, increased extraction rate of animals to cater food requirements, need to address income security among rural farming families, and address malnutrition problems in the rural areas due to lack of nutritious food, application and use of these technologies to produce more genetically superior animals that can cater all these above issues is important. Improving the current efficiencies by enhancing the skills of technicians, recruiting and training additional manpower to carry out the techniques, provision by the government of needed logistic supports, careful field application trials to assess the efficiency and determine the problems that need to be addressed to improve efficiencies are necessary.

CONCLUSION

The embryo *in vitro* production, vitrification and transfer techniques is a potential tool to produce live healthy calves and allows international transport of genetically superior germplasm eliminating the high risk of disease transmission and high cost of live animal importation for genetic improvement programs. This technology offers a high potential in facilitating livestock genetic improvement, rescuing endured genetically superior females, and optimizing the reproduction performance. Further studies to improve efficiencies could lead to profitable buffalo production activities.

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BUFFALO REPRODUCTIVE BIOTECHNOLOGIES: THE CURRENT STATUS IN CHINA

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ABSTRACT

China ranked the third in buffalo population in the world, but most are swamp types with low performance in meat and milk production. In recent years, Chinese government launches program to improve the buffalo genetic to boost its beef and milk production in south China. Along with this program, some reproductive biotechnologies has been widely used in buffalo breeding, while some other are under intensive research or in field trail. Here, we summarize the reports of the research and application of buffalo reproductive biotechnologies as well as some unpublished research advance that we collected in buffalo breeding and reproductive biotechnologies in China.

Keywords: Buffalo, biotechnology, China

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GENETIC STUDIES IN WATER BUFFALO IN CHINA

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ABSTARCT

Firstly, we discuss about the research progresses in buffalo conducted our research team, including (1) Identification genes & markers associated with traits of water buffaloes by GWAS, (2) Diversity and origins of domestic buffaloes by 90K SNP-Array, (3) Establishment of detecting methods of recessive hereditary diseases of dairy cows and water buffaloes, following by brief introduction of our main research works in buffalo and Huazhong Agricultural University (HZAU) in China. Finally, In this presentation, we share the potential international research collaboration opportunities in buffalo and Co-education for the international students (Master, PhD). For investigation of transferability of BovineSNP50 BeadChip from cattle to water buffalo for genome wide association study: Cattle and water buffalo belong to the same subfamily Bovine and share chromosome banding and gene order homology. In this study, we used genome-wide Illumina BovineSNP50 BeadChip to analyze 91 DNA samples from three breeds of water buffalo (Nili-Ravi, Murrah and their crossbred with local GuangXi buffalos in China), to demonstrate the genetic divergence between cattle and water buffalo through a large single nucleotide polymorphism (SNP) transferability study at the whole genome level, and performed association analysis of functional traits in water buffalo as well. A total of 40,766 (75.5 %) bovine SNPs were found in the water buffalo genome, but 49,936 (92.5 %) were with only one allele, and finally 935 were identified to be polymorphic and useful for association analysis in water buffalo. Therefore, the genome sequences of water buffalo and cattle shared a high level of homology but the polymorphic status of the bovine SNPs varied between these two species. The different patterns of mutations between species may associate with their phenotypic divergence due to genome evolution. Among 935 bovine SNPs, we identified a total of 9 and 7 SNPs significantly associated to fertility and milk production traits in water buffalo, respectively. However, more works in larger sample size are needed in future to verify these candidate SNPs for water buffalo.

Keywords: Water buffalo, SNP-Array, genome, phenotypic divergence etc.

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UNDERSTANDING THE FERTILIZING ABILITY OF BUFFALO OOCYTES & SPERMATOZOA IN FUNCTIONAL GENOMICS PERSPECTIVE

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ABSTRACT

Sound reproduction performance of animals is the backbone of production and economic success with any livestock farming system. At least now, it is well documented that the reproduction efficiency of livestock in India and adjoining Asian subcontinent, are not up to the desired levels. For instance the reported calving to conception interval (service period) at field conditions extend for more than 250 days against the recommended period of 85-115 days. In majority of the cases the buffaloes calve twice in three years while the target/aim is to obtain one calf crop in every 13-14 months. Collectively it indicates that conception failure and repeat breeding are nagging problems leading to less number of life time calf crops per animal and reduced overall productivity. In dairy cows as well, clear evidences are available indicating decreased fertility over a period of time, particularly so under the increasing selection pressure for productivity. The percentage of estrus cows that stand to be mounted has declined from 80% to 50% and the duration of detected estrus has reduced from 15 h to 5 h over the past 50 years. This leads to difficulties in identification of animals in estrus and also poses problems in deciding proper timing of insemination. On a conservative estimate, it amounts to a cumulative loss of 20-30 million tonnes of milk annually on account of anestrus and repeat breeding in cattle and buffaloes which translates to a loss of nearly US\$ 750 b annually. Conventional approaches of dissecting reproduction ability of animals in endocrine way and designing strategies to correct problems have worked to some extent but it is miles to go to realize full reproduction potential of animals particularly the ones with high genetic merit. Functioning of the reproduction axis involves an orchestrated operation of hypothalamus, pituitary and gonads. Molecular determinants involved are the expression of correct genes at correct sequence and optimum intensity all of which are dependent on the genetic codes and beyond. Application of the tools in functional genomics in recent times have made it possible to track the events with enough precision which have resulted in alteration of existing protocols towards imparting artificial competence of key participating cells like oocytes and spermatozoa. Growing embryos also need to be dictated to follow correct development path resulting in successful recognition of pregnancy and growth of the foetus. For spermatozoa also it is getting clear that apart from the net DNA carriage they need to carry required epigenetic balance and carriage of the host of RNAs which contribute in the process of fertilization and subsequent development of embryos. Thus possibilities emerge that these genomics tools can be used to preselect sperms for higher fertility. The whole repertoire of genes expressed in oocytes and spermatozoa are far from complete. New genes and gene regulators which play crucial role in optimum fertility are required to be described along with non-invasive gene stimulation strategies. Together these genomics strategies will be the new generation manipulation approaches ultimately making possible "Calf-a-year" a realizable target in commercial animal production systems.

Keywords: Fertilizing ability, buffalo oocytes, spermatozoa

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ASSESSMENT OF X CHROMOSOME GENETIC VARIATION FOR POPULATION GENETIC ANALYSES OF DOMESTICATED WATER BUFFALOES IN NEPAL

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ABSTRACT

Water buffaloes represent an important livestock commodity in many countries around the world, in particular in Asian countries such as in Nepal, where over two-thirds of the milk and meat production come from domesticated buffaloes. A total of 24 farmed water buffalo females from the Agriculture and Forestry University (AFU) and 16 other farms from the surrounding areas of Rampur (Chitwan, Nepal) were genotyped using the Illumina's Bovine HD Genotyping Bead SNP (Single Nucleotide Polymorphism) chip. As part of a preliminary study assessing variation on the X-chromosome for future population-level genetic analyses, we analysed a total of 39,367 SNPs. Selection of SNPs for further analyses followed the criteria of $\geq 80\%$ call frequency, $MAF > 0.1$, any SNPs for which all individuals were heterozygous or homozygous removed, SNPs in Hardy-Weinberg equilibrium (HWE). The software PEAS was used to obtain several estimates of polymorphism and diversity as well as to test for any deviation from HWE. Relatedness between the individuals was assessed by 'individual identity-by-state (IBS) distance' implemented in PEAS and a Neighbor-joining tree constructed using the software MEGA. Identification of the position of the SNP against the cattle genome was conducted using Ensembl and BLAST. IBS distance estimated using 412 SNPs ranged from 0.185 to 0.363, with three genotype clades identified in the Neighbor-joining tree. Most of the individuals collected from AFU's farm formed a distinct group together and clustered with other individuals sampled in nearby farms from the north part of the study area. A total of 99 SNP markers had a single hit on the cattle X chromosome, five of which have been confirmed to be located in genes of the buffalo X chromosome. Further analyses will include autosomes' data to thoroughly assess genetic variation of this sample of buffaloes from the region.

Keywords: Genetic variation; Population genetics; Single Nucleotide Polymorphisms; X-chromosome markers.

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GENETIC IMPROVEMENT OF DAIRY BUFFALO: CONSTRAINTS AND PERSPECTIVES

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ABSTRACT

Over two-third of the buffalo population in Italy is included in the selection scheme for the genetic improvement of milk production. The Italian Ministry of Agriculture promoted, from the seventies, through appropriate funding, the development of milk recording systems and selection programs for increasing animal productivity. Selection is based on official milk recording, following the procedures of the International Committee for Animal Recording (ICAR), recording type A4, with technicians visiting the farm every four weeks. Twice every year genetic merit for milk, mozzarella yield, fat and protein content are calculated by an animal model. Therefore in Italy all recorded buffaloes (females and sires) possess a breeding value. Milk production of the recorded buffaloes (in 270 day lactation) increased from 1800 kg in 1990 to 2300 kg in 2014 (63,000 buffaloes). Beyond the genetic evaluation performed from milk recording data, bull progeny testing is performed to spread out as much as possible the genetic improvement through delivering semen of proven bulls. Progeny testing trials started in 1987, twenty-two campaigns were performed since then, and 76 positive bulls were issued for breeding. Young candidates to the progeny test must be sons of both dam and sire ranking over 80 for breeding value and over 80 for morphology. Every candidate bull goes first to a quarantine center for veterinarian controls. Then, young bulls are taken to the semen collection unit (there are three of them in Italy) where functional characteristics of the collected semen are analyzed. From each bull in test, 1000 semen doses are collected to inseminate approximately 400 females. Semen of the positive bulls is marketed in Italy through 13 artificial insemination centers. Breeding values are published twice a year. A printed catalogue reports the top 1% of Italian buffalo cows with the highest genetic merit for mozzarella and milk production, as well as the genetic merit of the positive AI bulls. Genomic selection is the new challenge: it improves the rate of genetic gain through the increasing of the accuracy and intensity of selection and shortening the generation interval. CREA is part of the Italian Consortium for buffalo genome sequencing, which produced a panel of 90 thousands polymorphic markers, at present under study for the use in the genomic selection.

Keywords: Milk recording, breeding schemes, progeny test, genomic selection

Milk Recording and Breeding Schemes: The Italian Example

Italy is traditionally a dairy country with over 3 million dairy cows and over 5 million dairy sheep. Remarkably, in the last forty years, numbers of buffaloes in Italy increased from 80,000 to almost 400,000 in 2014. The reason for this increase is merely the market pressure: the increased demand for the mozzarella cheese, both on the national and international market.

Because of the importance of the dairy sector, the Italian Ministry of Agriculture has promoted, from the seventies, through appropriate funding, the development of milk recording systems and selection programs for the improvement of milk production. Buffalo has taken most advantage from this activity which was coincident with the increase of the economic importance of buffaloes, to the extent that at present, out of 230 thousands adult female buffaloes, one third of them is milk recorded and participate to genetic improvement programs. Examples of milk recording systems organized at governmental level and aiming to genetic improvement of dairy buffalo can be found in many countries of the world. However, they often represent limited realities, performed either in nucleus herds or experimental farms and far to reach the majority of the buffalo population in the country. I think that in no other country but Italy the selection scheme is widespread to the extent that even in herds outside the Herdbook benefits from genetic improvement are attained.

Milk production of the recorded buffaloes (in 270 days lactation) increased from 1800 kg in 1990 to 2300 kg in 2014. The only breed of buffalo is the so called "Mediterranean Italian", similar to most of the buffaloes reared in Europe and Near East. It is characterized by higher fat content (almost 8% averagely) and protein content (4.7%) compared to other breeds.

Selection is based on milk recording, which follows the procedures established by the International Committee for Animal Recording (ICAR), and is the same used for dairy cows, i.e. the recording type A4, where the technicians visit the farm every four weeks. Twice every year genetic merit for milk, mozzarella yield (PKM), fat and protein percent, fat and protein kg, are calculated by an animal model. This means that in Italy all recorded buffaloes (females and sires) possess a breeding value.

Beyond the regular genetic evaluation performed from milk recording data, bull progeny testing is also

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performed to spread out as much as possible the genetic improvement through delivering semen of proven bulls. Progeny testing trials started in 1987, twenty-two campaigns were performed since then, and 76 positive bulls were issued for breeding. Pictures and traits of the bulls can be viewed at the web site www.anasb.it.

Young candidates to the progeny test must be sons of both dam and sire ranking over 80 for breeding value and over 80 for morphology. Every candidate bull goes first to a quarantine center for veterinarian controls. Then, young bulls are taken to the semen collection unit (there are three of them in Italy) where functional characteristics of the collected semen are analyzed. From each bull in test, 1000 semen doses are collected to inseminate approximately 400 females. Semen of the positive bulls is marketed in Italy through 13 artificial insemination centers.

Since all buffalo milk produced in Italy is used for the production of mozzarella cheese, the most important breeding goal for Italian buffalo is the estimated mozzarella yield (kg) per lactation (PKM), which takes into account 270 days milk yield (kg) weighed by coefficients estimated by cheese making trials, which consider the effect of milk components on this trait: $PKM = Kg\ Milk * \{ [(3.5 * protein\% + 1.23 * fat\%) - 0.88] / 100 \}$ (Altiero et al., 1989). Results of the genetic evaluation are published by the Italian Buffalo Breeders Association (ANASB) twice a year. A printed catalogue reports the top 1% of Italian buffalo cows with the highest genetic merit for mozzarella and milk production, as well as the genetic merit of the positive AI bulls.

Average buffalo herd size in Italy is 178.6 head per farm (Borghese et al., 2015). The proportion of breed able females to total buffaloes is about 45%. Artificial insemination, however, is not as widespread in buffalo as it is in dairy cows, for three main reasons: 1) estrus detection is more problematic than in cows; 2) seasonality of estrus makes uncertain the following calving and lactation; 3) herd size is over 100 females in many herds. Therefore, to favor the attainment of the benefits of the genetic improvement in all registered herds, on farm natural mating is allowed in the Herdbook. To assure high pregnancy rate, farmers use more than one bull in each mating group, because, since 1993, paternity test through DNA markers is available and it is compulsory for registering the new born in the Herdbook. Genetic merit of the natural breeding bulls is also calculated from the animal recording results, and it is provided on demand to the bull owner.

Almost 100% of the buffaloes are machine-milked. Because buffalo production registered an expansion in Italy in the past forty years, i.e. when all dairy farms were already equipped with milking parlors, the buffalo production system was adapted to the dairy cow system, by immediately separating the calf from the dam after calving, and directly milking the buffalo in the milking parlor. However, buffalo have longer and thicker teats with longer teat canals compared with dairy cows, which is important to consider when milking them with a machine. These pronounced differences partly explain why buffaloes are reported to be hard to milk. However, the research (Thomas et al., 2005) contributed to a deep knowledge of the anatomy and physiology of the lactation in buffalo to the extent that the major producers of milking machines provide now modern, effective milking parlors specific for buffalo.

Market Aspects

As I said before, Italy is a dairy country, with a huge number of brand-protected typical cheeses. This number is more and more increasing thanks to the movement of people within the country, as well of the tourism and the higher awareness of people towards local and genuine foods in contrast with industrial products. As an example, there are in Italy ten more dairy breeds beyond the most popular Holstein, maintained in local areas, mostly hilly and mountain areas, but participating to the official milk recording and modern selection schemes. These breeds are prosperous because of their milk and the brand-protected typical cheese they provide (De Marchi et al., 2007).

Buffalo cheese is the mozzarella. Mozzarella is the dairy product making the fortune of Italian buffalo, first in Italy and now all over the world. Peculiarity of the processing of this cheese is the stretching phase, consisting in stretching the curd in hot water after having cut the curd at the appropriate maturation stage (Addeo et al., 1996). The stretching gives the cheese the typical characteristic of being elastic, characteristic which all consumers like. During stretching, also fat globules are incorporated in the cheese, this making buffalo mozzarella highly different in texture, smoothness and taste from the mozzarella produced from cow milk (Faccia et al., 2009). With the expansion of buffalo farming and dairy processing plants, as well and with the expansion of the markets, several other cheeses are being produced from buffalo milk, like scamorza and treccia. However, these cheeses are produced basically with the same processing system used for the mozzarella, therefore with the stretching phase, but they are ripened for longer. The buffalo mozzarella sold as "Mozzarella di Bufala Campana" has been granted the status of DOC- "Controlled designation of origin" since 1993. Since 1996 it is also protected under the EU's Protected Designation of Origin or DOP as well as Protected Geographical Indication schemes. Requirement for obtaining the "Protected Origin Status" is that the cheese may only be produced from milk coming from buffaloes reared in restricted geographical areas of Central and Southern Italy (Campania, Lazio, Apulia and Molise) with the traditional procedure.



Genomic Selection

Genomic selection is the new challenge, because it improves the rate of genetic gain through the increasing of the accuracy and intensity of selection and shortening the generation interval. The application of genomic selection has been used in several breeds of dairy cattle (Holstein, Brown Swiss, Simmental, and Jersey) and has shown that it is possible to significantly increase the rate of genetic progress. Methods are now being developed to use genomic information for other applications, including controlling inbreeding and the management of genetic diversity. The research center where I work (CREA) is part of the Italian Consortium for buffalo genome sequencing. The Consortium has produced the reference buffalo genome sequence using two Next Generation Sequencing Technologies (combination of Illumina and 454 short-read sequencing technologies). Furthermore, the genomes of buffaloes of four different breeds have been sequenced and aligned against the bovine genome, to identify sequence variants among the buffalo genomes. 90,000 putative single nucleotide polymorphisms were selected to create an Axiom® Buffalo Genotyping Array 90K (Colli et al., 2016).

To our knowledge, two Genome Wide Association Studies targeting buffalo phenotypes connected with milk yield have been performed to date using the Axiom® Buffalo Genotyping Array 90K (Iamartino et al., 2017; De Camargo et al., 2015).

CONCLUSIONS

Although the majority of milking buffaloes are reared in India (46 million), Pakistan (10 million), China (8.5 million) and Egypt (1.5 million), countries in which they dominate cows in producing milk for human consumption, Italy, with less than half million of buffaloes, is worldwide awarded for the dairy products obtained from buffalo milk. This position has been achieved thanks to three components: the delicious taste of the mozzarella cheese accompanied by the marketing ability to let it known in local and international markets; the development and the application of milk recording systems and selection programs reaching all farming areas; the research applied in most Italian Universities and Research Centers focusing on all sectors of animal science – genetics, reproduction, physiology, machine engineering, which contributed to make the Italian buffalo production system modern and innovative, able to increase farmer's profits by empowering him to produce highest quality milk.

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FUNCTION AND MECHANISM OF MAP4K4 GENE ON MILK TRAITS AND SCS BETWEEN DAIRY COW AND BUFFALO

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ABSTRACT

This study explored the association of single nucleotide polymorphisms (SNPs) present in MAP4K4 gene with different milk traits in dairy cows and buffaloes. Association between MAP4K4 genotype, different traits and Somatic Cell Score (SCS) performed using General Linear Regression Model of R. Two SNPs at exon 18 (c.2061T>G and c.2196T>C) with genotype TT in both SNPs found significantly higher for somatic SCS. We found the significant effect of exon 18 (c.2061T>G) on protein percentage, milk yield and SCS. SNPs identified at different location of MAP4K4 gene of the cattle and many of them significantly associated with the SCS and different milk traits. LPS stimulation could increase the expression of MAP4K4 in bovine mammary epithelial cells, and this increment was time-dependent ($P<0.05$). pGL3 vectors with various truncated MAP4K4 promoters 5 for cow and 5 for buffalo designed and each fragment transfected into 293T cell, Bovine epithelial and CHO cells. The activity of each promoter regions analyzed using a Dual-Luciferase Reporter Assay System. The results showed that the promoter region from -1100 to -778 bp was essential for cow-MAP4K4 while -608 to +176 bp was essential for the buffalo-MAP4K4 that the two regions contained the core functional promoter to maintain the promoter activity. The core promoter activity of cow-MAP4K4 was significantly higher ($P<0.05$) than buffalo-MAP4K4, might imply the susceptibility of mastitis and other inflammatory disease more in cow than buffalo. Thus, MAP4K4 gene could be a useful candidate gene for assortment in dairy cows and buffalo especially for mastitis and the identified polymorphisms might potentially be strong genetic markers.

Keywords: Mastitis, milk production, MAP4K4, SNP, promoter

INTRODUCTION

Mitogen-activated protein kinase kinase kinase 4 (MAP4K4); also recognized as Nck-interacting kinase (NIK) and hepatocyte progenitor kinase-like/germinal centre kinase-like kinase (HGK)) is a serine-threonine protein kinase family member. MAP4K4 gene is responsible in key processes in initiation of innate response (Dror et al., 2007). Recently, MAPK has been investigated with various human diseases and potential therapeutic targets like heart failure (Fuller et al., 2015). Thus the role of MAP4K4 in immunity and inflammatory disease is still a subject of investigation especially for human and animal study. This study is to investigate whether naturally occurring genetic difference and promoter variations in the 5'-UTR of candidate genes alter the transcription factor binding site(s) for gene expression between cow and buffalo could be a new insight for molecular experiment.

MAP4K4 SNP at exon 18 rs209280849 (c.2061T>G) and rs211626246 (c.2196T>C) was found to be highly significant for SCS in associative in dairy cow (Bhattarai et al., 2017) showing MAP4K4 could be a candidate gene for inflammatory study in dairy cow. MAP4K4, SOCS7 and IL-17R are associated with NF- κ B signaling pathway directing to the transcriptional regulation of pro-inflammatory genes (Karin and Greten, 2005). Bovine MAP4K4 is a candidate gene for inflammation therefore more functional study can be done and comparative study of gene function in similar types of species can be performed. Through understanding of the role MAP4K4 in inflammation at the molecular level will ultimately improve dairy breeding for better lactation performance. The naturally evolved cattle and buffalo possess a unique gene pool and differ from their taurine counterparts in terms of adaptability, immunity and dairy traits. Studies comparing prevalence of mastitis in cow and buffalo revealed that mastitis is prevalent higher in cow than in buffaloes (Kee, 2012, Khanal and Pandit, 2013, Subedi and Dhakal, 2002, Joshi and Gokhale, 2006a). But these studies are limited only for clinical observations. Yet no study was performed that can explain this fact and identify the possible genetic mechanism. It would be worthy to perform a holistic profile of MAP4K4 promoter variants and their possible linkage with variants in the phenotypic traits in cows and buffaloes. In this essence, we hypothesized that MAP4K4 gene can have important role on immunity and could be associated with inflammatory disease like mastitis. Considering the significant role that the promoter region plays in influencing gene expression and gene transcription, the present study was undertaken to identify variations between cow and buffalo MAP4K4 promoter, its analysis and comparison.

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MATERIALS AND METHODS

Previous SNPs report of MAP4K4 in cow genome at exon 18 (c.2061T>G and c.2196T>C) was significantly associated with SCS in dairy cow. We tried to identify the similar mutation in buffalo genome. For the initial mutation screening for exon 18, a total of 100 blood samples including 50 Holstein cows and 50 Murrah cross buffaloes were used. Information from public database of NCBI (www.ncbi.nlm.nih.gov), sequence of MAP4K4 gene referring to *Bos taurus*_UMD_3.1.1 (GCF_000003055.6) for cow and UMD_CASPUR_WB_2.0 (GCF_000471725.1) for buffalo was annotated. Primer premier 5.0 software was used to design all the primers (Premier Biosoft International, Palo Alto, CA, USA), which was synthesized by Sangon Biological Engineering Technology (Shanghai, China). We used various bioinformatics software to analyze RNA and Protein sequence in cow and buffalo.

Bovine Epithelial Cell line (BMEC) was used to measure the mRNA expression level of the MAP4K4 gene. Extraction of RNA was made at various time duration (3 hrs, 6 hrs, 12 hrs and 24 hrs) after the LPS treatment. In order to clone the bovine MAP4K4 promoter region, we designed gene-specific primers to amplify a 2.0-kb genomic region upstream of the bovine MAP4K4 gene TSS. Primers were designed to amplify the target sequence of MAP4K4 gene. BMEC, Human embryonic kidney cell (HEK293T) and Chinese hamster ovary cell (CHO cell) were used for the culture experiments. One-way ANOVA was used and the unpaired student's t-test was used to detect significant differences ($P < 0.05$) existed in a group of data. Values were represented as the mean \pm SD, and statistical significance was indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Data were representative of at least three independent experiments.

RESULTS

We did the mega-blast to differentiate the mRNA sequence between cow and buffalo which revealed 53 differences in mRNA sequence of which 4 were responsible for amino acid change. The mRNA at 1169 (A>T) changes the amino acid from Glutamine (Q) to Leucine (L); 1382 (A>G) change Asparagine (N) to Threonine (T); 2183 (T>C) change Valine (V) to Alanine (A); 2360 (G>C) change Serine (S) to Threonine (T). These changes could be responsible for difference in the protein structure or functions of MAP4K4 between cow and buffalo which can be helpful for further analysis. We used -2000 bp upstream sequences (considering ATG as +1) to predict the possible promoter sequence, Transcription start sites, Transcription binding sites and CpG Island in cow and buffalo-MAP4K4, respectively. Our results revealed the sequence between -736 to -687 bp was predicted as possible promoter sequence in cow-MAP4K4 gene. Similarly, three regions was predicted as possible promoter sequence in buffalo-MAP4K4 sequence viz, (-473 to -424 bp), (-44 to +6 bp) (+46 to +95 bp). CpG islands were predicted (Figure 1).

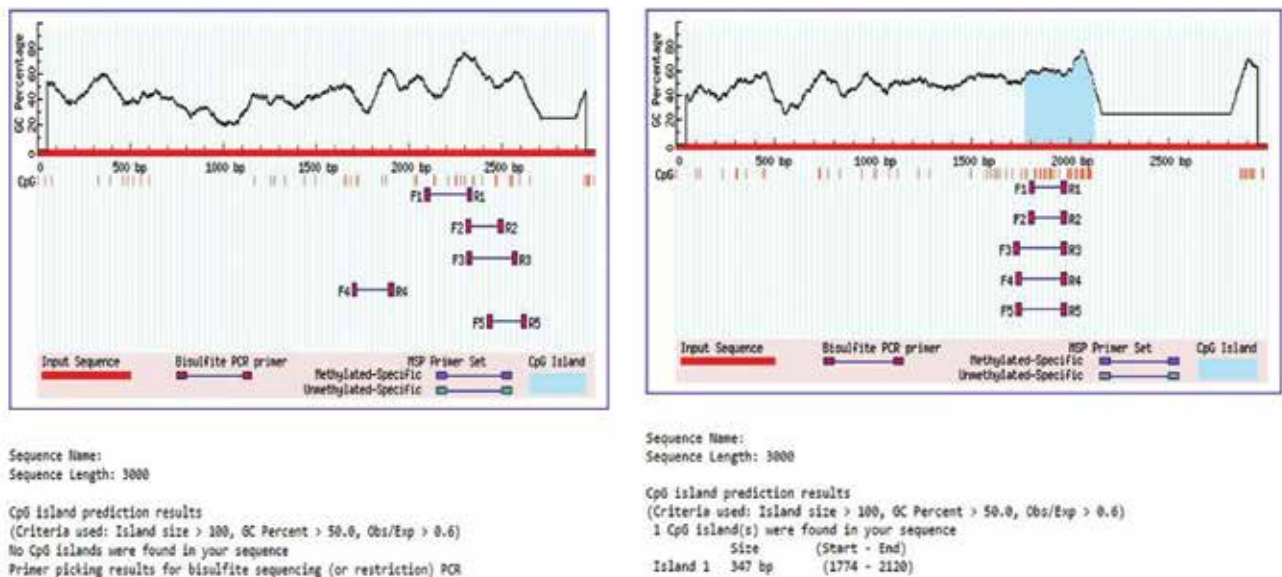


Figure 1. CpG island prediction in Cow MAP4K4 (left) and Buffalo MAP4K4 (right). The software <http://www.urogene.org/methprimer/> was used for the prediction. The results showed no prediction of CpG Island in cow MAP4K4 5' upstream sequence and 1 CpG Island is predicted in Buffalo MAP4K4 5' upstream sequence.

Relative expression of MAP4K4 mRNA was investigated in bovine mammary epithelium cell line. It was found considerably higher levels of MAP4K4 ($P < 0.01$) in response to a 24 h LPS (10 ng/ml) challenge. Significant difference of mRNA was observed with time dependent manner (Figure 2).

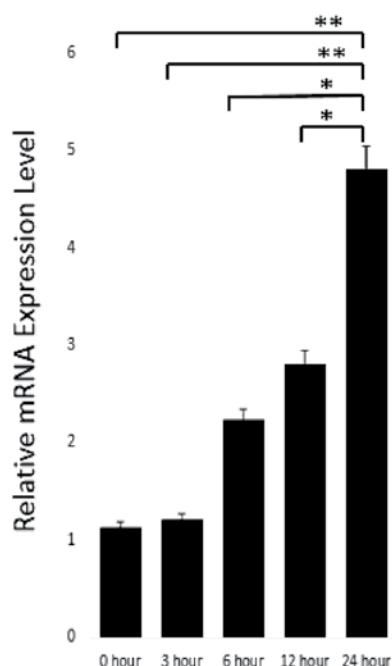


Figure 2. Relative expression of MAP4K4 mRNA in bovine epithelium cell line. Expression was found significantly differed with time dependent manner of LPS (10ng/ml) treatment. Error bars represent the standard deviation (SD) (n = 3).

Comparative SNP study at the exon 18, rs209280849 (c.2061T>G) and rs211626246 (c.2196T>C) in 50 cross dairy Murrah buffaloes and 50 dairy Holstein cows revealed that presence of only of GG genotype (in c.2061 T>G) and presence of 60% of CC genotype (in c.2196T>C) in buffalo. Higher frequency of TT homozygous and TC/TG heterozygous allelic distribution was found in dairy cows (Table 1).

Table 1. Distribution of TT/TG/GG/CC genotype in cow and buffalo population

mRNA position	AA Change	Genotype	Frequency	
			Cow	buffalo
T2061G	Ala691Ala	TT	0.30	0.00
		TG	0.50	0.00
		GG	0.20	1.00
T2196C	Pro736Pro	TT	0.20	0.10
		TC	0.50	0.30
		CC	0.30	0.60

Based on the predication and availability of suitable primers, 5 reporter vectors were constructed to test core promoter activity in cow MAP4K4 (viz; CV1, CV2, CV3, CV4, CV5) and 5 reporter vectors were constructed to test core promoter activity in buffalo MAP4K4 (viz; BV1, BV2, BV3, BV4, BV5). A series of 5 reporter constructs for cow-MAP4K4 and 5 reporter constructs for buffalo-MAP4K4 was constructed around the predicted area to compare the activity for minimum promoter sequence. The possessions of these alternate reporter constructs were estimated after transfection of the corresponding luciferase reporter plasmids into bovine epithelium cell, 293T and CHO cells. Results showed that the activity of these different promoter constructs was tested which were higher in bovine epithelium cell and 293T than in CHO cells. The promoter activity in pL-CV4 was significantly increased among the different cow-MAP4K4 promoters whereas pL-BV2 was significantly higher among the buffalo-MAP4K4 promoters comparing to other corresponding adjacent promoter sequences, it indicated that within the region of -1100 to -778bp was essential for cow-MAP4K4 and -608 to -452 bp was essential for the buffalo-MAP4K4 to maintain the promoter activity (Figure 3).

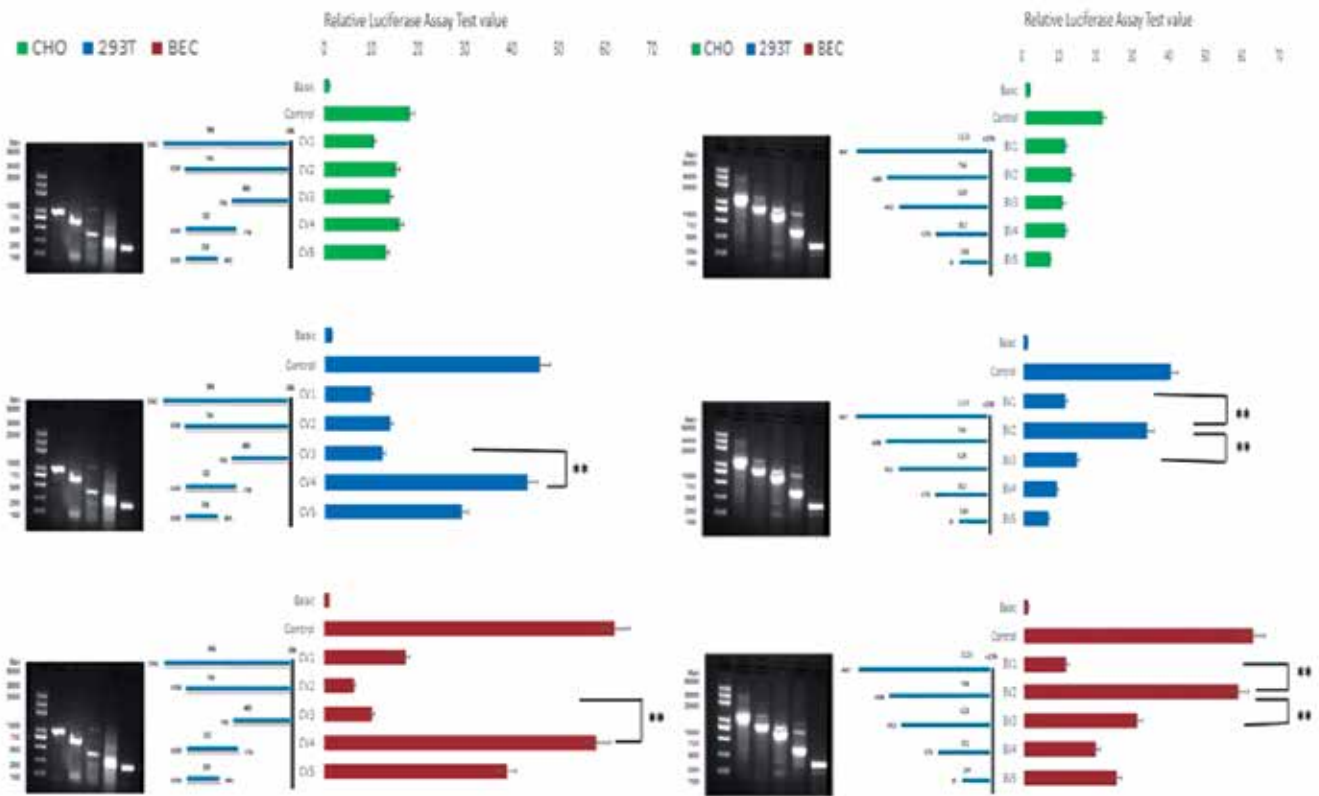


Figure 3 Identification of the MAP4K4 core promoter in cow (left). Identification of the MAP4K4 core promoter in buffalo (right). Bar represents the relative luciferase activities of the promoter fragments. Values are represented as Mean \pm standard deviations. The error bars denote the standard deviation. The unpaired Student's t-test was used to detect significant differences. The data shown are representative of average of three independent experiments.

The sequence in between -1100 to -778 bp region in cow and -608 to -452 bp region in buffalo is conserved region and it has several DNA binding sites like Sp-1, CAT box, c-Jun, STAT-1, NF-1, Myp-1 TATA box (Figure 4) that are essentially important for pro-inflammatory signaling mechanism. The consensus sequences of Sp-1, CAT box, c-Jun, STAT-1, NF-1, Myp-1 were predicted by JASPAR program (jaspar.genereg.net). Comparison of the strength of the identified minimal proximal part of relevant cow-MAP4K4 gene and buffalo-MAP4K4 gene was done in various cells. We identified the significant higher activity of cow functional promoter than buffalo ($P < 0.05$) (Figure 4)

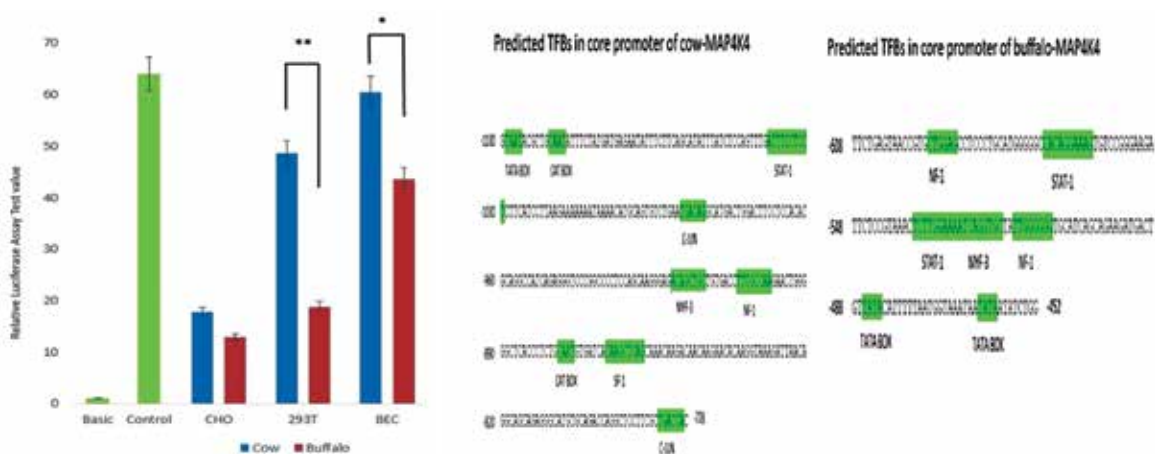


Figure 4 Comparison of activity of identified core promoter sequence of cow MAP4K4 and Buffalo MAP4K4 sequence (left). Sequence and identified TFB sites of the proximal minimal promoter of MAP4K4 gene in cow and buffalo.



DISCUSSION

MAP4K4 induced activation of NF- κ B signaling pathway in macrophage (Aouadi et al., 2009) and silencing of MAP4K4 inhibit the TNF- α -induced p65 nuclear localization and transcriptional activation (Rothflach et al., 2015) indicating there could be some additional mechanism of NF- κ B modulation by MAP4K4. The gene trans-skeleton and the prediction of protein interaction with other closely related protein revealed a significant difference between cow-MAP4K4 and buffalo-MAP4K4 protein which could be indicative for the difference in gene expression and consequently difference in signaling pathways. To isolate the possible functional sequence of promoter we tested various regions with truncated plasmid vectors and confirmed the region -778 to -1100 bp was essential for promoter activity in cow-MAP4K4 gene while -452 to -608 bp was essential region for promoter activity in buffalo-MAP4K4 gene (Figure 3). The comparison of the functional promoter region of cow-MAP4K4 and buffalo-MAP4K4 in Bovine mammary epithelial cells and 293T cells by luciferase assay revealed the noteworthy difference ($P < 0.05$) in the expression indicating that the identified promoter of cow-MAP4K4 is significantly higher than identified promoter of buffalo-MAP4K4 (Figure 4).

The SCS has been used as the criterion for improving mastitis resistance (Shook and Schutz, 1994). Since the cow-MAP4K4 at exon 18 rs209280849 (c.2061T>G) and rs211626246 (c.2196T>C) having TT genotype for higher SCS while GG genotype (c.2061T>G) and CC genotype (c.2196T>C) for lower SCS score (Bhattarai et al., 2017). We tried to explore the distribution of TT/TG/GG/CC genotype in this two location in cow and buffalo population (Table 3). Results indicate all buffaloes having GG allele (having lower SCS) in c.2061T>G and 60% of buffalo having CC allele (having lower SCS) in c.2196T>C implying lower chance of mastitis in them. We found the two major results one describing the significant difference in strength of expression of promoter activity between cow and buffalo and other the significant difference in allelic frequencies in cow and buffalo populations. These two results code for the similar conclusion that cow and buffalo reveals significant differences for immune response and inflammation when analyzed MAP4K4 as a marker gene. Moreover, our result is supported by the previous prevalence study of mastitis between cow and buffalo where the prevalence of mastitis is higher in cow compare to buffalo (Joshi and Gokhale, 2006b, Dhakal et al., 2007, Khanal and Pandit, 2013, Kee, 2012).

CONCLUSION

MAP4K4 has been correlated with many pathophysiological processes like immunity and inflammatory diseases as it play crucial role in inflammatory signaling governed by NF- κ B signaling. This result concluded that cows are more vulnerable for the disease like mastitis and could be vulnerable for other inflammatory reactions than buffalo. This provides the insight for better understanding the difference in physiology and immune response between cow and buffalo.

ACKNOWLEDGEMENT

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AUTHENTICATION OF BUFFALO MEAT USING MITOCHONDRIAL Cytochrome-b GENE

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ABSTRACT

Animal species identification is an important means to check food labeling that is required for exporting the meat. Moreover, authentication of meat (species of origin) has significance in view of religious, quality assurance, food safety, public health, conservation and legal concerns. There are various methods which are based on identification of species-specific proteins by means of electrophoresis and/or immunological methods (ELISA). However, these techniques are not reliable for identifying species in complex heat treated or cured products as a result of deterioration of the proteins. DNA is more stable than proteins during heat processing and although it can be fragmented by heat, modern DNA-techniques still allow us to identify DNA from the different species present in a sample. Furthermore, unlike specific proteins, DNA is present in all organs and tissues. In this present study, we have done the authentication of buffalo meat for the first time in Nepal. We performed mitochondrial DNA sequencing for PCR products targeting cytochrome-b gene region for the identification of meats of cooked and dried meat of common food animals (buffalo meat for this study). A pair of forward and reverse universal primers amplifying a conserved region of mitochondrial cytochrome-b (cytb) gene for targeted (buffalo) species was designed which yielded a 421bp PCR amplicon. The selected primers were confirmed for species specificity by blasting with NCBI database. Even though direct sequencing of PCR amplicon does not require verification, the cost per sample is relatively high. Direct sequencing therefore is recommended for testing small number of samples whereas alternative methods such as PCR-RFLP assay can be used for routine diagnostic laboratories to differentiate the meats. This study is the initiation in Nepal which helped to authenticating the meat samples ensuring food safety to consumers and also reinforces the laws related to meat and meat products.

Keywords: Meat, authentication, direct sequencing, cytochrome-b gene

INTRODUCTION

Identifying meat species used in meat products is a critical point in the quality control measures. It is an important means to check food labeling that is required for exporting the meat. Moreover, authentication of meat (species of origin) has significance in view of religious, quality assurance, food safety, public health, conservation and legal concerns reasons (Ayaz et al., 2006). Adulteration with single and multiple species are reported in various countries (Rashed et al., 2017). One of the most common cases is to illegally sell cheaper meat as meat from more profitable and desirable species. This issue, therefore, is of concern to researchers, consumers, retailers and governmental control authorities in all steps of the production system. In Nepal, the meat consumption is raising tremendously with day escalates the demand of the meat per day and thus becomes the most highly priced commodities. These encourage economic gain-oriented meat species adulteration especially in minced and comminuted meat products. Multispecies adulterations have been reported mainly in commercial meat products. Adulteration mixing mutton meat and buffalo meat in chevron is very common in Nepalese market while addition of inferior quality meat with superior one is also not less. Adulteration with offal and fat in minced meat has the consequences of health risks such as coronary heart disease (CHD) and other related diseases of the circulatory system (AHA, 1978). Nepalese people prefer to have spicy and well-cooked meat which makes people not conscious of good quality meat. Nevertheless, people are becoming relatively more aware than past. However, there is no legal action if the low quality meat and adulteration is sold in the market.

The adulteration of the meat in the meat and meat products hinders possibilities to export the meat in international market. Different countries have their own requirements for importing various meat products where the confirmation of the species is the most important. The present situation of the country warrants urgent survey of the adulteration in the meat in the market of the country. Despite of its immense necessity, there was no any government entities in Nepal (public quality control laboratories and inspection services authorities) thus failing huge losses during export, until recently Nepal Agriculture Research Council (NARC), who has the laboratory facilities to employ in permitting explicit species identification. Lately a huge consignment has been rejected by the importing party as the supplier could not provide the scientific evidence to verify the species composition of the cooked and dried meat (personal communication; Rubi Rana, young entrepreneur, MeatCo Pvt. Ltd., 2017) which was brought to the laboratory of NARC for speciation.

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Meat species can be identified by physical (Singh, 2011), anatomical (Sachan and Singh, 2010), serological (Reddy et al., 2000), histological (Tremlova, 2000), immunochemical (Rencova et al., 2000), enzyme-linked immunosorbent assay (ELISA) (Hsieh et al., 1997), or molecular biological (Zahran and Hagag, 2015) methods. Each method has its own effectiveness and disadvantages. But, most of the techniques are not reliable for identifying species in complex heat treated or cured products as a result of deterioration of the proteins. Among all, DNA-based techniques are regarded as the most pertinent methods for species identifications. DNA is existent in nearly all tissue types of an individual, stable under different circumstances (e.g. freezing, salting, drying, cooking and manufacturing) and permits for differentiating even very closely-related species due to the diversity afforded by the genetic code.

As the application of the polymerase chain reaction (PCR) seems to give the most satisfactory results (Teletchea et al., 2005), the objective of the present work was to use simplex PCR assays with a pair of forward and reverse universal primers amplifying a conserved region of mitochondrial cytochrome-b (cytb) gene for detection of three randomly taken samples of the consignment lot; one each of fresh buffalo meat and fresh chevron. The present work is done the first time in Nepal to identify the species of meat articles.

MATERIALS AND METHODS

Sample Collection and Preparation

Five meat samples: three randomly taken samples of the consignment lot (cooked, spice treated and dried); one each of fresh buffalo meat and fresh chevron were taken (Table 1). Each sample was immersed in vial containing ethanol. Samples upon receipt were frozen at -20°C.

Table 1. Number and source of meat samples collected

Species	Common name	Meat samples		
		Number	Source	State
<i>Bubalus bubali</i>	Buffalo	3	Consignment	cooked and dried
<i>Bubalus bubali</i>	Buffalo	1	Abattoir	fresh
<i>Capra hircus</i>	Goat	1	Abattoir	fresh

DNA Extraction and Evaluation

DNA was extracted from the received samples using a commercial animal tissue DNA extraction kit (DNeasy Blood & Tissue Kits, Qiagen) using manufacturer's instruction. For efficient digestion, tissue was lysed overnight in Proteinase K beforehand. Quality and quantity of extracted DNA was assessed using Nanodrop technique (NanoDrop@ND-1000 Spectrophotometer).

PCR amplification

PCR mix components and its performing steps were performed according to the manufacturer manual. PCR amplification of specific universal mitochondrial gene was carried out for each sample (Table 2) and thermocycling condition is given in Table 3. A negative extraction control (NTC) and positive control (POS) was included in the PCR for quality assurance. The amplicons of PCR were assessed by 1.5% agarose gel electrophoresis. Bright single band of target amplification was observed for all samples. The PCR amplicons are of 421 bp. Amplicons were purified using QIAquick PCR Purification Kit, Qiagen followed by direct sequencing (Intrepid-Nepal, Thapathali, Kathmandu, Nepal). Upon sequencing, the readings were analysed for quality assurance using Chromas software and identity was found by blasting the obtained DNA sequence in the NCBI database.

Table 2. Primer sequences and their annealing temperatures

DNA regions	Primer sequence 5' to 3'	Annealing temperature (°C)
mtDNA Cytochrome-b	Primer F: TACCATGAGGACAAATATCATTCTG	50
	PrimerR: CCTCCTAGTTTGTAGGGATTGATCG	



Table 3. Thermocycling condition

	temp	time	
Initial Denature	95°C	0:15:00	1X
Denature	95°C	0:00:30	40X
Annealing	50°C	0:00:30	
Extension	72°C	0:00:60	
Final extension	72°C	0:10:00	1x
pause	4°C	∞	

RESULTS AND DISCUSSION

Molecular techniques have been used elsewhere for meat species identification. The PCR assays targets genomic as well as mitochondrial DNA for the purpose of meat species identification. The conventional techniques allow the qualitative detection of different species with a defined limit of detection. However, Real-time PCR generally offers greater sensitivity and specificity and is a quantitative method for identification of species (Walker et al., 2013). Species-specific PCR assay was found to be rapid and cost effective for identification of meat species due to specific detection of target sequence without the need of further sequencing or digestion of the PCR products with restriction enzymes (Rodriguez et al., 2004), but it cannot be designed when species are very closely related (Kelly et al., 2003). On the contrary, PCR-RFLP could differentiate closely related meat species (Amjadi et al., 2012; Jaayid, 2013; Mane et al., 2014). In this present study, since we had an urgency to find the species detection to export the consignment to the destination on time, we have chosen simplex PCR assay and direct sequencing of the positive PCR amplicon which is detected by electrophoreses (Figure1) and compared the sequences with the GenBank database (Table 3). The amplification of mitochondrial DNA segment (cytochrome-b gene) in all samples taken yielded the same amplicon with a size of 421 bp and all the bands therefore are found in the same line of the agarose gel (Figure 1).

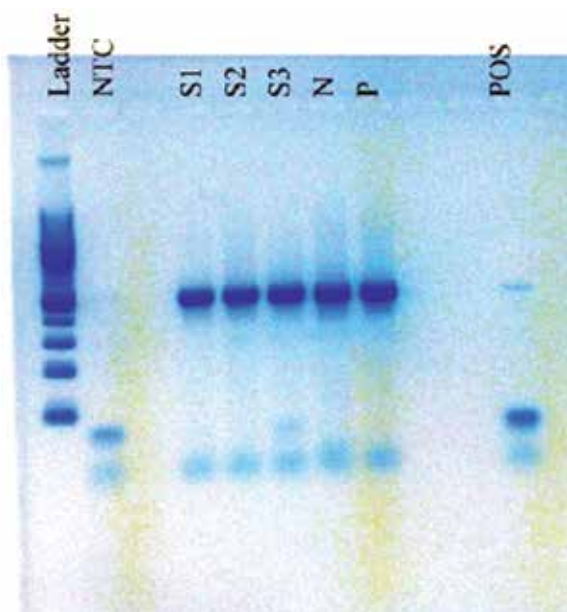


Figure1. Agarose gel electrophoresis of PCR products on 1.5% Agarose gel; NTC- Negative Template Control- A set of reverse primers was performed in PCR without template DNA; POS- Positive Control; S1, S2, S3- Three samples from consignment; N- known chevon sample; P- known buffalo sample; Ladder: 100 bp DNA ladder (Zymo Research)

DNA sequencing and BLAST Analysis

The sequences of the five samples were blasted with GenBank database and aligned. The BLAST analysis (using blast software in NCBI) of the samples gave 99-100% identity with 99-100% query coverage. The randomly sampled consignment samples (S1-S3) were 99% similar to that of the GenBank sequences of water buffalo (*Bubalus bubali*) where the known samples of fresh buffalo meat and chevon also proven to be the same species as expected (Table 4).



Table 4. DNA sequences and the identity of the meat articles

Sample ID	DNA sequence	Query coverage	Identity %
S1	ATCCCATACATTGGTACTAGTCTGGTTGAGTG AATCTGGGGAGGATTCTCAGTAGACAAAGCA ACCCTCACCCGATTCTTCGCATTTCACTTCATC CTCCCATTCAATTATCGCAGCACTTGCAATAGT CCACCTATTATTCCTCCACGAAACAGGATCCA ACAACCCAACAGGAATCTCATCAGACACAGA CAAAATCCCATTCCACCCCTACTACACCATTAA AGACATCCTAGGCGCCCTACTACTAATCCTAG CCCTGATACTATTAGTACTATTACACCCGAC CTCCTCGGAGACCCAGACA ACTACACCCGAGC AAACCCACTCAACACACCTCCCCACATCAAAC CTGAATGGTACTTCTTATTCGCATACGCAATC TTACGATCAATCCCT	100	99 (Water buffalo, <i>Bubalus bubali</i>)
S2	TCCCATACATTGGTACTAGTCTGGTTGAGTGAATCTG GGGAGGATTCTCAGTAGACAAAGCAACCCTCACCCG ATTCTTCGCATTTCACTTCATCCTCCCATTCAATTATCG CAGCACTTGCAATAGTCCACCTATTATTCCTCCACGA AACAGGATCCAACAACCCAACAGGAATCTCATCAGA CACAGACAAAATCCCATTCCACCCCTACTACACCATT AAAGACATCCTAGGCGCCCTACTACTAATCCTAGCCC TGATACTATTAGTACTATTACACCCGACCTCCTCGG AGACCCAGA	100	99 (Water buffalo, <i>Bubalus bubali</i>)
S3	TCCCATACATTGGTACTAGTCTGGTTGAGTGAATCTG GGGAGGATTCTCAGTAGACAAAGCAACCCTCACCCG ATTCTTCGCATTTCACTTCATCCTCCCATTCAATTATCG CAGCACTTGCAATAGTCCACCTATTATTCCTCCACGA AACAGGATCCAACAACCCAACAGGAATCTCATCAGA CACAGACAAAATCCCATTCCACCCCTACTACACCATT AAAGACATCCTAGGCGCCCTACTACTAATCCTAGCCC TGATACTATTAGTACTATTACACCCGACCTCCTCGG AGACCCAGACA ACTACACCCGAGCAAACCCACTCAA CACACCTCCCCACATCAAACCTGAATGGTACTTCTTA TTTCGCATACGCAATCTTACGATCAATCCC	100	99 (Water buffalo, <i>Bubalus bubali</i>)
N	TCCCATATATTGGCACA AACCTAGTCGAATGAATCTG AGGGGGATTCTCAGTAGACAAAGCCACTCTCACCCG ATTCTTCGCCTTCCACTTTATCCTCCCATTCAATCAC AGCCCTCGCCATAGTCCACCTGCTCTTCTCCACGAA ACAGGATCGAACAACCCACAGGAATTCATCAGAC ACAGATAAAAATCCCATTTCACCCCTACTACACCATTA AAGATATCTTAGGCGCCATGCTACTAATTCTTGTTCT AATATTACTAGTACTATTACACCCGACCTACTCGGA GACCCAGACA ACTATATCCCAGCAAATCCACTCAATA CACCCCTCACATTA AACCTGAGTGGTATTTCTTATT GCATACGCAATCCTACGATCAATCCCTAA	100	100 <i>Capra hircus</i> <i>cretica</i> (Cretan goat)
P	TCTCTCAGCATCCCATACATTGGTACTAGTCTGGTTG AGTGAATCTGGGGAGGATTCTCAGTAGACAAAGCA ACCCTCACCCGATTCTTCGCATTTCACTTCATCCTCCC ATTCATTATCGCAGCACTTGCAATAGTCCACCTATTA TTCTCCACGAAACAGGATCCAACAACCCAACAGGA ATCTCATCAGACACAGACAAAATCCCATTCCACCCCT ACTACACCATTAAAGACATCCTAGGCGCCCTACTACT AATCCTAGCCCTGATACTATTAGTACTATTACACCC GACCTCCTCGGAGACCCAGACA ACTACACCCGAGCA AACCCACTCAACACACCTCCCCACATCAAACCTGAAT GGTACTTCTTATTCGCATACGCAATCTTACGAT	100	99 (Water buffalo, <i>Bubalus bubali</i>)

Preventing adulteration of meat foods with less desirable or objectionable meat species is important for economic, religious (beef in case of Hindu religion) and health reasons (Ayaz et al., 2006). Beef is rare in Hindu countries such as Nepal but sometimes beef may use in meat products of some companies. In addition, determination of the species of origin of the meat components in meat products is an important task in food hygiene, food codex,



food control and veterinary forensic medicine (Ayaz et al., 2006). Adulteration and fraudulent of meat species is important legal issue as there are some animals (wild and domestic) are protected by laws for their conservation and patient right.

Several methods have been developed to identify different species of meat. The conventional methodology used for the determination of species origin in meat products had been predominantly based on immunosorbent assay (ELISA), immunochemical and electrophoretic analysis of protein. Electrophoresis requires several hours and presents low reproducibility. Additionally, through the acquisition of sequence data, DNA can potentially provide more information than type of protein content, due to the degeneracy of the genetic code and the presence of many non-coding regions (Partis et al., 2000). DNA hybridization (Jain et al., 2007) and PCR methods (Chikuni et al., 1994) have been used for the identification of meats and meat products. PCR thus is a helpful technique for meat species identification. Since we were using comminuted meat products, we had used Simplex PCR assay followed by direct sequencing of the amplicons for a rapid detection and identification of meat species in meat products of a company in Nepal which needs evidence of species identification to export.

This finding allowed us a direct and rapid identification and detection of adulteration of unwanted meat in meat products and allows certification to export the consignment to the destination. Although so far in Nepal, the adulteration is not punished seriously by laws, we could not find any report of these cases. None report of adulteration doesn't mean there is no adulteration. Many consumers suspect adulteration in meat while they buy meat for consumption. Despite of stringent laws and well developed system to identify the species, there were many reports of multi-species adulteration or fraudulent meat in different part of the world. Hsieh et al. (1995) reported that ground turkey meat sold in retail markets was contaminated by beef or lamb meat which might be because of improper cleaning of the grinder between each change of meat species prior to grinding (Hsieh et al., 1995). Meyer et al. detected 0.5% pork in beef using the duplex PCR technique proving that PCR was the method of choice for identifying meat species in muscle foods (Meyer et al., 1994). Jain et al. (2007) used multiplex PCR technique for detection of meat species via tracing of cytochrome-b gene (Jain et al., 2007). Ong et al. (2007) used three restriction enzymes in PCR-RFLP using the mitochondrial cytochrome-b region to establish a differential diagnosis which detect and discriminate between three meat species and they were showed this technique can be applied to food authentication for the identification of different species of animals in food products. Abdel-Rahman et al. (2009) successfully detected adulteration of more than three different meats using species-specific primers and PCR-RFLP technique.

CONCLUSION

The present study confirms the meat for consignment were from water buffalo by using simplex PCR assays with a pair of forward and reverse universal primers amplifying a conserved region of mitochondrial cytochrome-b (cytb) gene. The present work is done first time in Nepal to identify the species of meat articles. In addition, this study suggests an accurate, sensitive and rapid analytical technique for species identification of meat and meat products based on PCR analysis of mitochondrial cytochrome-b (cytb) gene followed by direct sequencing for enforcement of labeling regulations and ensuring that meats agrees the requirement of the importing country. These practices are also helpful in implementation of prevention of animal slaughter acts, wildlife conservation acts and some other international acts.

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RNAi-MEDIATED KNOCKDOWN OF INHIBIN α SUBUNIT ON GRANULOSACELL LED OOCYTE DEVELOPMENT IN TRANSGENIC MOUSE MODEL

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ABSTRACT

Inhibin α (INH α) Subunit gene plays a vital role in folliculogenesis, cell differentiation and oocyte development in mammals. However, the specific action of RNAi-INH α in folliculogenesis, oocyte development and other ovarian function remain largely unclear. To define its roles in mammalian reproduction, transgenic mice of RNAi-INH α that knock down the INH α expression by shRNAi were used. The action mechanism of RNAi INH α on Granulosa cells (GCs) apoptosis, GCs cell cycle regulation, hormonal control; puberty and super ovulation were investigated by using transgenic mice model. RNAi-INH α transgenic female mice were sub-fertile and correlated with the number of Oocytes ovulated after puberty with cellular and molecular alterations. The results showed that at 3 weeks, transgenic mice produced the increased number of Oocyte as compared to control mice. In contrast, Oocyte numbers were significantly reduced ($P < 0.05$) in 6 and 9 weeks of age even after with the PMSG and hHCG administration in transgenic mice. Serum INH α level was significantly decreased in both 3 and 6 weeks whereas, FSH was significantly up regulated in 3 weeks but not in 6 weeks. Furthermore, suppression of INH α expression significantly promoted apoptosis by up-regulating Caspase-3, Bcl-2, INH β B and GDF 9 and down regulated Kitl and TGF β RIII genes both at transcriptional and translational levels. Moreover, it also dramatically reduced the progression of G1 phase of cell cycle and declined the number of cells in S phase as determined by flow Cytometer. Knockdown of INH α by using RNAi-transgenic technology leads to disruption of normal ovarian regulatory mechanism in mammals. These results indicate that INH α subunit is an important regulator of GCs apoptosis, cell cycle progression, Oocyte development and other reproductive events in mammals.

Keywords: INH α ; granulosa cells; in-vivo study; apoptosis; RNA interference

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ENHANCING BUFFALO PRODUCTION BASED ON FEED RESOURCES DEVELOPMENT AND INNOVATIONS

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ABSTRACT

The buffalo (*Bubalus bubalis*) population of about 180 million heads are raised globally, but over 96% of them have been domesticated in the Asian region. Swamp buffaloes are mostly raised in South East Asia while riverine-type buffaloes are domesticated in South Asia where they are used for draft and meat; and for milk production, respectively. The buffaloes are considered as an important ruminant that attributes to the farmer's household livelihood generating draft, meat, by-products, milk and milk products, especially as a lucrative income provider. Researches have been revealing their remarkable ability in utilizing low-quality roughages, agricultural by-products and having the unique rumen ecology than those in cattle. The rumen $\text{NH}_3\text{-N}$ concentration and the ability to recycle nitrogen into the rumen as well as higher nutrient digestibility have been discovered in buffalo than in cattle. Furthermore, "Feeding the microorganisms, feeding the buffalo" is also true as the rumen of buffalo exhibits different rumen microorganisms and the fermentation end-products. The rumen's microbiome shows a close relationship with fermentation and subsequent productivity. Among a number of feed resources and feeding methods, the food-feed-system (FFS) is of potentially promising for establishment (E), development (D), utilization (U) for the sustainability (S) of buffalo production. Further research using good roughage sources with strategic concentrate supplements those containing plant secondary compounds (condensed tannin, saponins) could be of great concern and for possible implementation of fattening and milk production, especially both on-farms and on-commercial farming.

Keywords: Buffalo, rumen ecology, feed sources, food-feed-system, methane

INTRODUCTION

A number of books on buffalo have been compiled collecting research work and development globally, including that of FAO (2005), which has covered many good practices on buffalo production of both the riverine and swamp-types. Currently, the e-book (Wanapat et al., 2017; edited by Giorgio A. Presicce) has collectively compiled that covers different aspects of buffalo production and research. Wang et al. (2017) outlined the new finding regarding the history of swamp buffalo using whole mitogenomes showing their route from the glacial period into domestication. The global buffalo population is estimated to be approximately 180 million, spreading over 42 countries of which 177 million (97%) are found in Asia, while approximately 5.38 million (3%) are found in the rest of the world (FAO, 2008). The major source of meat, especially, in Asia where meat from ruminants constitute about 21.0% of the total meat production, buffalo meat contributes 11.5% of the total ruminant meat, and about 2.7% of all meat produced in the region (Cruz, 2010). In the past century, the buffalo meat was not accepted in the world by the consumers and there was no market. However, the actual trends in consumption require meat with low fat content thereby the non-traditional meat now considered an important source. Among them, buffalo meat has high protein level, low fat and cholesterol content compared to beef (Murthy and Devadason, 2003). Hence, researchers are trying to improve buffalo as potential breeds for qualities meat for human consumption. Heintz (2001) found that the young buffalo meat is superior to young cattle meat. Moreover, buffalo meat has immense demand with increased population, requiring of food in developing countries, particularly, trend of the market in the world, the price of buffalo milk is twice than that of cows' milk, buffalo hide is used in the leather industry and buffalo feces used for manures and fuel in rural areas. Global warming is a hot issue which affects environment and livestock production. Total emissions of greenhouse gases (GHGs) from agriculture, including livestock are estimated to be between 25–32%, depending on the source (USEPA, 2006; IPCC, 2007) and on the proportion of land conversion that is ascribed to livestock activities. Moreover, Goodland and Anhang (2009) reported that livestock production and its by-products are contributes at least 51% of global warming gases or account for at least 32.6 billion tons of carbon dioxide (CO_2) per year. While, CO_2 is the largest greenhouse gases (55-60%) followed by methane (CH_4) (15-20%). Therefore, livestock is the one sector that produces methane from the rumen. It has been estimated that global anthropogenic green house gas (GHG) emissions from the livestock sector approximate to between 4.1 and 7.1 billion tones of CO_2 equivalents per year, equating to 15-24% of total global anthropogenic GHG emissions (Steinfeld et al., 2006). Currently, researchers are trying to reduce methane production in the rumen by

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using many feed additives to inhibit methanogenesis. Meanwhile, plants produce a diverse array of plant secondary metabolites to protect against microbial and insects attacks (Wallace, 2004). These natural plant ecochemicals such as essential oils (EO), saponins, tannins and organo-sulphur compounds have been shown to selectively modulate the rumen microbial populations (Wallace, 2004; Patra and Saxena, 2009a), resulting in an improvement of rumen fermentation and nitrogen metabolism, and a decrease in methane production and nutritional stress such as bloat or acidosis, thus improving the productivity and health of animals (Wallace et al., 2002; Kamra et al., 2006; Rochfort et al., 2008). Recently, a number of studies have discussed the potential of plant bioactive as modifiers of rumen microbial fermentation and ruminant production (Wallace et al., 2002; Wallace, 2004; Hart et al., 2008; Calsamiglia et al., 2007; Patra and Saxena, 2009b).

Greenhouse Gas and Methane Emission by Livestock

Livestock are already well-known contributor of GHG emissions. Livestock's Long Shadow, the widely cited report by the United Nations Food and Agriculture Organization (FAO), estimates that 7,516 million metric tons per year of CO₂ equivalents (CO₂e), or 18% of annual worldwide GHG emissions, are attributable to cattle, buffalo, sheep, goats, camels, horses, pigs, and poultry. Livestock contribute about 9% of total CO₂ emissions, but 37% of CH₄ and 65% of nitrous oxide (N₂O) (Steinfeld et al., 2006). That amount would easily qualify livestock for a hard look indeed in the search for ways to address climate change. But new analysis shows that livestock and their byproducts actually account for 32,564 million tons of CO₂ per year, or 51% of annual (Goodland and Anhang, 2009). This report depicts an alarming call and has created high interest and remarkable debate on the statistics of its estimation. The estimation of CH₄ gas emission from various sources especially from the livestock enteric fermentation accounts for 28% of the total CH₄ emission.

Animal production, plays four important roles in the release of gases into the atmosphere (Leng, 2011) that are (i) directly through production of CH₄ in fermentative digestion of ruminants, (ii) indirectly when a proportion of the fecal materials decompose anaerobically, (iii) indirectly through CO₂ production from fossil fuels to provide the production and marketing infrastructure and inputs such as motorized transport, fertilizers, herbicides and insecticides, (iv) through the clearing of forests and range lands, the timber on which was a natural sink for CO₂.

Methanogens

Rumen ecology is the unique environment where anaerobic fermentation process occurs by the reaction of the rumen microorganisms namely bacteria, protozoa and fungi. The main fermentation end-products are those of volatile fatty acids, ammonia nitrogen (used for microbial protein synthesis), and CH₄ production. All feeds especially, roughages are degraded by the microorganisms by cellulolytic bacteria via the Embden Myerhof Parnas pathway from glucose to pyruvate and further to the synthesis of the short chain volatile fatty acids (VFAs); acetate (C₂), propionate (C₃), butyrate (C₄), valerate (C₅), caproate (C₆). These VFAs are considered as a major source of energy for the ruminants. In addition, hydrogen produced in the rumen is trapped by methanogens to produce CH₄ gas and later be eructated into atmosphere. As shown in Figure 2, fermentation efficiency could be manipulated by many possible ways including the nutritional feeding strategies (Wanapat, 2012). Boadi et al. (2004) reported that methanogens represent a unique group of microorganisms. They possess three coenzymes which have not been found in other microorganisms. The three coenzymes are: coenzyme 420, involved in electron transfer in place of ferredoxin, coenzyme M, involved in methyl transfer, and factor B, a low molecular weight, oxygen-sensitive, heat-stable coenzyme involved in the enzymatic formation of CH₄ from methyl coenzyme M. Five species of methanogens were reported to be isolated in the rumen. These include *Methanobrevibacter ruminantium*, *Methanosarcina barkeri*, *Methanosarcina mazei*, *Methanobacterium formicicum* and *Methanomicrobium mobile*. Only *Methanobrevibacter ruminantium* and *Methanosarcina barkeri* have been found in the rumen at populations greater than 10⁶ mL⁻¹, and are assumed to play a major role in ruminal methanogenesis. Methanogens are hydrophobic and therefore stick to feed particles as well as onto the surface of protozoa. The number of methanogens associated with protozoa reached a maximum (10 to 100 times pre-feeding levels) after feeding, when the rate of fermentation is the highest. It was shown that the symbiotic relationship of methanogens and protozoa might generate 37% of rumen CH₄ emissions.

Dietary Manipulation in Reducing Rumen Methane

There are several factors which can have great impacts on rumen CH₄ production namely level of intake, frequency of feeding, type of roughages, ratio of roughage to concentrate, type and concentration of non-structural carbohydrates etc. All these factors can play important roles on rumen pH, VFAs production, ammonia nitrogen and microbial protein synthesis and the consequences on rumen methanogens and CH₄ production, protozoa and cellulolytic bacteria. Boadi et al. (2004) and Hook et al. (2010) have proposed numerous potential ways as how to mitigate the rumen CH₄ production. The main approaches are as follows: improving animal productivity, nutritional



and management strategies (type of carbohydrates, level of intake, forages type and quality, feeding frequency, roughage treatment/processing, grazing management), management of rumen fermentation (propionate enhancers, use of fats and essential oils, use of plant secondary compounds: condensed tannins and saponins etc.

Plant secondary compounds

The secondary compounds of plant i.e. tannins and saponins are more important as ruminant feed additives, particularly on CH₄ mitigation strategy because of their natural origin in opposition to chemicals additives. Tannins containing plants, the antimethanogenic activity has been attributed mainly to condensed tannins. There are two modes of action of tannins on methanogenesis: a direct effect on ruminal methanogens and an indirect effect on hydrogen production due to lower feed degradation. The condensed tannins (CT) can reduce CH₄ emissions as well as reducing bloat and increasing amino acid absorption in small intestine. Methane emissions are also commonly lower with higher proportions of forage legumes in the diet, partly due to lower fiber contact, faster rate of passage and in some case the presence of condensed tannins (Beauchemin et al., 2008). Supplementation of PCH at 600 g/hd/d was beneficial in swamp buffaloes fed rice straw as a basal roughage, as it resulted in increased DM intake, reduced protozoal and CH₄ gas production in the rumen, increased N retention as well as efficiency of rumen microbial CP synthesis (Chanthakhoun et al., 2011). Legumes containing condensed tannin (e.g. Lotuses) are able to lower CH₄ (g kg⁻¹ DM intake) by 12-15% (Beauchemin et al., 2008; Rowlinson et al., 2008). Also, some authors reported that condensed tannins to reduce CH₄ production by 13 to 16% (DMI basis) (Grainger et al., 2009; Woodward et al., 2004), mainly through a direct toxic effect on methanogens. Woodward et al. (2004) carried out a similar trial with cows fed Lotus corniculatus, on methane was 24.2, 24.7, 19.9 and 22.9 g kg⁻¹ DMI for the respective treatments. The CT in lotus reduced CH₄ kg⁻¹ DMI by 13% and the cows fed lotus produced 32% less methane kg⁻¹ milk solids (fat+protein) compared to those fed good quality rye grass. It was reported that extracts from plants such as rhubarb and garlic could decrease CH₄ emissions. However, there is only limited information on the effect of different saponins on rumen bacteria.

Saponins are natural detergents found in many plants. There have been increased interests in saponin-containing plants as possible means of suppressing or eliminating protozoa in the rumen. A decrease in numbers of protozoa has been reported in the rumen of sheep infused with saponins or fed on saponin-containing plants. Decreased numbers of ruminal ciliate protozoa may enhance the flow of microbial protein from the rumen, increase efficiency of feed utilization and decrease methanogenesis. Saponins are also known to influence both ruminal bacterial species composition and number through specific inhibition, or selective enhancement, of growth of individual species. Saponins have been shown to possess strong defaunating properties both *in vitro* and *in vivo* which could reduce CH₄ emissions (Rowlinson et al., 2008). Beauchemin et al. (2008) recently reviewed literature regarding their effects on CH₄ and concluded that there is evidence for a reduction in CH₄ from at least some sources of saponins, but that not all are effective (Rowlinson et al., 2008). While extracts of CT and saponins may be commercially available, their cost is currently prohibitive for routine use in ruminant production systems. However, still required on the optimum sources, level of CT astringency (chemical composition), plus the feeding methods and dose rates required to reduce CH₄ and stimulate production. Moreover, there have been reports of decreased CH₄ emission by ruminants consuming plant secondary compounds (Carulla et al., 2005; Puchala et al., 2005). Supplementation of pellets containing condensed tannins and saponins (MP and soapberry fruit) influenced rumen ecology by significantly lowering CH₄ concentration in rumen atmosphere and reduced methanogen population (Pongchompu et al., 2009). However, high CT concentrations (>55 g CT/kg DM) may reduce voluntary feed intake and digestibility (Beauchemin et al., 2008; Grainger et al., 2009). Waghorn et al. (2002) reported a 16% depression in CH₄ emissions kg⁻¹ DMI (13.8 to 11.5 g kg⁻¹ DMI) due to the presence of CT in a diet of Lotus pedunculatus fed to sheep housed indoors.

Processing and preservation of feeds

Forage processing and preservation affect enteric CH₄ production but limited information with regard to these effects is available in the literature. Methanogenesis tends to be lower when forages are ensiled than when they are dried and when they are finely ground or pelleted than when coarsely chopped (Martin et al., 2007). Grinding or pelleting of forages to improve the utilization by ruminants has been shown to decrease CH₄ losses per unit of feed intake by 20-40% when fed at high intakes.

Roughage and Concentrate

The forage to concentrate ratio of the ration has an impact on the rumen fermentation and hence the acetate: propionate ratio (declines with F: C ratio). The CH₄ reduction is well in line with the observations of Bannink et al. (1997) that concentrate rich diets showed lower and higher coefficients of conversion of substrate into acetate and



propionate, respectively. However, many experimental databases suggest that a higher proportion of concentrate in the diet leads to a reduction in CH₄ emissions as a proportion of energy intake (Blaxter and Clapperton, 1965; Yan et al., 2000) due to an increased proportion of propionate in ruminal VFA. The scope for reductions in CH₄ emissions depends on the starting level of concentrates, as there are dietary limitations and there are large differences in current usage of concentrates in different regions of the world (Rowlinson et al., 2008). The poor tolerance to low pH by protozoa and cellulolytic bacteria decreases further hydrogen production. A positive correlation between cellulolytic and methanogens in the rumen of different animal species (cattle, sheep, deer) has been shown (Rowlinson et al., 2008), except in the buffalo. This disparity was explained by the fact that *F. succinogenes*, a non-hydrogen-producing cellulolytic species, was the major cellulolytic bacteria of this animal. On the contrary, Sejian et al. (2011) reported that higher proportion of forage to concentrate resulted in decreasing ruminal CH₄ production. They are stated that lower CH₄ production from high forage: grain diet can be attributed to the effect of the high content of fat in the diet which could potentially reduce fiber degradation and amount of feed that is fermentable as well as forage grinding effects. Yurtseven and Ozturk (2009) observed that amount of ruminal methane produced from corn was lower than that of barley grain in ruminant. This is may be due to higher starch content and slow starch degradability of corn vs. barley grain. With regard to the ingredient composition of concentrates, selecting carefully defined carbohydrate fractions, such as more starch of a higher rumen resistance and less soluble sugars could significantly contribute to a reduction in CH₄ emission (Tamminga et al., 2007). Sejian et al. (2011) reported that total mixed ration (TMR) feeding leads to decrease CH₄ production vs. separate forage-concentrate feeding. Wanapat and Wachirapakorn (1990) conducted feeding trial with buffalo fed on untreated and urea-treated rice straw and revealed the results with buffalo perform well especially when consumed urea-treated rice straw a major roughage sources at roughage: concentrate (50:50), respectively.

Plant Oils

There are five possible mechanisms by which lipid supplementation reduces CH₄: reducing fiber digestion (mainly in long chain fatty acids); lowering DMI (if total dietary fat exceeds 6-7%); suppression of methanogens (mainly in medium chain fatty acids); suppression of rumen protozoa and to a limited extent through biohydrogenation (McGinn et al., 2004; Beauchemin et al., 2008; Johnson and Johnson, 1995). Oils offer a practical approach to reducing CH₄ in situations where animals can be given daily feed supplements, but excess oil is detrimental to fiber digestion and productions. Oils may act as hydrogen sinks but medium chain length oils appear to act directly on methanogens and reduce numbers of ciliate protozoa (Machmuller et al., 2000). However, Kongmun et al. (2010) reported that supplementation of coconut with garlic powder could improve *in vitro* ruminal fluid fermentation in terms of volatile fatty acid profile, reduced CH₄ losses and protozoal population. In contrast, there is no response to diets containing 2.3, 4.0 and 5.6% fat (cottonseed and canola) fed over an entire lactation. Beauchemin et al. (2008) recently reviewed the effect of different levels of dietary lipid on CH₄ emissions and reported that with beef cattle, dairy cows and lambs, there was a proportional reduction of 0.056 in CH₄ (g kg⁻¹ DM intake) for each 10 g kg⁻¹ DM addition of supplemental fat. While this is encouraging, many factors need to be considered such as the type of oil, the form of the oil (whole crushed oilseeds vs. pure oils), handling issues (e.g., coconut oil has a melting point of 25°C) and the cost of oils which has increased dramatically in recent years due to increased demand for food and industrial use. In addition, there are few reports of the effect of oil supplementation on CH₄ emissions of dairy cows, where the impact on milk fatty acid composition and overall milk fat content would need to be carefully studied. Strategies based on processed linseed turned out to be very promising in both respects recently. Most importantly, a comprehensive whole system analysis needs to be carried out to assess the overall impact on global GHG emissions (Rowlinson et al., 2008).

Buffalo's Ability to Utilize Non-protein-Nitrogen (NPN) in the Diet

The swamp buffaloes have been shown to adapt well to high level of urea (NPN) especially at 4% of DM concentrate mixture. As a result, cellulolytic bacteria (*R. allbus*, *R. flavefaciens* and *F. succinigenes*) were found significantly increased measured by real-time PCR technique (Wanapat et al., 2016). This agreed with the reports of Kang et al. (2015) who have earlier reported that urea at high level can result in comparable results with SBM when the buffaloes were fed on rice straw increasing nutrient digestibility, propionic acid, N-balance as well as efficiency of microbial nitrogen synthesis. Whilst, Ampapon et al. (2016) additionally provided information that high level of urea supplementation with cassava hay, remarkably improved rumen fermentation by increasing microbial protein synthesis per day, propionic acid, C₂:C₃, and hence reducing rumen CH₄. Buffalo had higher nutrient digestibility than cattle when fed on rice straw and NH₃-N was limiting factor (Wanapat et al., 2013). Ratio of roughage to concentrate in the diets can remarkably influence rumen fermentation end-products due to the shift of rumen pH, microorganism, hence on the production of volatile fatty acid and NH₃-N production (Wanapat et al., 2014). Cassava



hay with high level of crude protein (25% CP) and tannin (3%) can improve rumen fermentation and nutrient utilization in buffalo (Chanjula et al., 2004).

The nutritional management for buffalo production especially for riverine-type buffalo was outlined in details using different kinds of feeds such as straw treatment urea-molasses block, leguminous supplements and other (Sarwar et al., 2009). Later, Patha (2013) has made a comprehensive review on prospect of nutrition and feeding for buffalo production, the findings of the higher ability of nutrient utilization of NPN and rumen by-pass protein, as well as, the efficient converter of low-quality roughage have been reiterated. Seasonal feed resources are of prime importance for swamp buffaloes to support the efficient production under the prevailing small-holder farming systems. Manipulations of rumen microorganisms, fermentation and subsequent absorption by the animals are essential. Current research work on locally available feed resources such as urea-treated rice straw, cassava hay etc. revealed significant improvement in rumen ecology with higher cellulolytic bacteria and fungal zoospores and subsequent fermentation end-products. However, investigation of rumen microorganisms diversity of swamp buffalo and their roles in fermentation using molecular technique especially the use of PCR–DGGE/Real Time-PCR warrant future research undertakings (Wanapat and Rowlinson, 2007).

Rumen Microorganisms and Anaerobic Fermentation

It has been stated that the rumen is a complex ecosystem but it can be better defined in terms of input or estuaries. Fermentation of carbohydrates in the rumen into VFAs and CH_4 via Embden Myerhof Parnas Pathway and were presented in details (Czerkawski, 1972; Leng, 1993). Ruminants require roughages in their diets to maximize production in maintaining health by sustaining a stable environment in the rumen. Relationship between fermentation on VFA production and physically effective fiber in the rumen are necessary. Understanding of stoichiometry of rumen fermentation has indicted an important approach to ameliorate the greenhouse effect that is, lowering of enteric CH_4 production per unit of feed intake or per unit of animal products from ruminants by strategic supplementation (Leng, 1993). Cheng et al. (1997) and Hungate (1966) was the pioneer in studying rumen microbial ecosystem, developing the roll-tube technique to culture anaerobic bacteria, protozoa and fungi and the author of the book “The Rumen and Its Microbes”. This book not only contributes to the understanding of the complicated microbial ecosystem of the rumen but also paves the way for rumen microbes studies up until now. Dehority (1991) showed that fiber digestion in the rumen was effects of microbial synergism of bacteria, protozoa and fungi living in the rumen. The term “YATP” was defined by Bergen (1977) as ruminal production of microbial cells as a function of energy availability to the microorganisms and the efficiency of cellular synthesis (g cells of formed/mole ATP used). The ruminal fermentation is a coupled process between carbohydrate degradation, VFA production and concomitant ATP generation and the process of microbial cell synthesis from nitrogenous precursors, mainly $\text{NH}_3\text{-N}$, carbon skeletons, sulfur and others. The ruminants utilize VFA as a major energy source whereas the microbial cells are as protein sources (amino acids) and vitamin B complex (Bergen, 1977; Dehority, 1991). Furthermore, the role of rumen fungi was found to be synergistically supporting the bacteria in the rumen of buffalo (Ho et al., 1988).

When comparing rumen microorganisms, Wanapat et al. (2000) reported that swamp buffalo had remarkable higher population of bacteria and fungi zoospores and lower protozoa population than those in cattle raised in similar condition (Figure 1, 2 and 3).

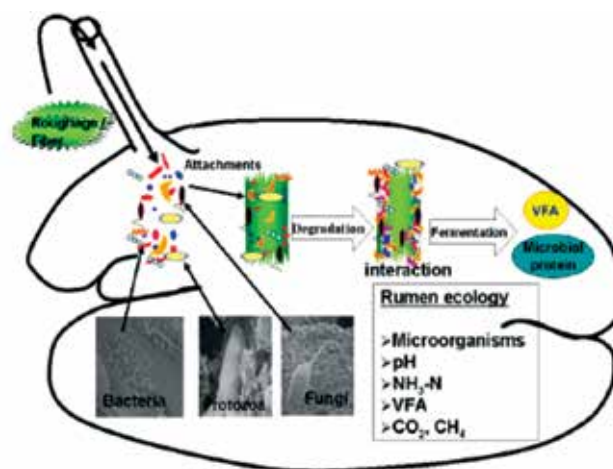


Figure 1. Illustration of rumen of swamp buffalo with ruminal bacteria, protozoa, fungal zoospores, fermentation and fermentation end-products

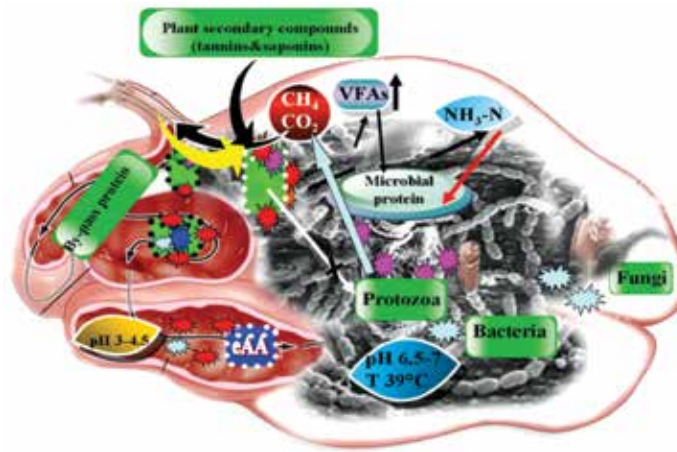


Figure 2. Role of plant secondary compounds (condensed tannins and saponins) on rumen fermentation process (Wanapat et al., 2012)

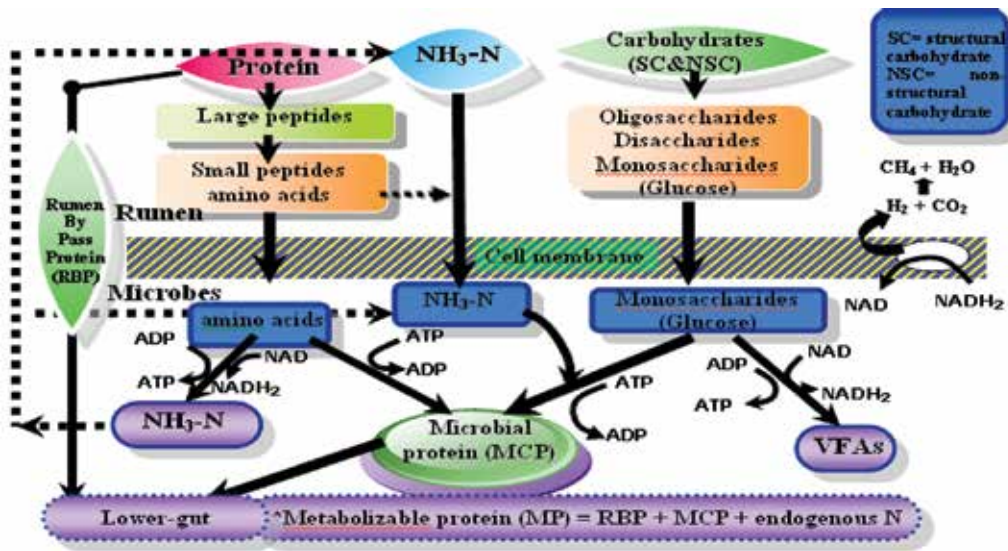


Figure 3. Energy and protein metabolism in the rumen microbial protein synthesis and methane production (Wanapat, 2012; modified from Nocek and Russell, 1988)

Food-feed-system

Food-feed-system (FFS) has been shown to produce both foods for human and feeds for animal. Moreover, intercropping with legumes can enrich nitrogen in the soil. Wanapat et al. (2007) found that yield of cassava foliage, when intercropped with legume, cowpea produced 5.96 ton/ha of green cowpea pod. It was found that productivities of intercrops were improved with a biomass of 6.83 ton DM/ha of cassava foliage, and 0.89 ton DM/ha of cowpea residues (initial cutting at 4 months and thereafter 4 cuttings at 2 months interval. In addition, a legume, *Stylosanthes*, was also intercropped in the cassava plot, and it produced 3.51 ton DM/ha. The practice of cassava-legumes intercropping also improves farm productivity. However, some farmers encountered problems with drying hay in the rainy season, therefore, the alternative strategies such as constructing solar-drying houses using simple materials such as plastic sheets and bamboo was recommended to farmers. As a result of FFS, green cowpea pods were used for household consumption, as a gift to neighbors and sold for generating higher incomes, while cowpea residues and *Stylosanthes* fodder were used as animal feeds (Wanapat et al., 2003).

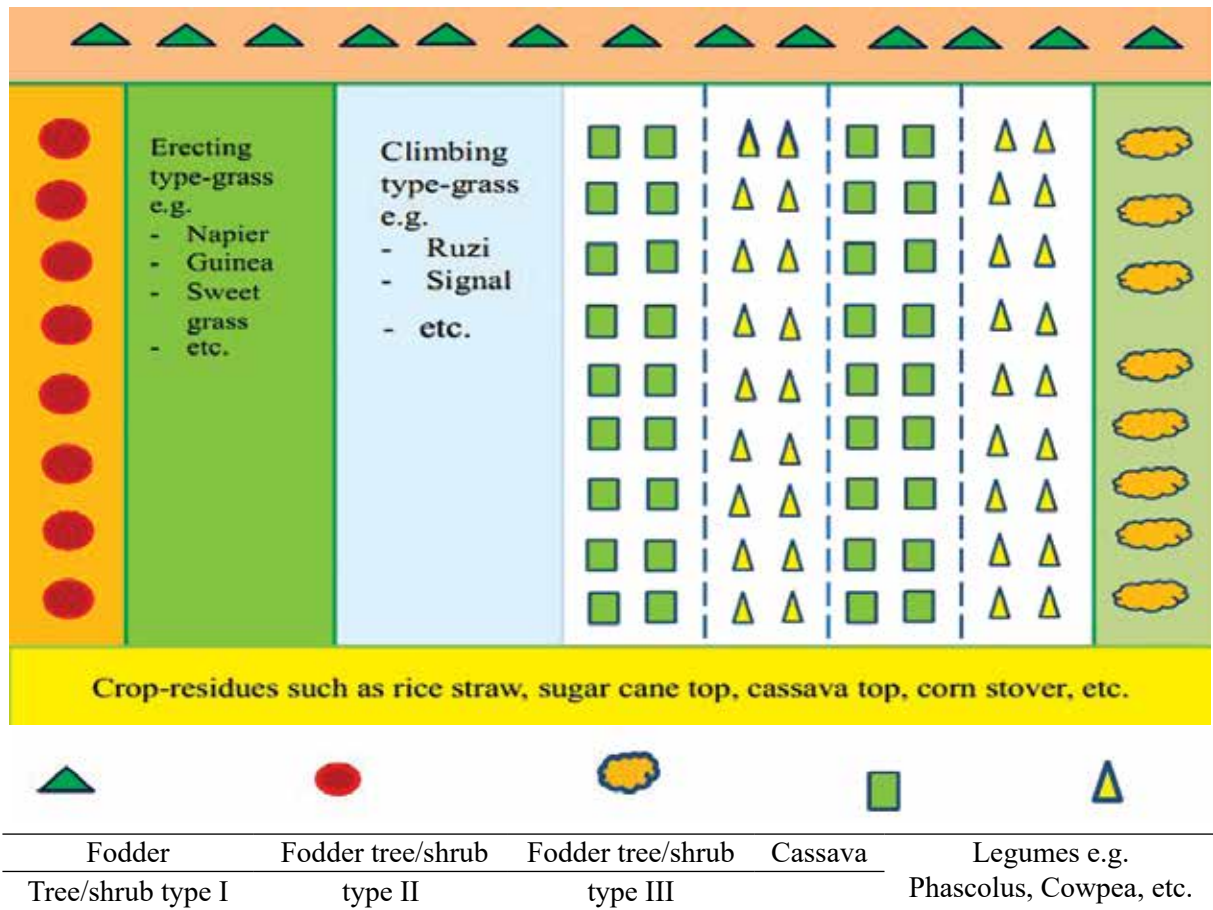


Figure 4. The proposed feeding system for ruminants, food-feed system (FFS) for the sustainable ruminant feeding system for smallholder farmers in the tropics

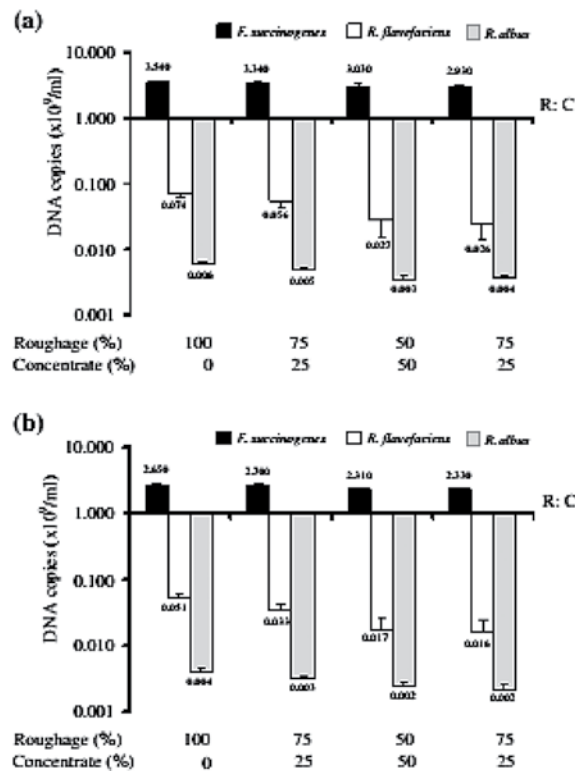


Figure 5. Population of the three representative cellulolytic bacterial species, *F. succinogenes*, *R. flavefaciens*, and *R. albus* in the digesta (a) and rumen fluid (b) of swamp buffalo fed different roughage (urea-treated rice straw)-to-concentrate ratios while values were averaged from samples taken at 0 and 4 h post feeding (Wanapat and Cherdthong, 2008).



Table 1. Effect of coconut oil and mangosteen peel supplementation on microbial abundance (Log copies/ml)

Item	Control	CO5	MP3	COM	SEM	P
Total bacteria	9.78 ^b	10.09 ^a	9.84 ^{ab}	9.92 ^{ab}	0.08	0.042
Protozoa	6.40 ^a	6.20 ^b	6.33 ^{ab}	6.14 ^b	0.06	0.048
Methanogen	6.07	5.92	6.12	5.98	0.08	0.089
<i>Fibrobactersuccinogenes</i>	9.04 ^a	7.14 ^c	8.88 ^a	7.44 ^{bc}	0.12	0.034
<i>Ruminococcusflavefaciens</i>	6.28	6.35	6.27	6.30	0.05	0.210
<i>Ruminococcusalbus</i>	6.43	6.49	6.44	6.44	0.05	0.419

CO5, coconut oil 5% dry matter intake; MP3, mangosteen peel 3% dry matter intake; COM, combination of CO5 and MP3; SEM, standard error of the means.

a–c

Mean values in the same row with different superscripts differ significantly at p values given in the last column (n = 4).

CO5, coconut oil 5% dry matter intake; MP3, mangosteen peel 3% dry matter intake; COM, combination of CO5 and MP3; SEM, standard error of the means.

a–c

Mean values in the same row with different superscripts differ significantly at p values given in the last column (n = 4).

CO5, coconut oil 5% dry matter intake; MP3, mangosteen peel 3% dry matter intake; COM, combination of CO5 and MP3; SEM, standard error of the means. a–c mean values in the same row with different superscripts differ significantly at p values given in the last column (n = 4) (Pilajun et al., 2004).

Table 2. Rumen adaptation for urea on rumen fermentation in swamp buffaloes

Items	T0	T1	T2	T3	T4	SEM
Ruminal pH	6.9	6.8	6.8	6.7	6.9	0.12
Ruminal Temperature, °C	38.5	38.8	38.9	39.1	39.3	0.18
Ammonia nitrogen, mg/dl	7.0 ^a	14.6 ^b	15.7 ^b	23.8 ^c	24.5 ^c	1.36
Blood urea nitrogen, mg/dl	1.2 ^a	13.4 ^b	12.5 ^b	19.1 ^c	20.4 ^c	2.56
Total VFA, mmol/l	74.1 ^a	80.3 ^b	84.6 ^c	95.4 ^c	101.2 ^c	1.04
Acetic acid, %	76.3 ^a	72.6 ^b	73.2 ^b	65.6 ^c	65.8 ^c	0.56
Propionic acid, %	12.4 ^a	18.8 ^b	19.4 ^b	25.2 ^c	26.9 ^c	0.41
Butyric acid, %	11.3 ^a	8.6 ^b	7.4 ^b	9.2 ^c	7.3 ^c	0.21
Acetic acid/ Propionic acid ratio	6.2 ^a	3.9 ^b	3.8 ^b	2.6 ^c	2.4 ^c	0.36

Means in the same row with different superscript letters differ (P<0.05). T0, without concentrate; T1, concentrate containing urea at 20 g/kg DM for a period of 2 weeks; T2, concentrate containing urea at 20 g/kg DM for a period of 4 weeks; T3, concentrate containing urea at 40 g/kg DM for a period of 2 weeks and T4, concentrate containing urea at 40 g/kg DM for a period of 4 weeks (Wanapat et al., 2016).

Table 3. Rumen adaptation for urea on ruminal microbes in swamp buffaloes

Items	T0	T1	T2	T3	T4	SEM
Direct count × cell/ml						
Bacteria, ×10 ⁹	5.2 ^b	5.8 ^b	6.3 ^b	7.7 ^c	8.6 ^d	0.43
Protozoa, ×10 ⁵	4.3	4.6	4.2	4.7	4.5	0.09
Fungal zoospore, ×10 ⁴	2.7 ^a	3.0 ^a	3.4 ^a	4.9 ^b	5.3 ^b	0.92
Microbe by real-time PCR						
Total bacteria, ×10 ⁸	1.1 ^a	1.7 ^a	1.5 ^a	6.6 ^b	7.4 ^c	0.36
<i>R. albus</i> , ×10 ⁷	3.6	3.4	3.2	3.3	3.7	0.02
<i>F. succinogenes</i> , ×10 ⁷	2.3 ^a	2.5 ^a	2.7 ^a	5.4 ^b	6.3 ^c	0.32
<i>R. flavefaciens</i> , ×10 ⁶	5.6 ^a	5.7 ^a	5.4 ^a	6.8 ^b	7.5 ^c	0.32
Methanogens, ×10 ⁴	2.2	2.3	2.3	2.4	2.6	0.02



Means in the same row with different superscript letters differ ($P < 0.05$). T0, without concentrate; T1, concentrate containing urea at 20 g/kg DM for a period of 2 weeks; T2, concentrate containing urea at 20 g/kg DM for a period of 4 weeks; T3, concentrate containing urea at 40 g/kg DM for a period of 2 weeks and T4, concentrate containing urea at 40 g/kg DM for a period of 4 weeks (Wanapat et al., 2016).

CONCLUSIONS

Buffaloes seem to be an important ruminant globally serving in many aspects for the world population engaging the integrated farming systems. Their ability in utilizing high fibrous feeds especially those of agricultural crop-residues and by-products, through the presence of diverse microorganisms in the unique rumen ecology, providing energy and protein for their productive functions have been significantly discovered. Manipulation of the rumen ecology by dietary factors such as the use of plant secondary compounds could have an impact on rumen microorganisms, microbiome and fermentation end-products. Furthermore, with advancement of molecular techniques, their applications in rumen buffalo research, would offer additionally useful data with regards to rumen ecology, particularly pertaining to microbiome, CH_4 production and mitigation, feed degradation and utilization, enzyme production, as well as meat quantity and quality. Most importantly, the EDU-S for buffalo production to increase production aspects, as well as to maintain the friendly-environmentally atmosphere and to enhance the livelihood of the farmers are profoundly implemented.

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EFFECT OF DIFFERENT SILAGE ON MILK PRODUCTION OF INDIGENOUS BUFFALO IN WESTERN HILLS OF NEPAL

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ABSTRACT

An attempt was made to study on effect of feeding of different silage on lactating indigenous buffalo (*Bubalus bubalis*) at mid hills of Nepal. Maize silage was prepared from maize stover after harvesting of maize and forage silage was prepared with Napier (*Pennisetum purpureum*) and Teosinte (*Zea maxicana*). Multifarious indigenous buffalo were fed a concentrate at 0.68% of their body weight (BW) on a concentrate dry matter (DM) basis daily while having *ad libitum* access to rice straw; rice straw with the concentrate (T1), with 33% maize silage (T2), 67% of maize silage (T3) and 100% maize silage (T4). Similarly another experiment was performed by substitution of 67% rice straw with forage silage (T1), substitution of 67% rice straw with maize silage (T2), substitution of 67% rice straw with 33.5% of forage silage and 33.5% maize silage (T3) and substitution of 100% rice straw with 50% of forage silage and 50% of maize silage (T4) along with concentrate of 0.68% of body weight. The nutritive values of rice straw, fodder, concentrate feed, maize silage and forage silage 3.68%, 10.26%, 15.33% 6.14% CP and 7.41% CP respectively. The milk composition did not differ among the treatment. Result of study revealed that substitution of 67% rice straw with maize silage and substitution of 100% rice straw with 50% of forage silage and 50% of maize silage produced highest milk production.

Keywords: Dry matter, maize silage, forage silage, indigenous buffalo

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EFFECT OF CHALLENGE FEEDING DURING PREGNANCY ON THE PRODUCTION AND REPRODUCTION PERFORMANCE IN DAIRY ANIMALS- A STUDY

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ABSTRACT

A study was conducted on 200 healthy pregnant average yielding (1500-2000 Litre/day) cows and buffaloes in Dharwad milk shed area. These animals were randomly divided into four groups (each group containing 50 animals) on the basis of age, stages of pregnancy, previous lactation yield and number of lactations. These animals were provided with normal ration in the beginning of pregnancy. During 7 to 8 months of pregnancy, challenge feed (for 3 months) were provided to these animals. The post partum feeding for all animals were kept uniform. These animals were hand milked scientifically in farmers' cowshed. The observation was made that resulted in increased milk production to 15-20 percentage, the body weight of the calves to 30-40 percentage increased and calf mortality decreased to less than 10 percentage, calves diseases decreased to 15-20 percentage. There was an apparent reduction in the interval for first estrus post partum by 45-55 days in animals under challenge feeding. Thus, it was inferred that challenge feeding during pregnancy, after 7 and 8 months of pregnancy was beneficial effect towards maintaining the milk production and as well as reproduction in cows and buffaloes of Dharwad milk shed area.

Keywords: Challenge feeding, body weight, post partum, estrus, milk yield.

INTRODUCTION

In Dharwad milk shed area, cattle and buffaloes were generally fed on wheat, jowar, paddy and other straws and stovers. These were supplemented with small quantities of grass available from scanty grazing land or cut grass. Since farmer receives the immediate returns on their investments through saleable milk to co-operative societies, generally no balanced cattle feed mixture (concentrate) was fed to the growing, milking pregnant and dry animals. Only lactating animals were given better feeding through supplementation of by-products concentrate such as oil cakes, barns and milled pulses. In pregnant animals the nutritional needs and dry matter intake plays very important role. Usually, the animals are fed diet as crops residues available in farmland, but these residues are deficient in energy, minerals and vitamins that restricts intake of feed quantity as well as digestibility. In turn, it leads to less milk production and delay in reproductive activities. The dry matter intake varies according to the density of energy in the diet, digestible amount of crude fiber, the physiological nature of the feed, succulence, odor and texture. Ambient temperature also plays very important role in feed intake. Feed formulation of ration is carried out based on physiological needs for specific functions such as, body maintenance, growth, quantitative milk production and quality of milk yield, pregnancy and drought condition. These various physiological functions requires adequate amount of energy, protein, minerals and vitamins. In normal dairy animal feeding practice, Importance is given to the energy and proteins. Idealdairy Cow/Buffalo should calve once in a year (i.e., 12 to 18 months intervals and should have a lactation length of about 300 days). But in practice calving intervals are often longer or lactation period is shorter hence, dry period up to 8 to 10 months (sometimes one year). During this period, animals should gain body weight as their fat is lost in the early lactation periods. It is required for the growth of the fetus also for the regeneration of mammary tissues.

In practice for "steaming up" of pregnant dry cows/buffaloes, balanced cattle feed concentrate of high energy is given and it is gradually increased during the last trimester of pregnancy. By the time of calving, in the early lactation period, the amount of concentrate given is about 75 percentage of the quantity the animal is expected to require. Steaming up is claimed to increase milk production, in part of preparing the animal for high intakes of concentrate that should be fed in early lactation. During the last 60 -90 days of pregnancy live weight increases by about 20-30 kg. The response to steaming up probably depends on the body condition at the beginning of the dry period. Restoring the reserves of the thin animal will probably have a greater effect in subsequent milk production than increasing the reserve of a fat in animal body. Normally 50 percentage of Digestible Crude Protein (DCP) and 25 percentage of Total Digestible Nutrient (TDN) of the maintenance requirements are fed to the animal. In order to make up these requirements 2-3 kg of additional balanced cattle feed concentrate mixture over the above maintenance ration should be given to pregnant dry cows and buffaloes. The objective of study was to know the effect of challenge feeding during pregnancy on the production and reproduction performance in dairy animals.

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MATERIALS AND METHODS

Selection and Grouping of Animals

The study was conducted for selected two hundred healthy pregnant cows and buffaloes of Dharwad milk shed area. These randomly selected animals were cross breed, local buffaloes/upgraded buffaloes. These animals were grouped into four groups; in each group 50 animals were selected. These animals were managed by the farmers according to their farm practices of their own. All the selected animals were allowed to graze 2-3 grazing hours daily and were fed dry and locally available green forages but concentrate feeding was not a common practice in this area. However, pulse based by products and some homemade concentrate was fed to the animals prior to the experiment.

These animals were selected through Artificial Insemination Records of dairy co-operative societies. Rectal Examination of these animals for pregnancy diagnosis was conducted by the mobile Veterinary doctors weekly. Selected animals were dewormed at the 5th and 6th month pregnancy. One day orientation training programme were conducted for these animal owners and feeding bags and reading materials kits were distributed to them through dairy co-operative societies.

Factors Studied

The observations were made on milk yield produced, body weight of the calves, and other quality factors such as FAT & Solid Non Fat (SNF) percentage, Calf mortality, calf diseases and interval for first post partum estrus. Selected animals' breed, age, body conditions score, body weight were measured and their previous milk yield factors were recorded such as FAT and SNF also stages of pregnancy considering all these factors.

Study Duration and Monitoring

The Milk productive data were collected over period of 5-6 months and reproductive data were conducted for 10-12 months. After an acclimatization period of 2-3 weeks, field visits were conducted closely and frequently to monitor by the mobile veterinary routes weekly. General health checkups and necessary advices were given to animal owners as and when required by them. Farmers were given advice on animal management, milk production, reproduction and data recording of the individual animals.

Raw Materials used in the Cattle Feed Preparations

The following raw materials used for preparation of balanced cattle feed mixture. Various combinations of ingredients can be compounded depending upon the availability, cost of the ingredients and cost per unit protein and cost per unit energy.

- Grains- Maize, Jowar, Broken rice, Wheat, Ragi.
- Brans- Ricepolish, Deoiled Rice bran, Wheatbran
- Cakes & Deoiled cakes- (doc)
 - Cakes- Coconut cake, Cotton seedcake.
 - De oiled cakes- soybean doc, Rapeseed cake, Cottonseed cake, Groundnut cake, Coconut cake, Safflower cake and sunflower. (doc)
- Premix Material;
 - Common Salt - 1.5%
 - Calcite Powder – 15%
 - Mineral Mixture – 2%
 - Urea – 1 %
- Molasses – 10-12 % and it contains 50-55 % of sugar & trace minerals.

Quality specification of Balanced Cattle Feed

1. Moisture (Max)	11.0%
2. Crude protein (Min)	25.0 %
3. Ether Extract (Min)	2.50 %
4. Crude fiber (Max)	0.80 %
5. Sand silica (max)	3.00 %
6. Total Digestible Nutrients (Min)	55-60 %
7. Rumen Degradable Protein (Min)	6.80%
8. Rumen Un-Degradable Protein (Min)	15.0 %
9. Metabolizable energy (MJ/kg)	9.50%



Tools and Methods for Data Collection

The data were collected personally by the author through participant observations, focused group discussions, key information technique as per the necessity during the study and personal interviews with the animal owners. With the help of semi structured interview schedule farmers' perception towards challenge feeding during last trimester pregnancy were collected. During the interview, care was taken to get the accurate information from the animal owners. A simple formulated register was supplied to each farmer to record the data.

The farmers were initially asked them to feed their animals the balanced cattle feed mixture $\frac{1}{4}$ kg to $\frac{1}{2}$ kg and later on advised to increase up to 1 kg in the morning and 1 kg in the evening. The quantity of balanced cattle feed mixture was gradually increased.

RESULT AND DISCUSSION

The author and mobile route veterinarians of milk Unions carried out regular weekly visits to the selected villages. At the end of the gestation period of cows and buffaloes, they were calved normally without any much complication such as dystocia, retention of placenta and ill health of the calf.

Body Weight

During the study conducted, the weight measurement of the calf taken was at farmers doors step and body weight immediately after birth in normal condition was 18-22 kgs. But in the animals which are given the balanced cattle feed mixture, the body weight of the calves were increased by 30-40% and daily weight gain of cow calves was around 250 gm and 400 gm per day in case of buffalo calves.

Milk Yield

Previous milk yield was recorded for these experimental animals and found that milk yield in cows was 6-8 liters/day and 3-4 liters/day in buffaloes. After the challenge feeding to these animals during 4-5 months, daily milk yield after calving these animals were recorded twice a day in co-operative milk societies was recorded and observed that not only there was milk yield but also there was little increase in FAT & Solid Non Fat (SNF) content in the milk. Hence, after the calving it was observed that milk yield was increased to 15-20%.

Calf Mortality

Calf mortality is very high in Indian condition which is around 60-70% due to internal parasites, diarrhea, respiratory and infectious diseases. In these challenge feeding the animal groups which are calved, the calf mortality was less than 10% due to 5th month dewormed the pregnant animals and regular checkup were held. After calving these animals, calves were fed sufficient quantity of colostrums as per the body weight and regular de worming and feeding of other feed stuffs after 21 days, and after 3 months, foot and mouth diseases (FMD) vaccination and after 4 months hemorrhagic septicemia (HS) and black quarter (BQ) vaccination were given to these calves.

Disease

After calving these challenged feed experimental animals, they did not suffer from metabolic diseases like ketosis, milk fever and there were no history of retention of placenta (ROP) and other uterine infections such as metritis, endometritis.

Post Partum Estrus

Farmer's perception stated regarding the supplementation of challenge feeding during last trimester of pregnancy most of these experimental animals showed estrus symptoms within 30-45 days and 75% of cows conceived after 3-4 months. Similarly 10-15% of cows conceived after 4- 6 months and 8-10% conceived after 6-8 months.

Nearly 70-80% of farmers expressed happiness about challenge feeding that helped them to get the increased in the milk yield, healthy calf, no problem during calf born and their animals came to heat within a prescribed period. In some cases 20-30% farmer fed the feed to the other lactating animals in their cow shed.

But 10-20% farmers expressed their opinion about challenge feeding process is laborious, little costly and non availability of the balanced cattle feed regularly. Private cattle feed is expensive and non-palatable.

CONCLUSION

Thus the study revealed that average milk yield increased by 15-20%, body weight of the calves were increased to 30-40%, major diseases were not found much in these animals, estrus reduction in the interval for first



estrus post partum by 35-55 days to achieve '**calf a year**'. It was inferred that challenge feeding during pregnancy (last trimester) was most beneficial effect towards high milk production as well as reproduction in dairy animals like cows and buffaloes.

RECOMMENDATIONS

1. Regular training programme of dairy animal management (DAM) is must.
2. Low cost feed formula for the benefit of economically backward dairy farmers.
3. Regular supply of balanced cattle feed to dairy co-operative Societies.
4. Supplement free of cost cattle feed or subsidy to the cattle feed.

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WEANING INDUCED STRESS ALTERS IMMUNE AND ENDOCRINE FUNCTIONS IN BUFFALO-CALVES

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ABSTRACT

A total of 60 buffalo calves of either sex aged 3 months, were used in this study. Total contingent was divided into three groups viz., treated, placebo and untreated comprising of 10 calves (5 male and 5 female) for each type of weaning protocol. The blood was collected 2 and 1 day before and 1 and 14 day after weaning in all groups. Levamisol® was given orally two days before weaning after collection of first blood sample. Statistical analysis revealed that gradual weaning method did not affect any of the parameters. Contrary to the gradual method, abrupt weaning method affected seven parameters, namely PCV, Hb concentration, RBC, leukocytes, lymphocytes, neutrophils and eosinophils. These parameters however, turned to normal by the day 14 after weaning. The values of leukocytes, neutrophils, red blood cells, Hb conc., PCV, rose on day 1 after weaning, while the percentages of eosinophils, lymphocytes decreased one day following weaning. These parameters returned to preweaning values on day 14 post weaning. Abrupt weaning protocol affected significantly ($P < 0.05$) six parameters including 4 metabolites and 2 electrolytes. The values of activity of γ -GT and concentrations of cholesterol, Na and Cl rose on day 1 after weaning, while serum concentration of triglycerides decreased one day following abrupt weaning. These parameters returned to preweaning values on day +14 post weaning except serum cholesterol, which declined to a significantly lower level on day 14 post weaning than preweaning value. Serum AP declined markedly on 14 day after weaning. Sex made significant effect only on cholesterol concentration among all serum biochemical parameters. Abrupt weaning caused an acute rise in serum conc. of cortisol, triiodothyronine (T_3) and thyroxin (T_4) in buffalo-calves on day 1 post weaning. Females presented higher values of serum cortisol and triiodothyronine as compared with preweaning values. Immune measures lymphocyte stimulation assay and Ig-A remained unaltered throughout study.

Keywords: Weaning, stress, haematochemistry, metabolites, immune function, hormones

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BUFFALO PRODUCTION AND MANAGEMENT AT NATIONAL BUFFALO RESEARCH PROGRAM, NEPAL

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ABSTRACT

Production practices and some recent research achievements of NBRP has been presented in this paper. The average performance of Murrah buffaloes recorded in 2015 were: lactation yields (1229.7±380.7 kg), calving interval (387.6 ± 31.5 days), calving to conception (49.4±14.0 days), dry days (65.3±12.7 days). For fattening purpose, the supplementation of 20-30 % crude protein, 50-60% green forage and 20% straw showed beneficial effect in body weight gain, feed consumption and feed conversion efficiency. In another study, body weight gain of 540 g/day was observed in heifers with additional ration in comparison to 337 g/day gain with maintenance ration. Reconditioning young male buffalo for at least 30 days after 1-2 days of transportation was found to be beneficial for meat production, where, reconditioning for 15, 30 and 45 days showed increase in quantity of edible meat by 70.4 kg, 76.72 kg, and 81.3 kg, respectively. We found that calves can be raised successfully by replacing whole milk feeding with unconventional milk replacer after the age of 21 days. Feeding milk replacer based on buttermilk and whole milk can save Rs. 2284 (~22 USD) and Rs. 2141 (~21 USD) per calf, respectively during the rearing period of four months. Mastitis and uterine prolapse were occasionally observed. The presented guidelines will be useful for buffalo farmers to increase their productivity and profit. Future research plan includes development of suitable breeds of buffalo for hill and Terai belts, and maintenance of selected genotypes of buffalo breeds, in collaboration with Animal Breeding Division, NARC, Khumaltar Lalitpur.

Keywords: NBRP, buffalo production and management

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COMPARATIVE HOOF VOLUME ASSESSMENT OF NILI-RAVI BUFFALO BEING REARED ON DIFFERENT FLOORING SYSTEMS

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ABSTRACT

Age, breed, season, nutrition, and type of flooring affect bovine hoof volume. Abrasive floors increase the wear and tear of horn tissues. Also, growth rate of claw horn is faster in yearlings as compared to mature animals. Considering such effects of age and flooring systems on hoof morphology, present study was designed to estimate hoof volume and weight bearing capacity of immature and adult buffaloes reared under different flooring systems. The study was carried out on concrete (Buffalo Research Institute –BRI) and soft (backyard-BY) flooring consisting of 15 immature (< 2 years) and 15 mature (2<11 years) animals in each flooring system. For assessment of hoof volume three measurements (cm) (coronary band, base, and abaxial) were taken from each claw of each hoof. Measurements of lateral and medial claws of same hoof were pooled and the volume (cm³) was calculated using the formula: (17.192 x Base) + (7.467 x AbaxGr) + 450270 x (CorBand) – 798.5. Data were analyzed using one way ANOVA and independent t-test. Differences were considered significant at P<0.05. Volume of fore-hooves of mature BRI (851.26±50.47) and BY (915.23±39.31) buffaloes was higher than hind-hooves of immature BRI (774.43±65.47) and BY (823.33±48.35) buffaloes, respectively. Volume of fore-hooves of mature BY (915.23±39.31) and immature BY (823.33±48.35) was higher than mature BRI (851.26±50.47) and immature BRI (774.43±65.47), respectively. Weight bearing capacity of fore-hooves was higher than hind-hooves, and of mature BY-reared buffaloes than that of mature BRI-reared buffaloes. In conclusion, hoof volume and therefore weight bearing capacity in buffaloes is affected by the flooring system. Smaller hoof volume observed in buffaloes reared on concrete floor, increases the risk of lameness due to decreased weight bearing capacity. Greater hoof volume observed in buffaloes reared on soft flooring results in increased weight bearing capacity which is a protective factor with reference to lameness.

Keywords: Claw growth, concrete floor, welfare, lameness, weight-bearing, horn tissue

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MORPHOLOGICAL AND PRODUCTIVE TRAITS OF BUFFALOES OF EASTERN TERAI, NEPAL

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ABSTRACT

A field study was conducted during October, 2016 at Kosaha-2, Shreepur-7 and Laukahi-5 of Sunsari district to understand the productive performance, morphometric measurements and qualitative traits of 20 adult buffaloes in Eastern Terai of Nepal. Productive performance of buffaloes was recorded based on direct observation in the field. Qualitative information regarding existing production system were recorded based on personal interview with the farmers/owners. Data were analyzed by simple statistical tool using MS Excel and SPSS.v16. Results of present study reflected that the age at first calving was 3.75 ± 0.14 years, whereas, total number of calves born per buffalo was observed 1.75. Similarly, number of lactations in this study was recorded 3.2 times, daily milk yield (1.6 liters), lactation length (9.5 months), and peak yield (3.8 liters). Hand milking was common practice for majority of the farm households. Majority of the buffalo population in the study areas were naturally mated. Morphometric traits such as body weight was recorded as 331.5 ± 7.6 kgs, chest girth (170.5 ± 4.5 cm), body length (128.5 ± 3.0 cm), height at withers (132.5 ± 4.1 cm), hock circumference (19.9 ± 0.4 cm), tail length (79.6 ± 6.0 cm), ear length (28.5 ± 0.9 cm), and horn length (32.76 ± 2.1 cm). Regarding to other morphological traits, majority of the buffaloes were observed plain, black and pigmented coat color, skin, muzzle, eyelid, eye, and hooves (with white spots on forehead, legs and tail switch). Similarly, almost 95% buffaloes had fixed horn attachments with black (70%) and grey (30%) horn color. Haemorrhagic septicaemia (HS), tapeworm infestation, weak legs were the major problems prevalent in the study areas. Whereas inbreeding, repeat breeding, low milk productivity and shortage of high quality local buffalo bull for breeding were other important problems for buffalo herders in the region. Thus the information observed and reported in this study would have great importance in developing effective buffalo improvement plans focusing terai regions in the future.

Keywords: Terai buffalo, productive performance, morphometric measurements, inbreeding

INTRODUCTION

Buffaloes are significant livestock commodity with total population of about 5.16 million in the country (DLS, 2016). Buffalo's contribution to the total Agriculture Gross Domestic Products (AGDP) is highest among other livestock commodities. They are the major source of milk and meat contributing 65% and 58 % respectively to the total production (DLS, 2016). Apart from providing food to human beings, they also provide power for agricultural operations (draught and pack), manure for agricultural growing crops, hides, bones and hairs for multiple uses. They have cultural, social and religious values. Their versatile role in rural households and national economy seems tremendous (Neopane et al., 2007). Indigenous buffaloes are known for their hardiness, disease resistance, survival on little inputs and adaptability to variable environments (Kohler-Rollefson et al., 2009). Initially, there were three breeds of buffaloes identified as indigenous in Nepal namely; Gaddi (Far western mid-hill region), Parkote (western and central mid hill region) and Lime (western mid-hill region) as shown in Figure 3. However, there exist other buffalo breeds in different parts of the country showing typical qualitative, phenotypic, productive traits which help to initiate this study to discover new breeds or strains.

Terai buffaloes are typical and distributed east to west across Terai regions of the country. They are acclimatized in the hot terrain environment of the country. Few scientific works have been done on phenotypic characterization, production performance and karyotyping work by Pokharel et al. (1998); Rasaili et al. (1998); Neopane et al. (1999); Amatya, et al. (2000); and Neopane et al. (2007) on indigenous buffaloes in specific regions. Phenotypic characterization of western terai buffalo from Banke district has been completed and they also belong to *Bubalus bubalus* and type riverine (Neopane et al., 2007). To support and compare the former results, this study was conducted at eastern terai region focusing various municipalities to collect information regarding the buffaloes found in that region. The objective behind this study was to understand the productive performance, morphometric measurements and qualitative traits of adult buffaloes in Eastern terai of Nepal which would be an asset in developing effective buffalo improvement plans.

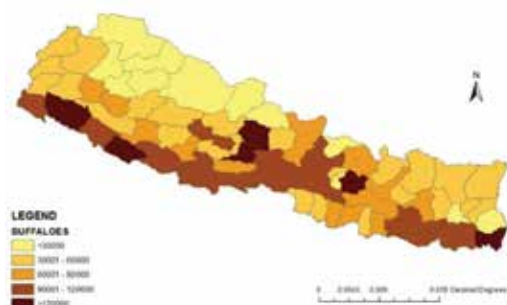


Figure 1. Distribution of buffalo population across the country (Adapted from ABD, 2014) and

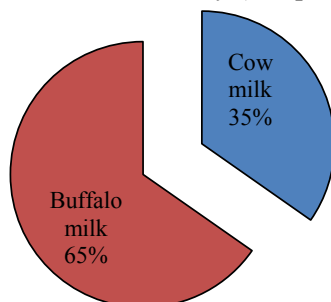


Figure 2. Contribution of milk by species (Source: DLS, 2016)



Figure 3. Indigenous buffalo breeds of Nepal (Adapted from Pokharel et al., 2012)

MATERIALS AND METHODS

Study Area

Sunsari District, a part of Province No. 1 in Terai plain, is one of the seventy-five districts of Nepal in plain of Eastern Nepal (Figure 4). The district covers an area of 1,257 km² and at the 2011 census had a population of 763,487. It is bounded by Morang district on the east, Saptari and Udayapur districts on the West, Dhankuta district on the north and India border on the South. Kosaha-2, Shreepur-7 and Laukahi-5 of Sunsari district were the site selected. The climate of Sunsari is humid tropical with predominantly hot and humid summer and a relatively cold misty winter. Annual maximum temperature is 38°C and minimum is 10°C, and annual precipitation of 2148 mm (DDC, 2016).



Figure 4. Map of Nepal indicating Sunsari district



Sampling and Data Collection

The study was conducted during October, 2016 at Kosaha-2, Shreepur-7 and Laukahi-5 of Sunsari district to understand the productive performance, morphometric measurements and qualitative traits of 20 adult Terai buffaloes of Eastern Nepal. Productive performance, phenotypic data and physical characteristics of buffaloes was recorded based on direct observation in the field. Qualitative information regarding to existing production system were recorded based on personal interview with the farmers/owners.

The data on reproductive performance and milk production parameters like age at first calving, total number of calves born per buffalo number of lactations, daily milk yield, lactation length, and peak yield was collected. Physical characteristics of Terai buffalo recorded were coat color, skin, muzzle, eyelid, eye, hooves and horn color. Morphometric traits such as body weight, chest girth, body length, height at withers, hock circumference, tail length, ear length, and horn length were measured.



Figure 5. Indigenous Buffaloes of Eastern Terai

Statistical Analysis

Data were analyzed by simple statistical tool using MS Excel and SPSS.v16.

RESULTS

The results of Productive performance, phenotypic data and physical characteristics of buffaloes found in Sunsari district is presented here under.

Physical Characteristics

The physical characteristics of Buffaloes found in eastern terai were presented in Table 1. The coat color of the buffaloes was dominantly black (100%). Skin, muzzle, eye and eyelid are all predominantly pigmented (black-100%). Hoof color was 90% black and 10% grey. Tail color are all black with switch having 40% white and 60% black as well as 40% medium and 60% long in length (observed as they are above, in or below the hock). Horns are all fixed with 35% grey and 65% black in color and are medium length with curve at the tip. Forehead is wide and all are black in color. Apart from this, Arna crossbreds buffaloes were also prevalent in the region (Koshi Tappu Wildlife Reserve is famous for Arna buffaloes) (*Bubalus arnee*) with coat color variation from light to grey, whitish color mark present below the knee and hock joint with long horn, curved laterally and extended directed backward with heavier body weight.

Table 1. Physical characteristics of Buffalo found in eastern Terai

S.N.	Body parts	Color Pattern	Percentage
1	Coat	Black	100
2	Skin	Pigmented (black)	100
3	Muzzle	Pigmented (black)	100
4	Eye	Pigmented (black)	100
5	Eyelid	Pigmented (black)	100
6	Hoof	Pigmented (black)	90
		Pigmented (Grey)	10
7	Tail	Black	100
8	Horn	Grey	35
		Black	65
9	Forehead	Black	100



10	Switch	White	40
		Black	60
11	Horn shape	Medium length with curve at the tip	100
12	Horn attachment	Fixed	100
13	Tail Length	Medium	40
		Long	60

Morphological Traits

Morphometric traits are the important parameters for indicating breed characteristics. Mean values with standard errors for the traits such as body weight (331.5 ± 7.6 kg), chest girth (170.5 ± 4.5 cm), body length (128.5 ± 3.0 cm), height at withers (132.5 ± 4.1 cm), hock circumference (19.9 ± 0.4 cm), tail length (79.6 ± 6.0 cm), ear length (28.5 ± 0.9 cm), and horn length (32.76 ± 2.1 cm) of indigenous buffaloes of eastern terai region were calculated as presented in Table 2.

Table 2. Morphological measurements of indigenous buffalo (Mean \pm SE) of Eastern Terai

Parameters	Body Weight (Kg)	Chest Girth (cm)	Body Length (cm)	Wither Height (cm)	Hock Circumference (cm)	Tail Length (cm)	Ear Length (cm)	Horn Length (cm)	Horn Root thickness (cm)	Horn root width
Values	331.5	170.5	128.5	132.5	19.9	79.6	28.5	32.76	21.5	21.5
(Mean \pm SE)	± 7.6	± 4.5	± 3.0	± 4.1	± 0.4	± 6.0	± 0.9	± 2.1	± 0.8	± 0.6

Note: SE: Standard Error; kg: kilogram; cm: centimeter

Productive Traits

Milk Production

Milk production is one of the economically important traits in case of milch animals. Profitability of every farm depends on the quality of animals, management system and ultimately the production (milk and meat in case of buffaloes). Milk production traits of buffaloes under study at Sunsari, eastern terai of Nepal is presented in Table 3. Traits recorded for milk production are number of lactations in this study was recorded 3.2 times, daily milk yield (1.6 liters), lactation length (9.5 months), and peak yield (3.8 liters). Hand milking was common practice for majority of the farm households. Farmers' in this region are still rearing these buffalo breeds in larger groups (50-60 adult she-buffaloes) instead of lower milk yield due to its demand for meat within and outside the country.

Table 3. Milk production traits of buffaloes under study at Sunsari, Eastern Terai of Nepal

Traits	Mean values
Number of lactations	3.2 times
Daily milk yield	1.6 liters
Lactation Length	9.5 months
Peak yield	3.8 liters
Method of milking	100% hand milking

Reproductive Traits

The reproductive traits considered in the study were age at first calving was 3.75 ± 0.14 years, whereas, total number of calves born per buffalo was observed 1.75. Apart from above productive performance, morphometric measurements and qualitative traits of the buffaloes found in eastern terai, Haemorrhagic septicaemia (HS), tapeworm infestation, weaker legs were the major problems prevalent in the study areas. District Livestock Service Office has been working in these problems along with their routine services on vaccination (FMD), drenching, deworming and artificial insemination. Whereas inbreeding, repeat breeding, low milk productivity and shortage of high quality local buffalo bull for breeding were other important problems for buffalo herders in the region. Hence, a complete package of practice including breeding, feeding, health, housing and marketing along with technologies on product diversification and value addition (milk and meat) need to be delivered to the farmers of the locality.

DISCUSSIONS

Information on morphological characteristics will helpful in ensuring effective management and conservation of animal genetic resources with a view to obtaining phenotypically pure local genetic resources for future selection and breed improvement strategies (Yakubu et al., 2010). Average body weight for the buffaloes of eastern terai



(331.5±7.6 kg) is slightly lower in comparison to the mid-western terai buffaloes (357±14.2 kg). However, body length, chest girth and wither height were observed similar for both eastern and mid-western regions terai buffalo (ABD, 1995). Other Nepalese indigenous buffaloes (Lime and Parkote) also have nearer values for body weight (311 and 342kgs), body length (125 and 128 cms), chest girth (174 and 176 cms) and wither height (119 and 124 cms) respectively with Gaddi (buffaloes from far-western hilly region) having somewhat higher values for above parameters (Rasaili et al., 1998; Amatya et al., 2000; Pokharel et al., 1998). Gaddi breed is the heaviest among other identified indigenous buffaloes of Nepal. Other body part measurements for tail, ear and horn length were in accordance with three identified Nepalese indigenous breeds of buffaloes.

Buffaloes are found across the Terai belt of Nepal. However, limited study has been done so far in these buffaloes breed therefore, still remained as non-descript. Some study showed that the buffaloes from eastern and western Terai region are morphologically similar in many parameters. However, a complete study with larger sample size comprising both phenotypic and molecular characterization need to be done to validate them for a new breed.

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EFFECT OF NON-GENETIC FACTORS ON MILK PRODUCTION TRAITS OF INDIGENOUS BUFFALOES (*Bubalus bubalis* L.) IN THE WESTERN HILLS OF NEPAL

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ABSTRACT

Understanding the influence of non-genetic factors on the phenotypic expression of milk production traits of buffaloes is important to develop selection/evaluation criteria with better accuracy. A research was carried out in the Ramjhathati, Parbat and Faliyagaun; of Myagdi district Nepal during October to November 2016 with the objective of evaluating the effect of parity on the different milk production traits of the Indigenous Lime and Parkote buffaloes, where breed and parity were considered as non-genetic factors. Altogether 98 buffaloes including 57 Lime and 41 Parkote were considered as the sample population. The milk production traits considered for the evaluation were day 1 milk yield (DMY), peak milk yield (PMY) and average daily milk yield (AMY). Data were analyzed by Henderson's Least Square Mixed Model and Maximum Likelihood (LSMMML PC-2) computer program using Harvey-1990 software. Results indicated that the pooled means for the DMY, PMY and AMY were observed 1.60 liter, 3.31 liter and 2.11 liter, respectively. Results revealed that breed and parity of indigenous buffaloes were not the important sources of variation with respect to the traits under study. However, higher value for the DMY, PMY and AMY were observed in the mid parity (4th-6th) as compared to that of early (1st-3rd) and late (above 7th) parity for both breeds. The peak milk yield in the Mid Parity was recorded higher in the Lime buffaloes (3.85±0.40 ltr) as compared to that of the Parkote buffaloes (3.32±0.52 ltr). Besides, the correlation coefficient between DMY and PMY, DMY and AMY, and AMY and PMY was determined 0.622, 0.498, and 0.623 respectively. Regression analysis of DMY, PMY and AMY indicated that PMY of Lime and Parkote buffaloes in this study is fluctuated by +1.0168 liter and +0.829 liter per liter fluctuation of DMY and AMY, respectively. Thus there is great scope of improving indigenous Lime and Parkote buffaloes through selection within the population. The breed and number of parities could be the important non-genetic factors with respect to milk production traits, though they did not found significant in present study. High positive correlation and the results of regression analysis reflected that selection for increased DMY could increase PMY and AMY and vice versa.

Keywords: Indigenous breeds, milk production traits, parity, lime, parkote

INTRODUCTION

Nepal is characterized by crop-livestock mixed farming system to sustain the rural livelihood in the country. Buffalo is one of the most important multi-purpose species contributing around 65% and 54% to the total milk and meat production and ranks in the first position in both cases, despite the fact that this species constitutes only 41% of the total dairy population including cattle and buffaloes (MoAD, 2016). Lime, Parkote and Gaddi are the precious indigenous breeds of Nepal distributing in the west and far-west region of Nepal, respectively having great scope of genetic improvement. Importantly, Murrah and its crossbred population comprises of about 35% of the total buffalo population in Nepal.

Phenotypic expression of quantitative traits such as day 1 milk yield (DMY), peak milk yield (PMY) and average daily milk yield (AMY) are mostly governed by polygenes and are associated to several non-genetic factors (Lasley, 1963). Thus, it is important to know the strength and dimension of influence of such non-genetic factors on the expression of economic traits of buffaloes that helps in developing selection/evaluation criteria with better accuracy.

In these connections, present study was undertaken to investigate the influence of the non-genetic factors including genetic group (breed) and parity of the animal on milk production traits such as DMY, PMY and AMY that have been scarcely studied to date.

MATERIALS AND METHODS

This study was carried out in the western hilly villages including Ramjhathati, Parbat and Faliyagaun, Myagdi over a period of October to November, 2016. Study mainly aimed at evaluating the effect of parity on the different milk production traits of the Indigenous Lime and Parkote buffaloes under farmers managed production system. Altogether 98 lactating buffaloes with their production records were identified and considered as the study

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population. For analysis of each traits considered, the number of buffaloes involved in the collection of production data is presented in Table 1. The non-genetic factors such as breed and parity were considered in the study.

Table 1. Distribution of sample buffaloes under different non-genetic factors in the analysis of milk production traits

Breed	Number	Parity	Number	Parity Vs. Breed	Lime	Parkote
Lime	57	Early (1 st – 3 rd)	72	Early (1 st – 3 rd)	43	29
Parkote	41	Mid (4 th – 6 th)	19	Mid (4 th – 6 th)	12	07
		Late (Above 7 th)	07	Late (Above 7 th)	02	05

The milk production traits under study were day 1 milk yield (DMY), peak milk yield (PMY) and average daily milk yield (AMY). To study the effect of non-genetic factors on production traits and to overcome the difficulties of disproportionate sub-class numbers, data were analyzed by Henderson's Least Square Mixed Model and Maximum Likelihood (LSMML PC-2) computer program using Harvey-1990 software. The following model was used to understand the influence of breed and parity on the milk production traits with the assumptions that different components being fitted into the model were independent and additive.

$$Y_{ijk} = \mu + B_i + P_j + (BP)_{ij} + e_{ijk}$$

Where, Y_{ijk} is the observation on the k^{th} parity of i^{th} breed; μ is the pooled (overall) mean; B_i is the fixed effect of i^{th} breed of buffalo; P_j is the fixed effect of j^{th} parity; $(BP)_{ij}$ is the effect of interaction between i^{th} breed and j^{th} parity; and e_{ijk} is the random error that is assumed to be normally and independently distributed [$\sim \text{NID}(0, \sigma^2 e)$].

RESULTS

Day One Milk Production (DMY)

Results of this study reflected that the overall mean DMY was 1.60 ± 0.08 liters with the range of 0.28 to 4.54 L (Table 2). Accordingly, breed had no significant effect on day one milk yield (DMY). However, DMY of Parkote was slightly higher (1.65 L) as compared to that of Lime buffaloes (1.54 liters). Similarly, parity also had non-significant effect on DMY of Lime and Parkote buffaloes in this study (Table 2). However, it was observed that the phenotypic value of the trait was slightly higher (1.79 L) for the buffaloes of mid parities (4th to 6th) as compared to early (1st to 3rd) and late parities (above 7th). In addition, DMY was not significantly influenced by the effect of interactions between breed and parity (B×P) of the lactating buffaloes (Table 2). However, comparatively higher value of DMY was observed in case of the Parkote buffaloes with mid parities (4th to 6th) as compared to other breed x parity combinations.

Peak Milk Yield (PMY)

The overall mean PMY was 3.31 ± 0.14 L with the range of 0.85 to 6.81 L (Table 2). Peak milk yield was not significantly influenced by parity of the animals. However, higher value (3.63 L) of PMY was observed for the lactating animals of mid parities (4th to 6th parities) as compared to that of early (3.22 liters) and late parities (3.17 L) (Table 2). In contrast, Thiruvankadan *et al.* (2014) observed the significant effect ($P < 0.01$) of parity with respect to peak milk yield of Murrah buffaloes in Tamil Nadu, India, who reported the lower value of PMY at 1st parity and increased from 2nd to 4th parity and gradually decreased above 4th parity.

Similarly, breed of buffaloes in present study also had no significant effect on peak milk yield (Table 2) indicating the similar values of PMY (3.30 L) for both breeds under consideration. In contrary, Dahama and Malik (1991), Birader (1990) and Chaudhary *et al.* (2000) reported the significant influence of breed on PMY of buffaloes.

Moreover, the effect of interactions between breed and parity in present study was also found non-significant with respect to PMY of Lime and Parkote Buffaloes (Table 2). However, Lime breed of mid parity (4th to 6th) was reported superior as compared to that for other breed-parity combinations.

Average Daily Milk Yield (AMY)

The overall mean average daily milk yield (AMY) in this study was recorded 2.11 ± 0.12 L with the range of 0.28 to 5.11 liters (Table 2). In contrast, Shah (2011) reported higher value (3.22 L) of AMY in Lime buffaloes at Lumle. Moreover, Pokharel and Tiwary (2007) observed higher value of AMY in another indigenous breed of Nepal i.e. Gaddi. It was found that the average daily milk yield of Gaddi buffaloes in Western Nepal for the first three months after calving was 4.69 L from two teats and in the next three months AMY was 4.62 L from three teats. Shrestha *et al.* (1994) studied the milk production traits of local and Murrah crossbred buffaloes in eastern Nepal where he observed the AMY of local buffalo as 2.55 L for local buffalo and 3.26 L for Murrah crossbred buffaloes. Hence, it is established that Murrah crossed local breeds in Nepal are found to have increased their milk productivity.



The mean AMY of Lime and Parkote buffaloes slightly resembled to the Anatolian water buffaloes of Turkey where the overall means AMY was reported as 2.76±0.051 L (Kul et al., 2015). Similar result was also reported by Şekerden et al. (1999) for Turkish Anatolian water buffaloes. On the other hand, Park (2002) reported significantly higher value of AMY (7 to 10 L) of the buffaloes in India.

Findings revealed that there was non-significant effect of breed was on the AMY of buffaloes under present study (Table 2). However, Parkote buffaloes were reported slightly superior as compared to Lime for the expression of this trait.

Likewise, parity of buffaloes also had no significant influence on AMY of indigenous buffaloes in this study (Table 2). However, the higher value (2.52 L) of AMY was recorded for the buffaloes of mid parities (4th to 6th) as compared to those of early (1st to 3rd) and late parities (above 7th) parities. In contrary, Khosroshahi (2011) reported significant effect (P<0.01) of parity with respect to average daily milk yield (AMY) of Azarbaijan native buffaloes. Similarly, Jamuna et al. (2015) also reported the highly significant influence (P<0.01) of parity on AMY of Murrah buffaloes reared at NDRI Livestock Farm, India.

Additionally, findings of present study indicated that the effect of interactions between breed and parity did not significantly influence the performance of the lactating buffaloes with respect to AMY (Table 2). However, Lime buffaloes having mid parity (4th to 6th) were found superior as compared to that for other combinations of breed x parity interaction.

Table 2. Least Square mean and standard error of milk production traits of Lime and Parkote buffaloes at different parity in Western Hills of Nepal, 2016

Non-genetic factors	Day 1 milk yield (L)	Peak milk yield (L)	Average daily milk yield (L)
Range	0.28 - 4.54	0.85 - 6.81	0.28 - 5.11
Pooled Mean	1.60±0.08	3.31±0.14	2.11±0.12
Breed			
Lime	1.54±0.17	3.30±0.29	2.12±0.24
Parkote	1.65±0.17	3.30±0.25	2.15±0.24
Significance	NS	NS	NS
Parity			
1 st – 3 rd Parity	1.52±0.10	3.22±0.16	2.04±0.14
4 th – 6 th Parity	1.79±0.19	3.63±0.32	2.52±0.26
Above 7 th	1.68±0.38	3.17±0.63	1.94±0.56
Significance	NS	NS	NS
Interactions			
Lime x 1 st – 3 rd Parity	1.48±0.12	3.17±0.21	1.96±0.17
Lime x 4 th – 6 th Parity	1.79±0.24	3.85±0.40	2.76±0.33
Lime x Above 7 th	1.42±0.60	3.12±0.98	1.84±0.81
Parkote x 1 st – 3 rd Parity	1.57±0.15	3.30±0.25	2.16±0.21
Parkote x 4 th – 6 th Parity	1.78±0.32	3.32±0.52	2.15±0.43
Parkote x Above 7 th	2.04±0.38	3.23±0.62	2.08±0.66
Significance	NS	NS	NS

Correlation Coefficients Among DMY, PMY and AMY

Results of this study reflected that there was moderate to high positive correlation between the milk production traits under consideration. Accordingly, the correlation coefficient between DMY and PMY, DMY and AMY, and AMY and PMY was determined 0.622, 0.498, and 0.623, respectively.

Relationship Between DMY, PMY and AMY

Positive linear relationship between DMY and PMY, DMY and AMY, and AMY and PMY was observed and is presented in Figures 1, 2 and 3. The regression equation of relationship between day 1 milk yield (DMY) and peak milk yield (PMY) was determined as $PMY=1.0168*DMY+1.6799$; with the adjusted R² value of 38.7% (Figure 1). Where, 1.0168 is the regression coefficient of DMY to PMY and 1.6799 is the constant.

Similarly, the regression equation for daily milk yield (DMY) and Average daily milk yield (AMY) was determined as $AMY=0.6802*DMY+1.0304$; with the calculated R² value of 24.22% (Figure 2). Where, 0.6802 is the regression coefficient of DMY to AMY and 1.0304 is the constant. Moreover, the regression equation for average daily milk yield (AMY) and peak milk yield (PMY) was determined as $PMY=0.829*AMY+1.5736$; with the calculated R² value of 48.33% (Figure 3). Where, 0.829 is the regression coefficient of AMY to PMY and 1.5736



is the constant.

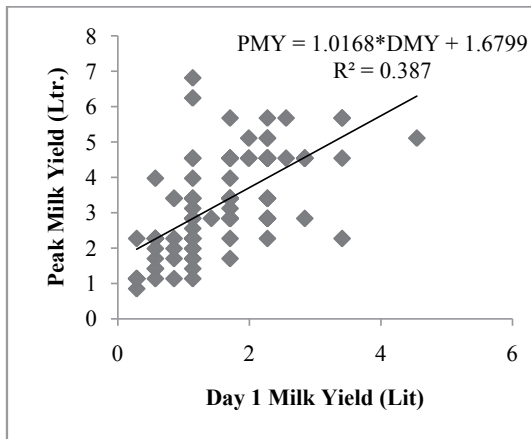


Figure 1. Relationship of day-1 milk yield and peak milk yield of Lime and Parkote buffaloes

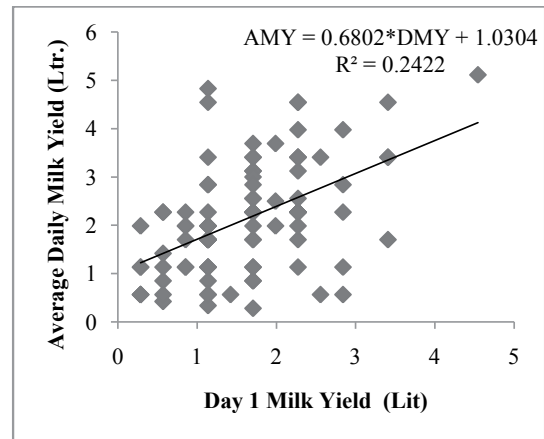


Figure 2. Relationship between day 1 milk yield and average daily milk yield of Lime and Parkote buffaloes

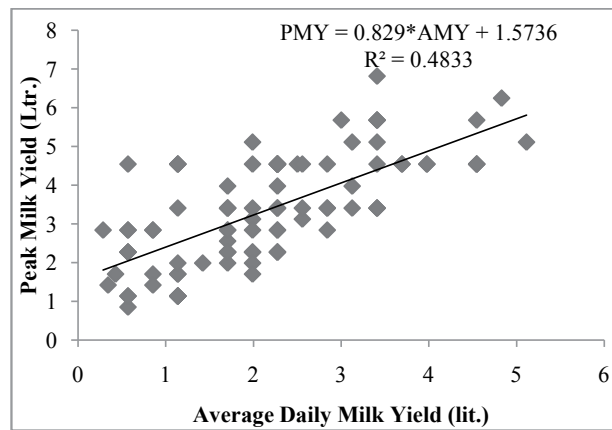


Figure 3. Relationship between average daily milk yield and peak milk yield of Lime and Parkote buffaloes

DISCUSSION

Results of present study reflected that the buffaloes expressed large variation (range) in the major economic traits under study i.e. day one milk yield (DMY), peak milk yield (PMY) and average daily milk yield (AMY) suggesting the greater scope of improvement in these traits through selecting the best and mating among the best individuals. These results could be supported by the findings of Pokharel and Tiwary (2007), Shrestha *et al.* (1994), Şekerden *et al.* (1999), Khosroshahi (2011), and Jamuna *et al.* (2015). The differences in AMY of different breeds of buffaloes in present study might be due to influence of different sires used for breeding and their own genetic potential as reported by Jamuna *et al.* (2015).

Moderate to high positive correlation coefficients calculated for DMY and PMY, DMY and AMY, and AMY and PMY indicated that selection in favor of DMY will give positive response towards PMY and AMY while developing improvement plan for these traits in the buffalo population considered in present study.

The regression equations reflected that there was a linear relationship between DMY and PMY (Adjusted $R^2 = 0.387$), DMY and AMY (Adjusted $R^2 = 0.242$), and AMY and PMY (Adjusted $R^2 = 0.483$), respectively. Similarly, results obtained through the analysis of the relationship between different milk production traits indicated that PMY of Lime and Parkote buffaloes is fluctuated by +1.0168 L and +0.829 L per liter fluctuation of DMY and AMY, Similarly, AMY is fluctuated by +0.6802 L per liter fluctuation in DMY of the indigenous Lime and Parkote buffalo population in the western hills of Nepal.

Based on the findings of present study, it can be concluded that Lime and Parkote buffaloes had high potentiality with respect to milk production traits with a greater scope of improvement through selection within the population. The breed and number of parity could be the important non-genetic factors affecting milk production traits such as DMY, PMY and AMY, though they did not influenced these traits significantly in present study. So, further study covering wider area and considering larger population is recommended for greater precision and



validity of this study.

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EFFECT OF FAILURE OF PASSIVE TRANSFER OF IMMUNITY IN BUFFALO CALVES

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ABSTRACT

Failure of passive transfer of immunity (FPT) due to several maternal or calf factors is considered as the most important risk factor for calf welfare. The present study aimed to investigate FPT with respect to risk factors, assessment and outcome in buffalo calves. Twenty normally born calves were assessed for vitality, passive transfer, acute phase response, health and growth from birth to 90 days of life. Serum IgG concentrations in the calves at 24-48 h of age was 10.27 ± 0.30 g/L and the incidence of FPT was 40.0 per cent. At 24-48 h, the FPT calves (serum IgG < 10 g/L) had significantly ($P < 0.05$) lower serum IgG, total proteins and gamma-glutamyltransferase activity than the normal calves (serum IgG ≥ 10 g/L). Significant correlations were observed between serum IgG and TSP ($r = 0.564$, $P < 0.01$), and serum IgG and GGT ($r = 0.622$, $P < 0.01$). Low volume of colostrum ingested at the first feed and total colostrum intake in 24 h after birth, and high colostral somatic cell count (SCC) were found to be the risk factors for FPT in calves while dam parity and calf body weight at birth did not influence the passive transfer in calves. The Ig index (serum $\text{IgG}_{21-28\text{d}} / \text{IgG}_{24-48\text{h}}$) was significantly ($P < 0.05$) higher in FPT calves indicating their poor health status. In the first 90 d of life, the incidence and severity of diarrhea, and days in treatment were higher while the age at disease onset and growth rate were lower in the FPT calves. It is concluded that FPT is prevalent in buffalo and crossbred cattle calves that adversely affects their health and growth.

Keywords: Passive transfer, buffalo calves, FPT, immunoglobulin, colostrum, risk factors

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IMPROVEMENT IN REPRODUCTION THROUGH SYNCHRONIZATION AND RESYNCHRONIZATION STUDIES IN DAIRY BUFFALOES

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ABSTRACT

Dairy buffalo has a unique significance in livestock agriculture system in many Asian countries. Despite its substantial contribution in milk and meat industry, the reproduction in this species is challenging because of poor estrus expression and low fertility. During the last one and half decade, our laboratory has been involved in series of systematic hormonal manipulations to address these issues. Pregnancy rates (PR) of buffaloes bred at detected estrus using prostaglandin (PG) F₂ α was higher (63%) compared to the Ovsynch protocol (36%) and differed significantly ($P < 0.05$) during the breeding season. In buffaloes, PR and estrus response did not differ ($P > 0.05$) either with used controlled internal drug release device (CIDR; 37% and 91%) or new CIDR (37% and 96%), respectively. Later on, incorporation of estradiol benzoate (EB) in conjunction with CIDR improved the estrus intensity without affecting the pregnancy per AI (P/AI) in nulliparous and multiparous anovular buffaloes. We then found that the optimal time of AI was between 60-72 h in CIDR protocol and between 48-60 h in CIDR-GnRH modified protocol in order to enhance fertility. Furthermore, administration of equine chorionic gonadotropin (eCG) at the time of CIDR removal significantly ($P < 0.05$) enhanced the fertility (56% vs 31%) as compared to controls. In another study, the PR was significantly higher ($P < 0.05$) due to human chorionic gonadotropin (hCG) than that of controls (62% vs 31%) when administered on day 7 post AI. In a unique study on inseminations performed at spontaneous estrus we discovered that P/AI was significantly higher ($P < 0.05$) at 24 h (53%) as compared to 0 h (26%) and 36 h (13%), after the onset of standing heat. More recently we found that resynchronization with GnRH on day 23 post AI resulted in higher cumulative pregnancy rate (81%) than control (59%), when determined after 1st and 2nd AI. Taken together, these data led us to conclude that the solutions to the problems of estrus expression, summer anestrus, acyclicity, and improper timing of AI are now clearly available. However, their application to small holder buffalo farming remains challenging.

Keywords: Synchronization, resynchronization, fertility, dairy buffalo.

INTRODUCTION

Buffaloes have an integral role in strengthening the agricultural economy, and improving food security to farmers in many Asian countries. They are excellent converters of low quality roughages and well adapted to harsh weather (Gordon, 1996). Nili-Ravi buffaloes are considered the best milk producers; they produce about 2,500 liters of milk per lactation with 6.5% butterfat (Hussain et al., 2006), and their home country is Pakistan. However, the buffalo has been traditionally considered as a slow breeder due to the delayed puberty, poor estrus expression, imprecise timing of ovulation, summer anestrus, prolong calving intervals, longer post-partum periods and higher incidence of embryonic and fetal losses (Singh et al., 2000; Baruselli et al., 2001). Therefore, in an attempt to overcome these problems hormonal treatments have been used for the optimization of reproductive functions.

Most of the estrus synchronization protocols in buffaloes are empirically based on those developed for cattle either by inducing premature luteolysis using prostaglandins (PG) (Kumaratillake et al., 1977) or prolonging the luteal phase using progestogens (Martinez et al., 1997) resulting in variable fertility. Synchronization studies have included the conventional use of PGF₂ α (Chohan, 1998) ovulation synchronization (OVS) protocol (Warriach et al., 2008), and new or used progesterone (P4) devices (Naseer et al., 2011); that ensued in acceptable fertility. Thereafter, P4 treatment was combined with estradiol benzoate (EB) in order to facilitate the follicular waves emergence (Naseer et al., 2012), with equine chorionic gonadotropin (eCG) that increased the size of the ovulatory follicles (Carvalho et al., 2013), or with induction of ovulation with GnRH or human chorionic gonadotropin (hCG) to enhance the reproductive efficiency (De Rensis and Lopez-Gatius, 2007).

However, the usage of these estrus synchronization protocols remain limited among buffalo farmers because they had to rely on signs of estrus for artificial insemination on detected estrus (AIDE). This was not the most efficient method for buffalo herds or at commercial farms. To overcome this issue Ovsynch protocol (Warriach et al., 2008), or CIDR-GnRH protocol (Haider et al., 2015) were replicated based upon the cow work and improvised for fixed timed artificial insemination (FTAI) in buffaloes that provided decent fertility results. Furthermore, these protocols were used to resynchronize the estrus and ovulation for a second or third round of FTAI without the necessity of estrus detection, particularly for those buffaloes which did not become pregnant to the first AI (Arshad

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et al., 2017). Despite, the emergence of new strategies for fertility improvement, this task has been continuously challenging due to embryonic and fetal losses in lactating dairy buffaloes (Wiltbank et al., 2016).

Embryonic loss, which has relatively been less studied and reported to be associated with heat stress, high milk production, lack of maternal recognition of pregnancy, uterine environment, corpus luteum (CL) size, and reduced circulatory P4 in buffaloes (Campanile et al., 2005b). Those buffaloes which maintained pregnancy had greater plasma concentrations of P4 on Day 10 and Day 20 after AI, which led to the finding that a decline in P4 secretion is a major cause of embryonic mortality (Campanile et al., 2005b). Therefore, several hormonal strategies have been devised to enhance P4 level and embryonic survival. There are a number of excellent reviews available on various aspects of buffalo reproduction (Singh 2000; Baruselli et al., 2001; Drost, 2007; De Rensis and Lopez Gatiús, 2007; Perera, 2008; Das, 2010; Warriach et al., 2015). The objective of this review is to present and describe the treatments available which have provided solutions for the commonly reported problems in buffaloes.

SYNCHRONIZATION PROGRAMS

Prostaglandin vs. Ovsynch Protocol

In buffaloes, one of the major problems is that most of the calvings takes place during July–September (Ahmad et al., 1981). However, very few calvings occurs during February–June, which results in lowered production and availability of milk in that period. Consequently, there is an increase in demand. In this background, buffalo workers have opportunity for synchronization during summer months (April–August) in order to program calving during the lean period. Initially, single PG injection was administered to induce estrus in buffaloes but the estrus response was highly variable (Dhaliwal et al., 1987) this necessitated the need for double PG regime.

Keeping in view the background information, Warriach et al., determined the effect of estrus synchronization methods (PG; detected estrus vs. Ovsynch) on estrus behavior, timing of ovulation and pregnancy rates in breeding and low breeding seasons in Nili-Ravi buffaloes. The durations of pre-standing estrus (9.2 ± 0.4 versus 7.3 ± 0.6 h), standing estrus (14.2 ± 0.8 versus 8.9 ± 0.6 h) and from termination of standing estrus till cessation of all signs of estrus (16.3 ± 1.6 versus 6.1 ± 0.5 h) were higher in PGF 2α induced estrus than Ovsynch protocol. The time to ovulation after the onset of standing estrus was also greater in PGF 2α induced estrus (30.6 ± 1.5 h) as compared to Ovsynch protocol (15.0 ± 0.8 h). Pregnancy rates of buffaloes bred at detected estrus after PG protocol during the breeding season (62.5%) remained similar from those inseminated during the low breeding season (55.5%). Similarly, pregnancy rates of Ovsynch buffaloes (36.3%) did not differ between two seasons (36.3 versus 30.4%, respectively). However, within the breeding season, the pregnancy rates after PG protocol were higher as compared to Ovsynch buffaloes (Figure 1).

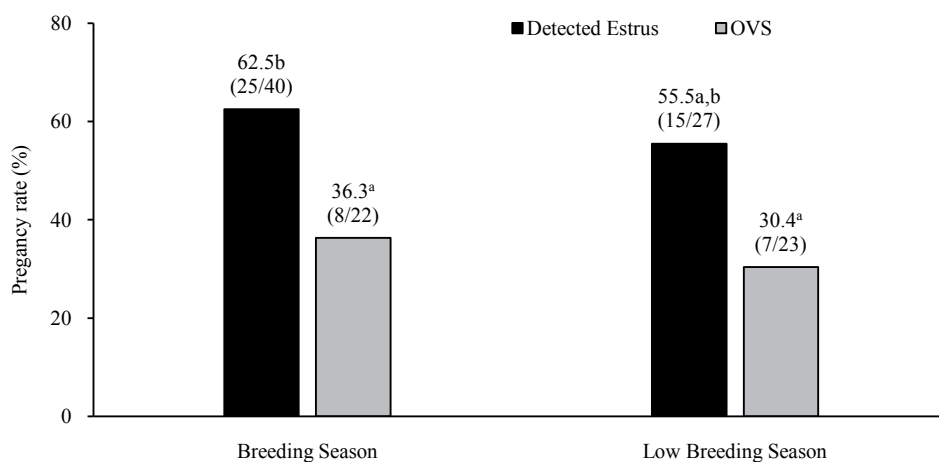


Figure 1. Effect of PG and Ovsynch protocols on pregnancy rate determined during breeding and low breeding season in Nili-Ravi buffaloes (Warriach et al., 2008).

PROGESTERONE BASED PROTOCOLS

Used vs. New CIDR Devices

In buffaloes, summer anestrus is a major problem that affects the estrus detection rate and decreases the AI submission rate. Consequently, fertility remains low in most of the buffalo herds during summer months. Different progestogens (mainly CIDR, PRID or CRESTAR1) preparations have been utilized for increased estrus expression in buffaloes (Barile 2005). Among the progestogens, the CIDR device was well utilized in synchronizing estrus and consistently had resulted in higher pregnancy rates in cattle (Colazo et al., 2004). The use of CIDR for reproductive



management to increase the fertility is becoming popular because of the short treatment period (7 day) and reduced incidence of persistent follicles (Ahmad et al., 1995). This popularity is evidenced by the fact that re-use of CIDR, though not recommended, is a common practice in dairy cattle herds (Colazo et al., 2004). Therefore, Naseer et al., (2012) compared the effect of used CIDR (UCIDR) and new CIDR (NCIDR) on estrus response and fertility rate in anestrus buffaloes. Estrus response and pregnancy rate remained similar between UCIDR and NCIDR devices.

P4-EB Based Protocols

During seasonal anestrus, Raut et al., (1988) reported low estrus intensity in buffalo and duration of estrus (8–10 h vs. 18 h) as compared to the rest of the year. To overcome this, administration of EB after removal of CIDR allows better estrus expression and fertility in buffaloes. In a study (Yusuf et al., 2015), estrus intensity, ovulation synchrony and pregnancy rate were determined following EB administration in CIDR-treated nulliparous and multiparous buffaloes. Overall, addition of EB improved estrus intensity as compared to buffaloes not treated with EB. Interestingly, when the EB treatment was not considered pregnancy rate per AI (P/AI) was greater in multiparous buffaloes as compared to nulliparous buffaloes.

P4-GnRH Modified Protocol

Estrus signs are less overt in buffaloes, leading to improper timing of insemination resulting in low fertility using AI (Anzar et al., 2003). Opportunities are now available which bypass heat detection and resort directly to the FTAI, either using Ovsynch or P4 based protocols with GnRH and EB in buffaloes (Neglia et al., 2003; Vecchio et al., 2010, Carvalho et al., 2013). With the use of the FTAI protocols in the buffaloes (Di Francesco et al., 2012), it was very important to know the time window of the ovulation to optimize the AI efficiency. Therefore, another study (Haider et al., 2015) was conducted to determine the optimum time of AI in relation to CIDR removal with or without GnRH to achieve the higher pregnancy rates in buffaloes. The pregnancy rates were higher in buffaloes inseminated either at 48 (50%) or 60 h (59%) than at 72 h (18%) in CIDR-GnRH protocol; whereas, they were higher in buffaloes inseminated either at 60 (37%) or 72 (40%) than at 48 h (10%) in CIDR treatment Figure 2 (A and B).

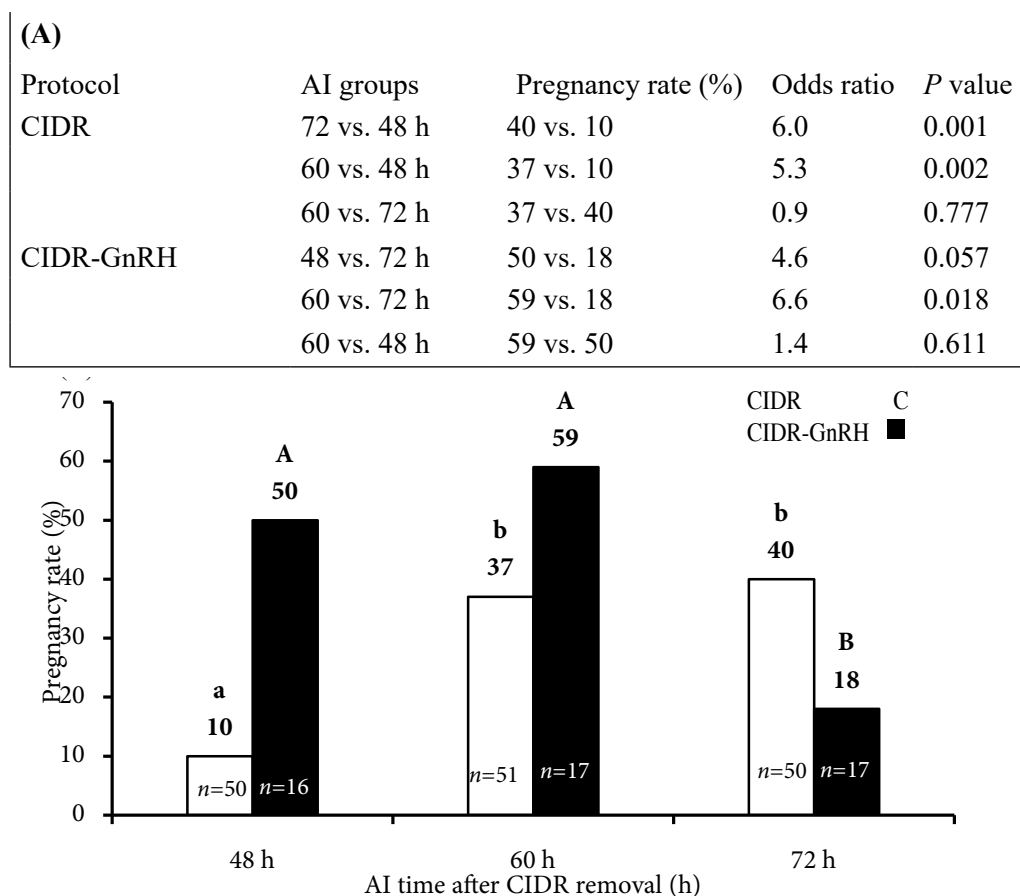


Figure 2. A) The effect of timing of AI on pregnancy rates after binary logistic regression analysis in buffalo synchronized with CIDR and CIDR-GnRH protocols. $P \leq 0.05$ shows the significant differences between AI groups. Odd ratios (OR) shows the occurrence to become pregnant in AI groups, if $OR = 1$: no effect on pregnancy, $OR > 1$: increased occurrence of pregnancy, $OR < 1$: decreased occurrence of pregnancy.



B) The effect of timing of AI on pregnancy rates in CIDR and CIDR-GnRH protocols in buffalo. ^{a,b} means blank chart bars with different superscripts differ ($P < 0.05$); ^{A,B} means black chart bars (■) with different superscripts differ ($P < 0.05$). The ovulations occurred at 76.13 ± 1.66 h and 68.40 ± 1.73 (after P4 device removal) in CIDR and CIDR-GnRH protocols respectively. The numbers in boxes indicate the number of buffalo.

P4-eCG Based Protocol

In a recent study (Khan et al., unpublished data), the effect of eCG on estrus response, ovulatory follicle development, ovulation rate and P/AI in CIDR-EB treated lactating Nili-Ravi buffaloes has been evaluated (Khan et al., unpublished data). Buffaloes ($n=87$), were treated with CIDR on random stage of estrus cycle (Day 0) and assigned into two groups; with eCG (+eCG; $n = 44$), or without eCG (-eCG; $n = 43$). Estrus response was higher in buffaloes treated with eCG (+eCG) than without eCG (-eCG) (98% vs. 74%), respectively. Mean ovulatory follicular growth rate per day was greater in buffalo treated with eCG than without eCG (1.62 ± 0.09 mm vs. 1.30 ± 0.05 mm; $P < 0.05$). The ovulation rate (93% vs. 74%) and P/AI (56% vs. 31%) were higher in eCG treated buffaloes as compared to without eCG, respectively.

P4-hCG Based Protocol

In buffaloes, despite the emergence and modification of synchronization studies, they do have generally lower fertility as compared to cows (Drost, 2007). The decrease in fertility is attributed due to embryonic death that is up to 45% between days 25 and 40 of gestation in buffaloes (Campanile et al., 2005a). In Italian Mediterranean buffaloes, tropic hormones, GnRH, hCG, and exogenous P4 on day 5 post AI failed to reduce embryonic losses between 26 and 40 days of gestation (Campanile 2007). However, buffaloes treated with GnRH, hCG, or P4 on Day 25 after AI showed significant reduction in embryonic mortality (Campanile 2008). This reduction in embryonic mortality tended to suggest that delayed treatment of buffaloes with tropic hormones or supplemental P4 is beneficial during the critical phase of embryonic development and placental attachment (Campanile et al., 2008). In a recent study (Husnain et al., unpublished data), buffaloes were treated with CIDR, GnRH, and hCG 7 days post AI to determine their effects on conception rate, and embryonic survival in buffaloes. The findings revealed that the effect of treatments (CIDR, GnRH, or hCG) on conception rate was higher in hCG treated (44/68, 65%) than control (32/92, 35%) and CIDR treated (24/53, 45%) buffaloes.

RESYNCHRONIZATION PROGRAM

Aggressive reproductive management systems consist of three strategies that can be implemented early during the breeding period: (1) inseminate all cows at the beginning of the breeding season, (2) identify the non-pregnant cows as early as possible, and (3) rebreed the non-pregnant cows as soon as possible (Bo et al., 2016). Arshad et al., 2017 recently investigated to determine the effect of resynchronization with either GnRH or P4 on Day 23 post AI on pregnancy rate, cumulative pregnancy, and embryonic and fetal losses in CIDR-GnRH synchronized Nili-Ravi buffaloes.

The pregnancy rate in resynchronized buffaloes remained similar between the OVS and CIDR groups, whereas the cumulative pregnancy rate in GnRH + OVS buffaloes (81%) after the 1st and 2nd FTAI when determined on Day 64 was higher than that in CON + AIDE (59%) buffaloes (Figure 3). The embryonic losses were significantly lower ($P < 0.05$) in GnRH-treated (18%) buffaloes than in CON (42%) buffaloes on Day 45 post 1st AI. Fetal losses were fewer due to treatments on Day 60 or 90 post- 1st AI.

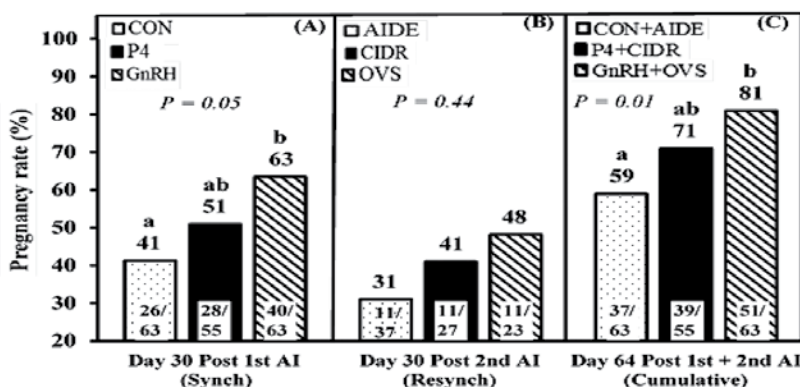


Figure 3. (A) Pregnancy rate after 1st AI in CIDR-GnRH synchronized Nili-Ravi buffaloes. (B) Pregnancy rate after resynchronization (2nd AI) in AIDE, CIDR, and OVS groups. (C) The cumulative pregnancy rate after synchronization (Synch) and resynchronization (Re-Synch) in Nili-Ravi buffaloes. ^{a,b} percentages with different superscripts within each time point differ ($P < 0.05$). The cumulative pregnancy rate in buffaloes treated with GnRH leading to OVS was significantly higher ($P < 0.05$) than in the CON + AIDE groups. The number in boxes indicates the number of buffaloes pregnant out of treated after synchronization and resynchronization.



TIMING OF ARTIFICIAL INSEMINATION FOR SPONTANEOUS ESTRUS

Owing to the anatomical and physiological similarities, a natural consequence was the adoption of AI methods in water buffalo from cattle protocols. Consequently, without gaining basic knowledge of timing of ovulation, AM-PM rule was adopted in the buffalo (Drost, 2007). This practice resulted in reduced number of pregnancies leading to less adaptability of AI by the farmer community (Anzar et al., 2003). Paradoxically, whether or not, this AM-PM rule holds true in water buffalo has been systemically investigated (Riaz et al., unpublished data). Maximum pregnancy per AI, 53% (15/28), was achieved in buffalo inseminated at 24 h after the onset of standing heat. This was followed by 37% (10/27) in those buffalo which were inseminated at 12 h after the beginning of standing estrus.

CONCLUSION

Taken together, Ovsynch and PG protocols improve the fertility in breeding and low breeding season, but presence of CL and follicle is mandatory. CIDR alone or in combination with GnRH or eCG or EB can be efficiently re-used in buffaloes without affecting estrus response and P/AI. Moreover, resynchronization strategies also proved favorable to enhance the overall herd fertility. However, large scale applications of these strategies are difficult, due to poor farmer understanding, socio economic constraints, small holder farming systems, and lack of availability of resources.

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CHARACTERIZATION AND TREATMENT OF INFERTILITY IN BUFFALOES OF HILLS AND TERAI REGIONS OF MID NEPAL

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ABSTRACT

Infertility is probably the major reason for culling/slaughtering buffaloes in Nepal. With the main goal of minimizing the culling of productive buffaloes, the infertile buffaloes of four districts of Nepal were treated after diagnosing the form of infertility. Total 52 households of buffalo resource centers of hill (Kaski, Syanja and Palpa districts) and Terai region (Nawalparasi district) were visited by a technical team and total 68 infertile buffaloes were examined clinically, and the reproductive organs were examined using transrectal palpation/ultrasonography. All the buffaloes were in good body condition score (BCS ≥ 2.75). Infertile buffaloes were categorized and treated based on the forms of infertility. Majority (91.2%) of infertility cases were anestrus followed by repeat breeding (8.8%). Within anestrus group, 54.8% buffaloes were in true anestrus characterized by inactive ovaries, and 45.2% were in silent estrus having major structure either dominant follicle (DF) [18/28] or corpus luteum (CL) [10/28]. All infertile buffaloes were treated with broad-spectrum anthelmintic (oxyclozanide and levamisole), mineral-vitamin mixtures, and specific treatment. Specific treatment included: phosphorus injections i.m. for true anestrus buffaloes (group 1, n=34), buserelin acetate 20 μ g i.m. for silent ovulators having DF (group 2, n=18), cloprostenol 500 μ g i.m. for silent ovulators having CL (group 3, n=10), and buserelin acetate 20 μ g i.m. at the time of breeding for repeat breeders (group 4, n=6). Out of total 62 anestrus buffaloes treated, 45.2% had positive response by showing estrus within one month of treatment. Of these, 29.4% (10/34) buffaloes in group 1 and 55.6% (10/18) in group 2 came into estrus within one month of treatment; and 80% (8/10) in group 3 came into estrus within a week of treatment. Four out of six (66.7%) repeat breeder buffaloes did not return to estrus within one month of mating. In conclusion, true anestrus was the major reproductive problem; anthelmintic and nutritional supplementation had moderate response to induce estrus in such buffaloes. Silent ovulator buffaloes having DF had good response with GnRH analogue treatment, and those having CL had excellent response with cloprostenol treatment. Routine reproductive examinations and necessary treatments may help to reduce the problem of infertility in buffaloes.

Keywords: Infertility, anestrus, repeat breeding, silent ovulation, buffaloes

INTRODUCTION

Nepal is an agricultural country with about 65.6% people depending on agriculture for their livelihood. The agriculture sector in Nepal contributes approximately 35% to the national gross domestic product (GDP) (Government of Nepal, 2014). Livestock is an integral and important component of the Nepalese agriculture sector, contributing approximately 24% to the agricultural GDP (ADS, 2012). Within livestock sector, the buffalo is the most important livestock species in Nepalese livestock system; it contributes more than two-third of the total milk production and about 60% of the total meat production in the country (Government of Nepal, 2014).

However, the production and productivity of buffaloes in Nepal is very low mainly due to subfertility and infertility. As a result, buffalo production in the country has not fulfilled the demand of milk and meat within the country, and a large number of buffaloes are being imported from India every year. Another major problem in buffalo farming in Nepal is: if buffaloes do not get pregnant timely, the buffalo owners sent them for slaughtering once their lactation period is over. As a result, buffaloes with high genetic values are being rapidly depleted from the country. It is alarming that in near future, there will be critical shortage of breeding buffaloes in the country. To address this problem, we need to solve the infertility problem of the buffaloes that will ultimately minimize or stop the unnecessary slaughtering of buffaloes of high genetic values. To solve the infertility problem of buffaloes, first we need to rule out the forms of infertility and its causes. Therefore, the objectives of this study were to characterize infertility and to determine the effectiveness of treatment for various forms of infertility in buffaloes of hills and Terai region of mid Nepal.

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MATERIALS AND METHODS

This study was conducted in various buffalo resource centers of hill (Kaski, Syanja and Palpa districts) and Terai (Nawalparasi district) regions of mid Nepal during June-July 2017. Those buffalo resource centers were monitored and supported by Central Cattle and Buffalo Promotion Office (CCBPO). Based on the information provided by the field technicians and farmers of concerned areas, total 52 households (farms) having infertile buffaloes were visited by a technical team from Department of Theriogenology, AFU and CCBPO. Information on history of last calving, deworming, and dates of mating was collected from the concerned herd owners. Total 84 buffaloes from these farms were examined clinically and the problematic buffaloes were treated accordingly.

Clinical and Gynecological Examination of Buffaloes

Infertile buffaloes were examined clinically regarding their general health condition, nutritional status, and the reproductive health including ovarian and uterine conditions. Nutritional status of each buffalo was noted in the form of body condition score (BCS, 1-5 scale). Reproductive organs of each buffalo were examined by transrectal palpation. Ovaries having any suspected follicle or corpus luteum were examined using transrectal ultrasonography for confirmation.

Classifying the Forms of Infertility

Based on the history and clinical findings, all infertile buffaloes were classified into following sub-groups.

- **True anestrus:** Buffaloes that did not come into estrus at least for six months since last calving, and had very small inactive ovaries without any palpable structures were considered as true anestrus buffaloes.
- **Silent estrous:** Buffaloes in which estrus was not noticed at least for six months since last calving, and having dominant follicle (DF; >10mm in diameter) and/or corpus luteum (CL) were considered to have silent estrous.
- **Repeat breeding:** Buffaloes that were mated repeatedly at least for three consecutive estrous cycles without having any clinical abnormalities in the uterus and ovaries were considered to be repeat breeding buffaloes.

Treatment of Various Forms of Infertility

All buffaloes in the study were not drenched against internal parasites at least for last one year. Based on the form of infertility, all infertile buffaloes were treated with broad-spectrum anthelmintic, mineral-vitamin mixture, and specific treatment. Broad-spectrum anthelmintic bolus containing oxclozanide and levamisole (Zanide-L forte, Qmed Formulation, Nepal) was administered orally @ 1 gm per 100 kg body weight. Mineral-vitamin mixture powder containing vitamin A, D3, B12 and various metho-chelated minerals (Nutrimed chelated powder; Medvet Pharma Ltd., India) was given orally @ 50 gm per day for 20 days. Specific treatment was performed as mentioned below.

Specific Treatment

- (i) True anestrus buffaloes (group 1; n=34) were treated with phosphorus injection (Tonoricin, Vetoquinol India: Each ml contained sodium salt of 4-dimethylamino-2- methylphenyl-phosphinicacid 0.2 gm) @10 ml i.m. alternate days x 3 injections.
- (ii) Silent ovulators having DF as a major structure (group 2; n=18) were treated with buserelin acetate (Gynarich, Intas Pharmaceutical Ltd., India) 20 µg i.m.
- (iii) Silent ovulators having CL as a major structure (group 3; n=10) were treated with cloprostenol (Interchemie, the Netherlands) 500 µg i.m.
- (iv) Repeat breeders (group 4; n=6) were treated with buserelin acetate (Gynarich, Intas Pharmaceutical Ltd., India) 20 µg i.m. at the time of breeding.

Criteria for Treatment Response

Buffalo owners were asked to detect estrus in treated buffaloes at least three times a day until one month from the onset of treatment. Treatment response in case of anestrus was considered positive if the treated anestrus buffalo expressed estrus within one month from the onset of treatment. Treatment response data were collected with the help of field technicians.

Data Analysis

Various forms of infertility were expressed in percentage. Effectiveness of treatment for various forms of anestrus was also expressed in percentage, and compared using Chi-square test in SPSS (version 16.0). P-value ≤0.05 was considered as significant.



RESULTS

Nearly 90% (46/52) farmers mentioned that they practiced to send their buffaloes for slaughter after cessation of lactation once their buffaloes did not get pregnant timely. All the buffaloes in the study were in good body condition score (BCS ≥ 2.75).

Forms of Infertility

Out of 84 buffaloes examined, 16 buffaloes were found to be pregnant, and thus, remaining 68 buffaloes were used for treatment. The proportion of various forms infertility has been shown in Figure 1. Out of 68 infertile buffaloes, majority (91.2%) were anestrus followed by repeat breeding (8.8%). Within anestrus group, 54.8% buffaloes were in true anestrus characterized by inactive ovaries, and 45.2% were in silent estrus or missing estrus having major structure either dominant follicle (18/28) or corpus luteum (10/28).

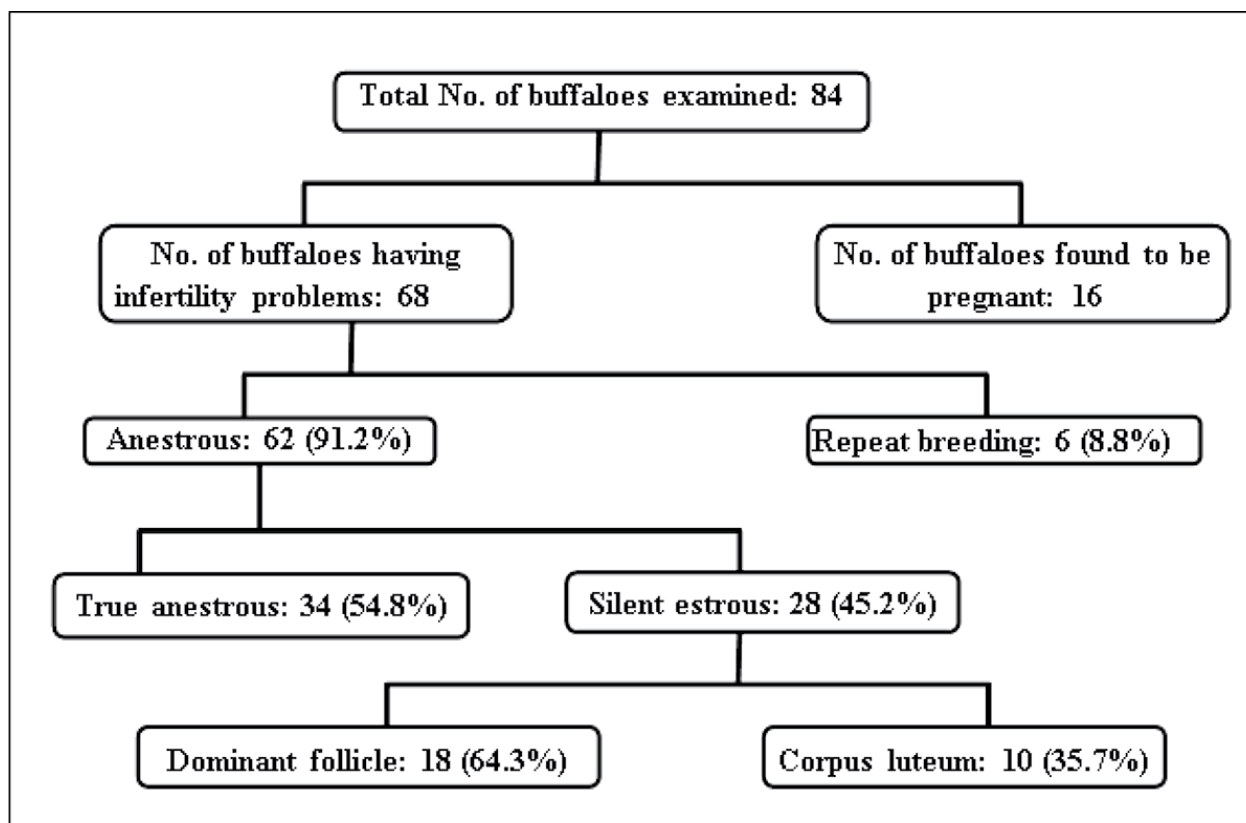


Figure 1. Forms of infertility in buffaloes of four districts

Treatment Response

Response of treatments for various forms infertility in buffaloes has been shown in Table 1.

Table 1. Response of treatments for various forms of infertility in buffaloes

Forms of infertility	Treatment used	No. of buffaloes treated	No. of buffaloes came into estrus	Estrus expression rate (%)
True anestrus	Tonoricin* 10 ml i.m. alternate days x 3	34	10	29.4
Silent estrus with DF	Buserelin acetate 20 µg i.m.	18	10	55.6
Silent estrus with CL	Cloprostenol 500 µg i.m.	10	8	80.0
	Total	62	28	45.2

*Tonoricin (Vetoquinol, India): Each ml contained sodium salt of 4-dimethylamino-2- methylphenyl-phosphinic acid 0.2 g.

Out of total 62 anestrus buffaloes treated, 45.2% had positive response by showing estrus within one month of treatment. Of these, total 29.4% (10/34) in group 1 and 55.6% (10/18) in group 2 came into estrus within one month of treatment; and 80% (8/10) in group 3 came into estrus within a week of treatment. Treatment response was significantly ($P < 0.01$) different among three groups of anestrus buffaloes. Four out of six (66.7%) repeat breeder buffaloes treated with GnRH at the time of breeding did not return to estrus within one month of mating (data not shown in Table 1).



DISCUSSION

This study characterized the forms of infertility and determined the effectiveness of treatment for various forms of infertility in buffaloes of hills and plain region of mid Nepal. Because of small number of animals, buffaloes from all four districts were pooled. More than 90% cases of infertility were attributed to anestrus and remaining cases were due to repeat breeding. This finding of the present study was similar to that reported by previous studies (Das and Khan, 2010; Devkota et al., 2012; El-Wishy, 2007; Sah and Nakao, 2010) those indicated that anestrus was the most important cause of poor reproductive performance in buffaloes. Within anestrus category, true anestrus characterized by small ovaries without any corpus luteum or dominant follicle was the major cause of anestrus. Similar findings were reported by the previous studies (Kumar et al., 2013; Sah and Nakao, 2010). There were 9% cases of repeat breeding and no cases of ovarian cyst; although Sah and Nakao (2010) found 5.2% cases of infertility were due to ovarian cyst. Results of the present and previous studies indicate that interventions to induce ovarian cyclicity in true anestrus buffaloes would be the key for solving the infertility problem in buffaloes. It was alarming that almost all farmers used to send their buffaloes for slaughter if the buffaloes did not get pregnant until the end of current lactation period.

For the treatment of anestrus buffaloes, since all the buffaloes in the study were not drenched against internal parasites at least for one year, all buffaloes were first treated with anthelmintics and vitamin-mineral mixtures. Treatment of true anestrus buffaloes with phosphorus injection along with anthelmintics and vitamin-mineral mixtures showed moderate (29.4%) treatment response within one month after onset of treatment. Treatment response in true anestrus buffaloes of the present study was lower than the findings of Sah and Nakao (2010) that reported 64.5% estrus expression rate within one month in true anestrus buffaloes treated with vitamin-mineral mixture for three weeks. Since the buffaloes in present study already had good BCS indicating good nutritional status, the treatment response with nutritional supplementation might have lower response than that of previous study (Sah and Nakao, 2010) because the buffaloes in their study were in moderate to poor BCS which might have responded well to nutritional supplementation.

About 56% anestrus buffaloes having DF came into estrus within one month after GnRH treatment. This treatment response was lower than the treatment response (100%) reported by a previous study (Sah and Nakao, 2010) but higher than that (28.6%) reported by another study (Devkota et al., 2013). Similarly, 80% anestrus buffaloes having CL in the ovary expressed estrus within one week after treatment with PGF2 alpha analogue; this finding was almost similar to that reported by previous studies (Devkota et al., 2013; Sah and Nakao, 2010). Estrus expression in buffaloes treated with PGF2 alpha analogue was over a shorter (within one week) period of time than that in buffaloes treated with GnRH analogue in which the estrus expression time ranged from one week to one month.

Although pregnancy diagnosis was yet to be done, two-third of repeat breeder buffaloes treated with GnRH at the time of breeding did not return to estrus within one month of mating. It can be assumed that GnRH treatment might have facilitated timely ovulation after breeding, which ultimately increases chances of pregnancy.

In conclusion, true anestrus was the major reproductive problem; anthelmintic and nutritional supplementation had moderate response to induce estrus in such buffaloes. Silent ovulator buffaloes having DF had good response with GnRH analogue treatment, and those having CL had excellent response with cloprostenol treatment. There was alarming situation that the farmers had been practicing to send their buffaloes for slaughter once their buffaloes did not get pregnant timely. It is recommended to organize regular infertility camps in buffalo farming areas to solve the problem of infertility in buffaloes so that it may contribute to stop the unnecessary slaughter of good genetic value buffaloes in coming days.

ACKNOWLEDGEMENTS

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REPRODUCTIVE PERFORMANCES OF WATER BUFFALOES IN SELECTIVE AREAS OF BANGLADESH

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ABSTRACT

The buffalo is the second most important dairy animal in Bangladesh. Data on reproductive performance of indigenous water buffaloes are very scanty. Therefore, a survey was conducted using questionnaire at Trashal, Islampur and Sonadangaupazilla in Mymensingh, Jamalpur and Feni districts respectively to know the productive and reproductive performance of different dairy breeds of water buffaloes. Total 320 buffaloes were included to evaluate the reproductive performance in terms of sexual maturity (months), estrous cycle length (day), estrus duration (hr), postpartum estrus interval (day), voluntary waiting period (day), gestation length (day), milk production (L/day), lactation length (days) etc. Significant differences were observed in different reproductive parameters in buffaloes of three districts. Late sexual maturity ($36.4.2 \pm 0.4$ months), shortest estrous cycle length (18.8 ± 0.3 days) and estrous duration (21.8 ± 0.3 days), late postpartum estrus interval (50.5 ± 1.3 days) and longest gestation period (315.5 ± 0.6 days) were observed in buffaloes of Jamalpur district. The shortest voluntary waiting period (47.9 ± 1.6 days) and lactation period (269.6 ± 2.7 days/year) were observed in buffaloes of Mymensingh district. The longest estrus duration (23.0 ± 0.1 days) and early postpartum estrous interval (45.2 ± 0.7 days), longest voluntary waiting period (72.8 ± 1.0 days) and lactation length (286.2 ± 2.7 days) were found in buffaloes of Feni district. Reproductive traits such as highest gestation length (316.0 ± 0.4 days), and late sexual maturity (36.8 ± 0.3 days), longest voluntary waiting period (47.91 ± 1.0 day) were observed in indigenous buffaloes. Milk production was the highest in cross breed (5.9 ± 0.3 L/day), and the lowest in indigenous buffaloes (4.0 ± 0.2 L/day). The longest lactation length was observed in Murrah (282.2 ± 2.2 Days). This study could help veterinarians and farmers to choose breed of buffaloes for improved and profitable farming system.

Keywords: Water buffaloes, reproductive performances, Bangladesh

INTRODUCTION

The buffalo is the second most important dairy animal in Bangladesh. Moreover, buffalo meat has gained importance in the recent years because of its domestic needs and export potential. Buffalo meat is well comparable to beef due to its physico-chemical, nutritional properties and palatability attributes (Anjaneyulu, 2004). The domestic water buffalo is an important animal in the agricultural economy of many tropical and subtropical countries (Suhail *et al.*, 2008). Buffalo, also known as 'Asian Animal' plays very important role in agricultural economy, being an integral part of farming system. Buffalo is contributing 12.1% to the world, 38.0% in Asia, 66.6% in Pakistan, 55.0% in India, 16.4% in China, 50.8% in Egypt and 65.2% in Nepal's total milk production (FAO, STAT, 2007). In addition to the milk, buffalo is also used as an important source of beef production in the form of culled adult females, males and unwanted male calves. Buffalo is contributing around 1.3, 2.8, 24.4, 26.9, 0.6, 21.2, and 51.8% of the total meat production in the aforementioned countries (Suhail *et al.*, 2008). It is a well-known fact that milk production increases with age at an ever till maximum production is attained and decreases later on (Khan *et al.*, 2009) have investigated the post-conception decline in milk yield and reported that dairy buffaloes in parity 3 had the least reduction in milk yield followed by parity 2, 4, 1, 5, and 6, indicating parity 3 as the best phase for milk production in dairy buffaloes. The reduction in milk was the smallest in summer followed by winter, spring and autumn, reported that economic returns from dairy buffaloes depend on lifetime performance (Balachandran, 2005). There are some factors that affect on the reproduction in the buffaloes in our country. These factors are silent estrous, resume of ovarian cycle, poor nutrition and body condition, suckling management, and climate etc. The duration of the estrous cycle in buffalo ranges from 17 to 26 days with a mean of around 21 days (Jainudeen and Hafez, 2003). However, there is greater variability of oestrous cycle's length in buffalo. So far, the studies on reproductive performance of indigenous water buffaloes are very scanty; therefore a survey was performed to know the productive and reproductive performance of different dairy breeds of water buffaloes the three districts in Bangladesh.

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MATERIALS AND METHODS

The study was conducted at Trishal in Mymensingh district, Sunadanga in Feni and Islampur in Jamalpur districts. Data were collected for a period of six months from December 2014 to May 2015 specially areas near to the Upzilla point.

Study Population

Total 320 water buffaloes were selected on the basis of milking period and breed. Buffaloes were divided into three genetic groups according to their genetic composition, such as indigenous (I, n=114) Cross breeds (C, n=140) and Murra (M, n=66). The buffaloes were in different lactation condition also near to same age. Only natural services were practiced.

Management of Animal

Outdoor system and both stall and group feeding were being practiced. The ingredients of feed were wheat bran, rice polish, sesame oil cake, grass pea, black gram and common salt, rice straw and green grass. Natural mating have been performed by own fixed stuffs. For more housed the dairy buffaloes in the shed and supplied roughage and concentrate occasionally. The milking buffaloes were entirely confined into the shed, whereas dry buffaloes and heifers were allowed for grazing. Unproductive buffaloes having fourth or fifth parities or any other incurable diseases were normally culled. Proper management, vaccination and preventive measures were also taken.

Methods of Data Collection

Reproductive data were collected for 6 months. A questionnaire was supplied to each farmer and closely and frequently monitored by visits. A questionnaire was designed in a simple manner to get accurate information from the dairy buffaloes owners. The questionnaire included questions to collect information on birth weight (kg), mature live weight (kg), age at sexual maturity (days), age at first calving (months), number of services per conception, first service conception rate (%), length of gestation period (days), time of postpartum first heat (days), calving interval (days), length of lactation period (days), highest milk production (L/day), average milk yield (L/lactation) and dry period (day). Characteristics such as estrous cycle length, estrous duration, age at sexual maturity, age at first calving, first service conception rate, length of gestation period, time of post-partum first heat, calving intervals were used to measure reproductive performance. Characteristics such as average milk yield (L/day) and of lactation length were used to measure productive performance of different buffaloes.

Statistical Analysis

The collected data were compiled, tabulated and analyzed in accordance with the objectives of the study. Data were expressed as Mean \pm SEM. To analyze the variance regarding all reproductive and productive performance paired "T-test" was used. All data were analyzed with SPSS software (version 20) and p-value ≤ 0.05 was considered as significant.

RESULTS

Reproductive performances of water buffaloes of rural areas in three districts in Bangladesh are presented in Table 1. Age at sexual maturity significantly ($P < 0.05$) varied among buffaloes of three districts. Longest estrous cycle length (20.8 ± 0.3 days) was observed in buffaloes of Mymensingh district. There was significant ($P < 0.05$) difference in estrous cycle length between buffaloes of Mymensingh and that of Jamalpur districts. It was observed that estrus duration varied significantly ($P < 0.05$) among buffaloes of Mymensingh, Jamalpur and Feni districts. Estrus duration was higher (23.0 ± 0.1 hours) in buffaloes of Feni District. When considered the time for post partum estrus interval, it was found that buffaloes of Feni showed estrus after 45.2 ± 0.7 days of parturition, whereas, buffaloes of Jamalpur showed estrus after 50.52 ± 1.3 days of parturition. Significant difference ($P < 0.05$) existed among buffaloes of three areas in term of postpartum parturition estrus interval. Voluntary waiting period was longest in buffaloes of Feni (72.8 ± 1.9 days) and shortest in buffaloes of Mymensingh (47.9 ± 1.6 days). There was significant ($P < 0.05$) difference in term of voluntary waiting period in buffaloes of these two areas. No significant variation was observed among buffaloes of three areas in terms of gestation length and daily milk production. In addition, longest lactation period (286.2 ± 2.7 days) was observed in buffaloes of Feni district and shortest lactation period (269.6 ± 2.7 days) was observed in buffaloes of Mymensingh.



Table 1. The overall values (mean±SEM) of reproductive parameters in water buffaloes observed in three districts

Parameter	Buffaloes of three districts		
	Mymensingh (n=40)	Jalalpur (n=40)	Feni (n=40)
Age at sexual maturity (month)	35.3±0.4	36.4± 0.5	35.2±0.4
Estrous cycle length (day)	20.8 ±0.3 ^a	18.80±0.3 ^b	19.92±0.4 ^a
Estrus duration (hours)	22.5± 0.2 ^{ab}	21.8± 0.3 ^b	23.0± 0.1 ^a
Post partum estrous interval (day)	46.3±0.9 ^a	50.52±1.3 ^b	45.2± 0.7 ^a
Voluntary waiting period (day)	47.9±1.6 ^a	68.5±1.6 ^b	72.8±1.9 ^b
Gestation length (day)	314.6±0.6	315.5±0.6	313.6±0.5
Milk production (L/day)	4.7± 0.3	4.9±0.3	4.6±0.2
Lactation period (days)	269.6±2.7 ^a	271.9±2.1 ^a	286.2±2.7 ^b

^{a,b} Values with different superscripts differ significantly.

Three breeds were found in study areas and breed difference was considered among reproductive parameters such as age at sexual maturity (months), estrous cycle length (day), estrous duration (hrs), post parturition estrous interval (day), voluntary waiting period (day), gestation length (day), milk production (day) and lactation length (days). Results are shown in Figures 1, 2, 3, 4, 5, 6, 7 and 8. It was found that sexual maturity occurred earlier in cross breed (33.8±0.3 months) in comparison to that of indigenous (36.8±0.2 months) and Murrah (35.0±0.5 months). There was no significant difference among three breeds in terms of estrous cycle length, milk production (L/day), post partum estrus interval and voluntary waiting period. On the other hand, significant differences existed in terms of gestation length, estrus duration and lactation length (days) among three breeds. Indigenous buffaloes had the shortest lactation length (270.9±2.1 days) and the lowest milk production (4.0±0.2 L/day) whereas, the highest milk production (4.8±0.4L/day) was observed in crossbred buffaloes. Longest lactation length (282.2±2.2 days) was found in Murrah breed.

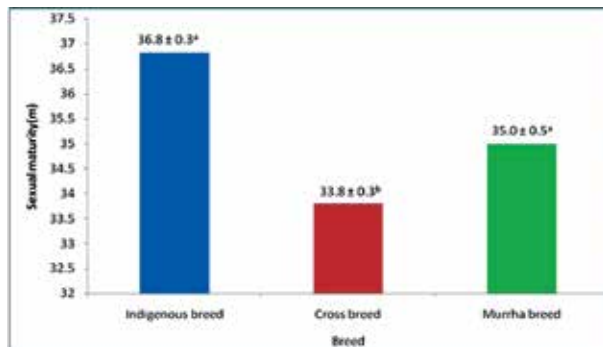


Figure 1. Age at sexual maturity (month) in buffaloes

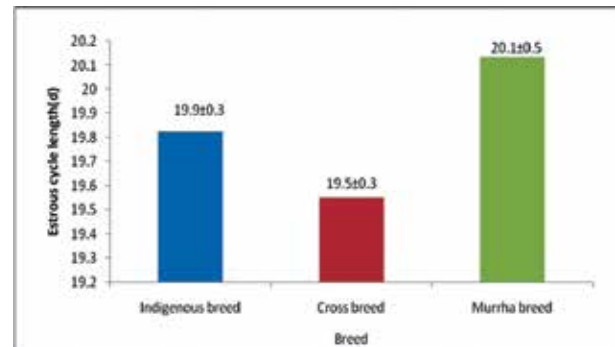


Figure 2. Estrus cycle length (days) in buffaloes

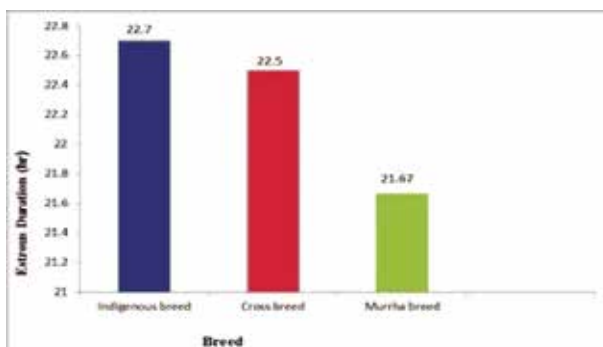


Figure 3. Estrus Duration (hour) in buffaloes

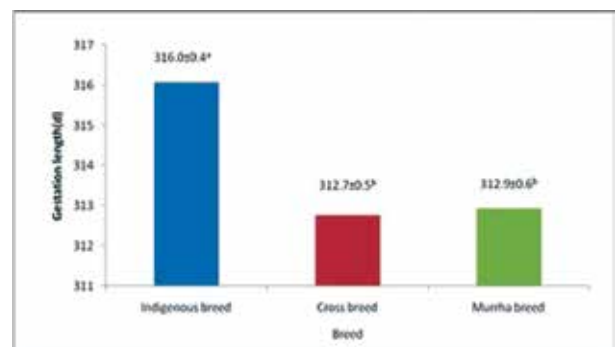


Figure 4. Gestation length (days) in buffaloes

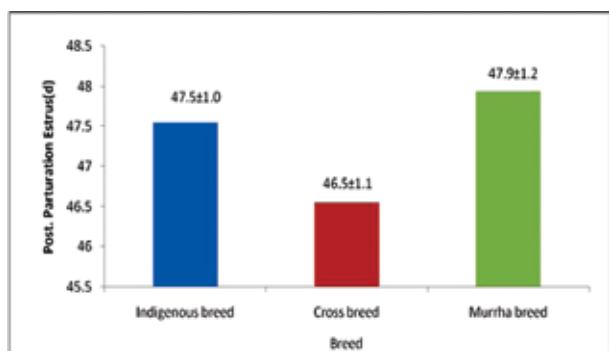


Figure 5. Post partum estrous interval (days) in buffaloes

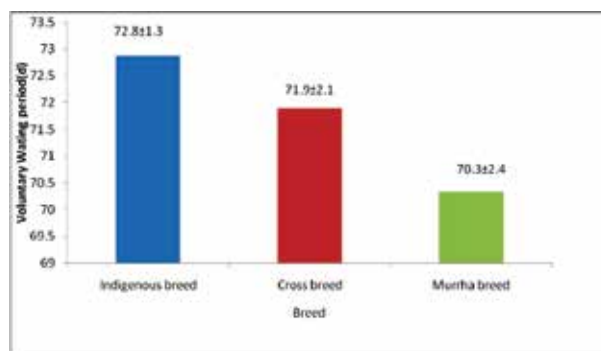


Figure 6. Voluntary waiting period (days) in buffaloes

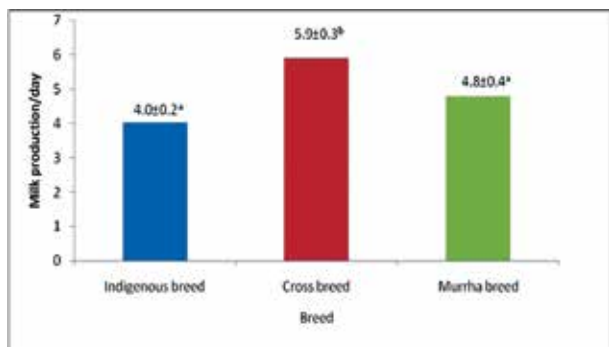


Figure 7. Milk production (L/day) in buffaloes

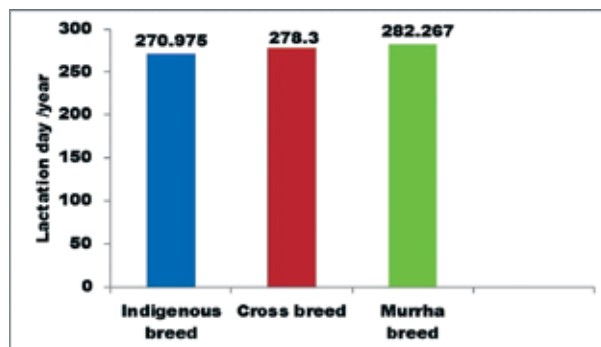


Figure 8. Lactation length (days) in buffaloes

During the study period, 10 reproductive diseases and disorders were recorded. These were abortion, still birth, dystocia, uterine prolapse, vaginal prolapse, retention of fetal membrane, pyometra, metritis, cystic ovary, non-functional ovary etc. Among these diseases, occurrence of retention of fetal membrane was the highest (12.0%), followed by cystic ovary (10.6%), pyometra (9.3%), dystocia (6.7%) and vaginal prolapse (6.7%).

Table 2. Occurrence of reproductive disorders

Reproductive disorders	Number affected	Occurance (%)
Retention of foetal membrane	9	12.0
Cystic ovary	8	10.64
Pyometra	7	9.34
Dystocia	5	6.67
Vaginal prolapse	5	6.67
Uterine prolapse	4	5.34
Metritis	4	5.34
Non-functional ovary	3	4
Stillbirth	2	2.65
Abortion	1	1.34

DISCUSSION

The age at sexual maturity in local, cross-bred and Murraha buffaloes was 36.8±0.3, 33.8±0.3 and 35.0±0.5 months, respectively. The variation in the sexual maturity among breeds were also statistically significant (P<0.05). These findings are inconsistent with that of Thatcher and Collier (2006). They reported that age at sexual maturity ranged from 35 to 43 months with a mean of 39.3±1.0 months. This finding was more or less similar with previous study (Islam, 2002). Estrous cycle length was 19.9±0.3 days in local, 19.5±0.3 days in cross-bred and 20.1±3.2 days in Murrah buffaloes. These values were higher than that observed by Khan *et al.*, (2000) who found 21.04±0.6, 23.1±0.9 and 23.04±0.5 days in indigenous, cross and Swamp buffaloes respectively. Estrous duration was 23.7±0.2 hrs in indigenous, 22.6±0.3 hrs in crossbred and 21.7±0.5 hrs in Murrah buffaloes. These values are in consistent with the findings of Olson and Deshpande (2001) that reported estrous duration of 21.6±1.1, 20.1±0.2 and 22.6±2.3



hrs in indigenous, cross-bred and Suri, respectively. Routine deworming and vaccination, accompanied by better nutrition, improved the health status of animal as revealed by improvement of fitness of animals. This variation might be resulted from diet, environment and management system followed in this selected areas. Ahmed *et al.*, (2000) studied the estrous behavior, oestrous cycle length, oestrous period, age at puberty, gestation length of indigenous buffaloes in Bangladesh. They found mean oestrous cycle length as 21.08 ± 3.08 days with a range of 15-28 days. The mean oestrous period, age at puberty, gestation length were 24.0 ± 0.1 hrs, 38.02 ± 2.1 days, and 319.73 ± 9.04 days respectively. Roi (2000) stated that there was no constant trend in estrous duration with level of cross buffalo inheritance. They also reported that estrous duration was lower in spring and summer than in winter; autumn and the rainy season. Moreover, Wagner, (2009) considered 21- 24 hrs of estrous duration as the optimum period for successful calving. It was observed that voluntary waiting period was highest (72.88 ± 1.0 days) in indigenous in this study, although there was no significant ($P > 0.05$) difference in the voluntary waiting period among different genotypes. The time of postpartum estrous is considered as an important economic reproductive trait for profitable dairy farming system. In this study, the shortest time for postpartum estrous was observed in cross breed buffaloes (46.5 ± 1.1 days). The time of postpartum breeding delays up to 60 to 90 days after parturition, when the uterus undergoes recovery and preparation for the next conception (Haresign *et al.*, 2003). The lengths of gestation period recorded in this study were 316.0 ± 0.4 , 312.7 ± 0.6 and 312.9 ± 0.6 days in indigenous, cross and Murrah respectively. There was marked ($P < 0.05$) variation in values between local and crossbred buffaloes.

The pure exotic breed (e.g. Murrha) is not suitable in context of Bangladesh in terms of environmental condition. It may require low temperature, better feeding and management. The disease prevention capacity is also lower than that of native buffaloes. On the other hand, the local breeds are well adapted as well as high disease resistance than exotic pure breed. The crossbred buffaloes performed better than that of exotic and native buffaloes in terms of adaptability and production. So, it is necessary to improve native buffaloes by selective breeding to increase the productive and reproductive performance. From the above perspective it could be concluded that crossbred buffaloes could be more suitable for sustainable and profitable buffalo production in Bangladesh contents.

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UPDATE ON ATTEMPTS OF DEVELOPING BREEDING STRATEGY FOR NEPALESE BUFFALOES (*Bubalus bubalis*) DURING LOW BREEDING SEASON

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ABSTRACT

Water buffalo (*Bubalus bubalis*) contributes almost two-third of the total milk and more than half of the meat supply in Nepal. Therefore, enhancing buffalo production is crucial for food and economy of the country. The compromised production capacity of buffaloes is mainly attributed to poor reproductive efficiency coupled with anestrus and a distinct seasonal breeding slog during low breeding season. Generally, Nepalese buffaloes breed well from August to November, and show intermittent breeding ability from December to February followed by either anestrus or poor breeding efficiency from March to June/July. If we combine this problem with summer infertility of dairy cows, it is obvious that country suffers a severe drop in milk production in spring and early summer months. In order to address this problem, research has been focused on understanding the factors causing anestrus or low breeding efficiency in buffaloes during low breeding season together with application of novel hormonal treatments to increase their fertility. The present paper is an update on attempts of developing breeding strategy for Nepalese buffaloes during low breeding season. The paper highlights the seasonal variation of anestrus conditions in Nepalese buffaloes and association of some nutritional and management factors on the incidence and hormonal treatment response of anestrus in buffaloes particularly during low breeding season. The results are highly encouraging and recently we are able to have a good pregnancy rate in buffaloes during low breeding season after addressing some nutritional and management issues. We have prepared a ground for more extensive future studies in this important area.

Keywords: Buffalo, fertility, poor breeding season, hormonal treatment, nutritional supplementation

INTRODUCTION

Water buffaloes (*Bubalus bubalis*) contribute >70 % to country's total milk pool and 60% to country's meat production in Nepal (Government of Nepal, 2013). Therefore, enhancing buffalo production is crucial for food and economy of the country. Nepalese buffaloes mainly suffer from breeding seasonality and overall low breeding potentiality reducing their total production capacity, and the reasons primarily include delayed puberty and prolonged calving interval that are associated with anestrus (Sah and Nakao 2006; Sah and Nakao, 2010; Devkota et al., 2013).

Incidence of anestrus in buffaloes is common during summer (Singh *et al.*, 1989, Das and Khan, 2010, Devkota *et al.*, 2012). Poor nutrition, management, stress and season are mainly responsible for this problem (Zicarelli, 1997, Qureshi et al., 2002, Borghese, 2005, Devkota et al., 2009, Devkota et al., 2013). Changes in seasonal biometeorological factors of photoperiod, ambient temperature and rain fall and related nutritional factors, either alone or in combination, play significant roles in causing seasonal reproductive pattern in buffaloes (Vale, 2007; Das and Khan, 2010; Perera, 2011). In Nepal, seasonal shortage of feed and fodder production is common during dry months of winter and spring (January to May) causing poor nutritional supply to animals during these seasons.

Nepalese buffaloes breed well from August to November (good breeding season), and show intermittent breeding ability from December to February (transition season) followed by either anestrus or poor breeding efficiency from March to June/July (low breeding season). If we combine this problem with summer infertility of dairy cows, it is obvious that country suffers a severe drop in milk production in spring and early summer months. In order to address this problem, we have focused on understanding the factors causing low breeding efficiency in buffaloes together with application of novel hormonal treatments to increase fertility in the low breeding season. A large number of hormonal regimens have been used with varying degree of efficacy in terms of estrus induction and conception rates (Barile, 2005; De Rensis et al., 2005; De Rensis and Lopez-Gatius, 2007), a combined strategy of improvement in environment, nutrition and management is prerequisite for hormonal manipulation in order to improve productivity in anestrus buffaloes (Das and Khan, 2010). The present paper is an update on attempts of developing breeding strategy of anestrus buffaloes during low breeding season in Nepal. The paper highlights the seasonal variation of anestrus conditions in Nepalese buffaloes and association of some nutritional and management factors on the incidence and hormonal treatment response of anestrus in buffaloes particularly during low breeding season.

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Seasonal Variation of Anestrus Conditions in Nepalese Buffaloes

The majority of buffaloes in the mid hill and lower mountain regions of Nepal consists of indigenous breeds such as Lime, Parkote and Gaddi, and in the southern plain Indian Murrah type or their crosses are major breeds of buffaloes. The environmental fluctuation of these areas is typical with cold and semi-dry to dry winter (December-February), rapidly increasing hot and dry spring (March-May), very hot and rainy monsoon summer (June-August) and moderate autumn (September-November) (Devkota and Bohora, 2009). In the buffaloes in Southern Nepal, silent estrus was a common problem round-the-year, although its higher incidence was observed during low breeding months. A distinct seasonal variation of anestrus conditions was observed in those buffaloes with >70% incidence of true anestrus from March to June that peaked (>80%) in April/May, which was $\leq 50\%$ in August and October-December (Devkota et al., 2012). This was almost similar to reports from other countries as well (El Wishy, 2007).

Nutritional and Management Factors Associated to Anestrus and its Treatment Outcome

The nutritional status of animals can be subjectively assessed in terms of their body condition score (BCS) (Edmonson et al. 1989). It is well established that reproductive efficiency in dairy cattle is related to the BCS (Markusfeld et al., 1997; Moreira et al., 2000; Amer, 2008). A strong association of true anestrus to poor BCS was observed in Nepalese buffaloes suggesting the effects of dry weather resulting shortage of feed and fodder availability during winter and spring months might have played a major role in causing higher incidence of true anestrus in the following months (Devkota et al., 2012). While comparing the BCS between cyclic and non-cyclic true anestrus buffaloes, BCS of cyclic buffalo was better than that of the non-cyclic buffaloes (Bohara and Devkota, 2009). The fact is also shown in other countries such as the phenomenon of higher incidence of ovarian inactivity during summer in Egyptian buffaloes was shown to be associated to poor BCS indicating nutritional cause with poor accessible nutrition in summer (Ali et al., 2009).

Another study conducted in Nepal revealed that low BCS affected negatively on pregnancy rate, and deficiency of Ca and protein and gastrointestinal parasitic infection reduced the pregnancy rate after the initiation of treatment with novel hormones such as PGF2 alpha in cyclic anestrus buffaloes (Devkota et al., 2013). Therefore, improvement of nutrition and control of parasitic infection could enhance the treatment effects on anestrus in buffaloes.

Application of Novel Hormonal Protocols to Increase Fertility in Anestrus Buffaloes

Since 2007 when the author introduced transrectal ultrasonography in animal reproduction for the first time in Nepal, it was possible to understand the ovarian dynamics of buffaloes and applying various hormonal protocols to address the fertility issues. In the year 2008-2009, the widely used Ovsynch treatment protocol was applied during active breeding season in anestrus buffaloes irrespective of their cyclic status and 40% pregnancy was obtained. Also, we could induce ovulation in 62.5% true anestrus buffaloes during low breeding season using double PG treatment in 13 days apart followed by Estradiol Benzoate in 24 h of second PG treatment, however, it was not possible to obtain pregnancy at that time (Devkota and Bohara, 2009). The studies in the following years revealed the strong association of nutrition and management factors to the treatment effects (Devkota et al., 2013; Devkota, 2015a; Devkota 2015b; Sah and Devkota, 2017). Based on these facts, we started categorizing animals based on nutritional status and applied more widely used progestin based CIDR protocols in anestrus buffaloes (Carvalho et al., 2013; Vecchio et al., 2013; Yousuf et al., 2015; Haider et al., 2015) during low breeding season. The preliminary findings were interesting that clearly indicated it was possible to obtain pregnancy in anestrus buffaloes having a good nutritional and management status during poor season by applying a CIDR-co synch protocol (Table 1) (Devkota 2015b). In this study, buffaloes in poor season were grouped into two different groups on the basis of their nutritional status reflected on BCS, < 2.5 and ≥ 2.5 , and all the buffaloes selected for good season had BCS of ≥ 2.5 . A CIDR-cosynch protocol was applied during poor season whereas ovsynch was applied during the good season. When the first AI pregnancy rate was compared between different BCS groups during the poor season, more buffaloes with ≥ 2.5 (good) BCS were pregnant ($P < 0.05$). There was an overall association ($P < 0.05$) of BCS during the two seasons on the first AI pregnancy rate (Table 1). It was understood that the CIDR co-synch protocol was effective to improve the reproductive performance of anestrus buffaloes, and poor BCS was associated to decrease the treatment effect. Parallely, a study was conducted to understand the role of nutritional and stress factors limiting the response of treatment with ovsynch in good season, and with CIDR-cosynch during transition and low season (Sah and Devkota, 2017). Ovsynch treatment during good season produced 42.9% (6/14) pregnancy whereas CIDR co-synch protocol produced 50.0% (7/14) during transition and 28.6% (4/14) during poor season. The physical condition (BCS) was associated to pregnancy outcome. Blood cholesterol and cortisol levels were different ($P < 0.05$) among three seasons. When compared to pregnant group, levels of glucose and total protein were lower ($P < 0.05$) and cholesterol level tended to be lower ($p = 0.07$) in non-pregnant group. It was clear that progestin based hormonal



protocols can be successfully applied in anestrus buffaloes during transition to poor season. However, the outcome depends largely on nutritional and stress factors.

Table 1. Association of BCS on the outcome of a CIDR co-synch protocol in anestrus buffaloes during the good and low breeding seasons

Breeding season	BCS group	No. of animals treated	No. of animals pregnant	Pregnancy rate (%)	P value
Low I	<2.5	7	0	0.0	0.013
Low II	≥2.5	7	3	42.9	
Good	≥2.5	6	4	66.7	

Source: Devkota (2015). Data presented in the 8th Asian Buffalo Congress 2015, Istanbul, Turkey.

Future strategy of breeding Nepalese buffaloes during low breeding season

Based on the findings of our past research, we have developed a strategy to address majorly the nutritional and management issues to achieve better pregnancy in low breeding season. We have recently developed a vitamin-mineral rich special nutritional supplement (under trial) to meet the nutritional requirement of anestrus buffaloes particularly during this season. We give a prior anthelmintic medicine at least one month before than the initiation of a novel hormonal protocol. The anthelmintic medicine is followed by the nutritional assessment and its supplementation so as to improve the BCS (minimum is 2.5) for applying progestin based hormonal treatment during low breeding season. The preliminary finding has revealed 57.1% and 28.6 % pregnancy rate after supplementing this formulation in gastrointestinal endoparasite eliminated and positive group, respectively (Result is presented in the International Buffalo Symposium 2017 in Nepal). The results of this holistic approach are highly encouraging. Thus, we have prepared a ground for more extensive future studies in this important area. The success will be a milestone on consistent production of milk round the year that will greatly contribute on raising the economic value of buffaloes as well as minimizing the current problem of scarcity of milk during spring to early summer months.

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TIMED ARTIFICIAL INSEMINATION (TAI) IN BANGLADESHI BUFFALOES DURING THE OFF BREEDING SEASON

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ABSTRACT

The study was designed to evaluate the efficacy of two different oestrous synchronization protocols in buffaloes for timed artificial insemination (TAI) during the off breeding season (March to September). A total of 60 lactating buffalo cows at ≥ 60 days postpartum were selected and were divided into two groups: CIDR-synch and Ovsynch protocols were conducted in Group 1 (n=20) and Group 2 (n=40), respectively. All buffalo cows were with a body condition score (BCS) ≥ 2.5 . Buffaloes in group 1 were synchronized by injecting GnRH on D0, inserting progestagen implant (CIDR) two days after GnRH injection, removing CIDR during injection of PGF_{2 α} on D7, injecting a second GnRH on D9 and TAI was performed 16 -20 h later (CIDR-synch protocol). Buffaloes in group 2 were treated by injecting GnRH on D0, PGF_{2 α} on D7, second GnRH on D9 and TAI was performed 16 -20 h later (Ovsynch protocol). TAI was performed with frozen-thawed semen of Italian Mediterranean buffaloes in all synchronized Bangladeshi buffaloes. Different epidemiological factors related to farm and animal information were considered and carefully recorded prior to the conduction of the experiment. Vaginal Electrical Resistance (VER) was recorded and ultrasound scanning of both ovaries was performed on D0, 7 and 10 to confirm estrus and to monitor ovarian follicular and luteal status. Pregnancy was diagnosed with transrectal ultrasonography after 35 days of AI. Higher pregnancy rate (45%) was observed in buffaloes of group-2 compared to that of group 1 (35 %) that was non-significantly different ($P > 0.05$). The findings of the study suggest that, choice of protocol for oestrous synchronization has prominent roles on oestrous induction and improvement of pregnancy rate of water buffaloes when inseminated with frozen-thawed semen during off breeding season.

Keywords: Buffalo, synchronization, timed artificial insemination, post partum.

INTRODUCTION

Buffalo has never been addressed in Bangladesh as an important animal and it is a neglected species. In Asia, the domestic water buffalo is generally classified into two principal sub-species (Yue *et al.*, 2013); the river type and the swamp type (Perera, 2008) and in Bangladesh also two types of buffaloes are found these are River type and Swamp type. Many buffaloes rear in saline coastal areas as herded animals, totally free as Bathan farming and mostly used for meat production. Bangladesh having 1.86 million buffaloes stands 9th in Asia in term of buffalo population (FAO, 1986). World's buffalo population is increasing day by day which is estimated to be 202 million represents approximately 11% of all bovine species in world (FAO, 2007); out of these, Asia alone represents 174.21 Million (FAOSTAT, 2008). Buffaloes play a prominent role in livestock production in many Asian countries including Bangladesh. The river buffalo is triple purpose species contributing milk, draft work and meat. Due to their contribution and importance, buffaloes are being concentrated in particular agro-ecological zones of Bangladesh. Despite their important role in Bangladesh national economy, buffaloes are always neglected animals in Bangladesh. The production system are characterized by small number of animal with no or minimal input, low output and periodic destruction of animals by disease and mostly maintained under scavenging system with little or no inputs for housing, feeding or health care. The native buffaloes are well adapted to the local environmental, low quality feed resources, housing facilities and scavenging system and the most important aspect is that their performance is also good in terms of feed efficiency. The feed resource base for these animals is scavenging and consists of crop residues, household wastage, tree fodder, roots and tuber, grain by-product and anything edible found in the immediate environment. However buffalo farmers usually face challenges in reproductive efficiency in female buffaloes by inherent late maturity, poor estrous expression in summer, distinct seasonal reproductive patterns and prolong inter-calving intervals (Madan, 1988; Madan and Raina, 1984). As a result fertility and pregnancy rates decrease dramatically. To overcome this type of problem and improve reproductive efficiency, different estrus and ovulation synchronization protocols like Ovsynch and CIDR Synch protocols have to be practiced in Bangladesh. Ovsynch plays an important role in improving conception rates in farm animals. Among two synchronization protocol Ovsynch is easier and reliable for the farmer which is very helpful for the improving livestock production. The experiment was carried out to study the efficacy of two different estrous synchronization protocols in buffaloes

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for timed artificial insemination (TAI) during off breeding season (March to September) in Bangladesh and to determine the vaginal electrical impedance and follicular dynamics at various days during synchronization protocol.

MATERIALS AND METHODS

The present study was conducted at a commercial Breeding Farm 'LalTeer Livestock Ltd., Mymensingh, Bangladesh' from March 2017 to June 2017.

Experimental Animals and Management

A total of 60 indigenous lactating water buffalo cows at ≥ 60 days postpartum from the mentioned breeding Farm were divided into two groups: Group 1 (CIDR synch, $n=20$) and Group 2 (Ovsynch, $n=40$). The buffalo cows which were in cyclic and characterized as in good health and without a history of reproductive illness were selected for experimental protocol. Body condition scoring (BCS) of all the cows were in between 2.5 and 3.5 out of 5.0. The animals were fed with paddy straw, concentrate mixture, cut-and-carry grass and milling by-product according to their body weight and milk production. Vitamin premixes (Powder Megavit-DB®, Elanco Animal Health, Dhaka, Bangladesh) were also supplied to the buffaloes with concentrate. All the selected buffaloes were dewormed by using Nitroxyline (Injection Nitronex®, Renata Animal Health, Mirpur, Dhaka, Bangladesh) 14 days before beginning of experiment.

Synchronization Protocol and Timed Artificial Insemination (TAI)

CIDR Synch and TAI

Buffalo cows in Group 1 ($n=20$) were synchronized by intramuscular injection of GnRH analogue (Buserelin 10 μ g; Receptal, Intervet, The Netherlands) on Day 0 and inserting progesterone implant containing natural progesterone 1.38gm (CIDR) two days after GnRH injection, removing of CIDR during the injection of PGF2 α (cloprostenol 500 μ g; Ovuprost®, Renata Animal Health, Mirpur, Dhaka, Bangladesh) on Day 7. On Day 9 the second GnRH (Buserelin 10 μ g; Receptal, Intervet, The Netherlands) was injected. TAI was performed 16-20h after the second GnRH injection using frozen semen from Mediterranean buffalo bull. Vaginal Electrical Resistance (VER) values were recorded at the time of FTAI, and ultrasound scanning of both ovaries was performed on D0, 7 and 10 to confirm estrus and to monitor ovarian follicular and luteal status.

Ovsynch and TAI

Buffalo cows in Group 2 ($n=40$) were synchronized by intramuscular injection of a GnRH (Buserelin 10 μ g; Receptal, Intervet, The Netherlands) on Day 0 followed by injection of PGF2 α (cloprostanol 500 μ g, Ovuprost, Renata Animal Health Ltd., Bangladesh) on Day 7. On Day 9 the second GnRH (Buserelin 10 μ g; Receptal, Intervet, The Netherlands) was injected. TAI was performed 16-20h after the second GnRH injection using frozen semen from Mediterranean buffalo bull. Vaginal Electrical Resistance (VER) values were recorded and ultrasound scanning of both ovaries was performed on D0, 7 and 10 to confirm estrus and to monitor ovarian follicular and luteal status.

Recording of Vaginal Electrical Resistance (VER)

The VER values of the buffalo cows were recorded on the protocol's Day 0, 7 and 10 using Electronic Oestrus Detector (Draminski Estrus Detector, Poland). The functional activity of the oestrus detector was tested and all hygienic precautions were taken before VER recording. The reading was taken three times and the mean value was calculated and noted.

Ultrasonographic Examination for the Estimation of Follicular Size and Pregnancy Diagnosis

Follicular development was monitored with B mode digital ultrasound equipment (Ultrasonic diagnostic instruments-vet (Digicare Biomedical Technology, Boynton Beach, Florida, USA) using 7.5 MHz high frequency linear array probe on Day 0 and 10, and pregnancy diagnosis at 35 days after TAI and data were recorded. All fecal materials from the rectum were removed before inserting the transducer. For orientation, the transducer was moved along the dorsal surface of the reproductive tract and then moved laterally to examine the ovaries. The transducer face was lubricated with a suitable coupling medium (Eco Gel™-250 mg, Eco-Med Pharmaceutical Inc., Ontario, Canada) and was usually covered by a lubricated plastic sheath before insertion. The transducer was then progressed cranially along the rectal floor to overlie the reproductive tract. The ultrasound monitor and operator's eye were at comparable level for accurate reading of ultrasound images. The enlarged Graafian follicle in ovaries with increased tonicity of uterus was regarded as buffalo in oestrus. Each ovary was scanned several times. The image was retained on the screen, and the size of the follicle was determined and recorded. Diagrams of the relative positions of the follicle as well as related ovarian structures were drawn.



Blood Sampling and Progesterone (P4) Assay

Blood samples were collected properly at the protocol's Day 0, 10 and 21 from jugular vein of each animal using non-heparinized blood collecting tube for serum collection. The harvested serum samples were stored at -20°C for further use to assay the progesterone profile.

Statistical analysis

Data from the research work were entered into Microsoft Excel-2007 work sheet and analyzed by Statistical Package for Social Sciences (SPSS) software version 20 using descriptive statistics. Continuous data from pregnancy rate were analyzed by chi-square test and VER values, follicular size were analyzed by paired T-test.

RESULTS

After CIDR Synchron and Ovsynch treatments, buffaloes showed different estrus signs like bellowing, frequent urination, swelling of the vulva and had mucus. The buffaloes had a variable degree of uterine tone and their cervixes were open enough for easy passage of AI gun during insemination.

Pregnancy Rate after Implementation of Synchronization Protocol

Pregnancy rate of the water buffaloes after implementation of synchronization protocol is presented in the Table 1. There was no significant variation of pregnancy rate in synchronized Group1-CIDR Synchron (35%) and Group 2 Ovsynch (45%) (P>0.05).

Table 1. Pregnancy rate after implementation of synchronization protocol

Experimental Group	Experimental buffaloes	Pregnant buffaloes	Non-pregnant buffaloes	P value
Group-1 (CIDR Synchron)	20	7 (35%)	13 (65%)	0.459 ^{NS}
Group-2 (Ovsynch)	40	18 (45%)	22 (55%)	

^{NS} indicates non-significant, where P>0.05.

Vaginal Electrical Resistance (VER) Values of Pregnant and Non-pregnant Buffaloes During the Synchronization Protocol

VER values of pregnant and non-pregnant cows during the synchronization protocol is presented in the Table 2. Result showed that at Day 0 and Day 7 the VER values were non-significantly lower in the buffaloes which were getting pregnant after TAI than the buffaloes of non-pregnant in both the Experimental Groups. But on Day 10 the VER values were significantly (P<0.05) lower in the buffaloes which were getting pregnant after TAI than the cows of non-pregnant both in Group 1 and Group 2.

Table 2. VER values of pregnant and non-pregnant buffaloes during the synchronization protocol

Experimental Group	VER (Ω)								
	Day 0			Day 7			Day 10		
	Pregnant	Non-pregnant	P-value	Pregnant	Non-pregnant	P-value	Pregnant	Non-pregnant	P-value
Group-1 (CIDR Synchron)	417.14 ±32.93	473.08 ±26.78	0.218 ^{NS}	235.71 ±11.30	265.38 ±17.50	0.262 ^{NS}	204.29 ±6.85	230.00 ±2.26	0.000 ^{**}
Group-2 (Ovsynch)	398.89 ±15.92	426.82 ±23.75	0.358 ^{NS}	260.00 ±12.02	277.73 ±18.81	0.455 ^{NS}	210.56 ±4.16	226.36 ±5.57	0.035 [*]

^{**}indicates highly significant, where P<0.01. ^{*} indicates significant, where P<0.05. ^{NS} indicates non-significant, where P>0.05.

Follicular Size of Pregnant and Non-pregnant Buffaloes During the Synchronization Protocol

Follicular size of pregnant and non-pregnant buffaloes during the synchronization protocol is presented in the Table 3. Result showed that at Day 0 the follicular size was more or less similar in all the buffaloes of both Experimental Groups. But on Day 10 the follicular sizes were significantly (P<0.01) higher in the buffaloes which were getting pregnant after TAI than the buffaloes of non-pregnant in both the Experimental Groups.



Table 3. Follicular size of pregnant and non-pregnant buffaloes during the synchronization protocol

Experimental Group	Follicular size					
	Day 0			Day 10		
	Pregnant	Non-pregnant	P-value	Pregnant	Non-pregnant	P-value
Group-1 (CIDR Synch)	4.07±0.22	4.08±0.25	0.989 ^{NS}	11.36±0.45	8.87±0.51	0.005**
Group-2 (Ovsynch)	3.87±0.14	3.86±0.17	0.971 ^{NS}	10.65±0.37	8.60±0.29	0.000**

** indicates highly significant, where $P < 0.01$. ^{NS} indicates non-significant, where $P > 0.05$.

DISCUSSION

Silent oestrus is a common problem in buffaloes even under good management and non-stressful periods of the year (Kamboj and Prakash, 1993; Abdallah, 2003) and the farmers cannot easily identify the cyclic buffaloes due to the nature of showing silent heat. CIDR Synch and Ovsynch with TAI were practiced in the present study in indigenous water buffaloes, where oestrous detection is the main restriction for satisfactory reproductive performance.

Result in the present study revealed that 45% buffaloes became pregnant when oestrus was induced by Ovsynch protocol and 35% buffaloes become pregnant when oestrus was induced by CIDR Synch protocol. The pregnancy rate obtained in Ovsynch protocol was more or less similar with the results of other experiments which ranged from 27.2 to 42.4% (Baruselli *et al.*, 1997; Irikura *et al.*, 2003; Neglia *et al.*, 2003; Paul and Prakash, 2005). Neglia *et al.* (2003) observed that a conception rate of 36% after using the Ovsynch protocol in postpartum lactating dairy buffalo cows. Similarly, Paul and Prakash (2005) obtained a conception rate of 33.3% and Warriach *et al.* (2008) obtained 36.3% conception rate in buffaloes when oestrus was induced at breeding season. However, the obtained pregnancy rate of the present study is consistent with the earlier study conducted by Berber *et al.* (2002) who reported 56.5% pregnancy rate in buffaloes. The pregnancy rate obtained in CIDR Synch protocol is considered more or less similar with the findings of Xu and Burton (2000). In the CIDR Synch and Ovsynch protocol the first GnRH injection can successfully synchronize a new follicular wave 1-3 days after treatment (Neglia *et al.*, 2003; Ali and Fahmy, 2007) and this wave results in the development of a new dominant follicle, which may be attributed to the subnormal P4 concentrations. Sub luteal circulating P4 levels have been reported to increase the frequency of LH pulses, and a prolonged growth phase of the dominant follicle (Bridge and Fortune, 2003). The subsequent injection of PGF2 α increases the percentage of synchronized animals by lysis of both the cyclic CL and the CL resulting from ovulation of the dominant follicle (Pursley *et al.*, 1995). After the PGF2 α injection, if the dominant follicle did not ovulate, a new wave of small follicles needs several days to grow and become able to produce estradiol-17 β , leading to an induction of the preovulatory LH-surge (Bridge and Fortune, 2003). It has been previously suggested that high P4 levels at the time of PGF2 α application may be an important factor to improve conception rates for subsequent inseminations (Hussein, 2003; De Rensis *et al.*, 2005). In order to increase synchrony of ovulation, a second dose of GnRH is injected to ovulate the preovulatory follicle at a precise time (Wiltbank, 1998). The second GnRH injection on Day 9 of the protocol causes an induced LH surge responsible for ovulation of the dominant follicle and formation of a new CL (Senger, 2003). Moreover, in the CIDR Synch the removal of CIDR device was intended to improve the precision of oestrus (Xu and Burton, 2000). However, the cause of difference in pregnancy rates among studies may be due to differences in breed of buffaloes, follicular number and size, oestrous induction protocols and agro-climatic conditions of study areas in different studies which emphasizes the requirement of selecting a suitable oestrous induction protocol for buffaloes that may result in satisfactory pregnancy rate without paying much time for oestrous detection.

Result revealed that VER values were significantly lower on the protocol's Day 10 (at the time of AI) in the buffaloes which were getting pregnant after TAI than the buffaloes of non-pregnant, which is similar to the findings of Gupta and Purohit (2001), although the values of VER in the present study were higher than the findings of Gupta and Purohit (2001). Gupta and Purohit (2001) stated that VER can be used successfully to predict the stage of oestrous cycle, ovarian status and ovulation; and insemination at a low VER distinctly improves the conception rates in buffaloes.

Results showed a significant ($P < 0.01$) effect of follicular size in the pregnant buffaloes after the application of CIDR Synch and Ovsynch protocol, which is more or less similar with the findings of other experiments (Berber *et al.*, 2002; Baruselli and Carvalho, 2005; Bartolomeu *et al.*, 2007). The time to ovulation after GnRH injection depends mainly on the diameter of the largest follicle at the time of injection (Hussein *et al.*, 2002; Hussein, 2003) and it is a determining factor for the successful synchronization of ovulation and high conception rates (De Rensis *et al.*, 2005). In a recent study, Campanile *et al.* (2008) have stated similar size of follicles in ovulated and non-ovulated buffaloes. However, the stage of follicular development (growth or regression phase) greatly affects its



response to GnRH treatment (Dharani *et al.*, 2010). Therefore, understanding and considering follicular waves as a functional unit, rather than the estrous cycle, could help in the proper synchronization protocol for the enhancement of reproductive efficiency in river buffaloes. A good knowledge about follicular wave dynamics could facilitate the development of a methodology for influencing ovarian function and oestrous in both cyclic and non-cyclic animals.

In conclusion, ovsynch and CIDR synch protocols both resulted in satisfactory pregnancy outcome in buffaloes. Larger follicular size at the time of insemination significantly improved the pregnancy rate. Ultrasound monitoring of the reproductive organs in buffaloes can be a suitable diagnostic tool for monitoring cyclicity and early pregnancy in Bangladesh.

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FOURIER HARMONIC ANALYSIS AS TOOL IN PREDICTING BULL FERTILITY FOR IMPROVED BUFFALO BREEDING

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ABSTRACT

Fourier Harmonic Analysis or FHA for short is a sperm-DNA analytical tool involving a computerized imaging system that describes the curvature of the perimeter of sperm head shape using measures of length, width, and area expressed as harmonic amplitudes. It finds the perimeter of the sperm head through the intensity of the DNA by epifluorescence microscopy and staining technique and explains the curvature of sperm perimeters by Fourier functions, which contain multivariate shape measures. Earlier studies in cattle and boar showed that these measurements are related to *in vivo* fertility. Initial studies in water buffalo showed that FHA is an efficient tool in predicting and classifying bulls according to fertility. Bulls classified by FHA as High-fertile had significantly higher *in vitro* penetration rate than those classified as Low-fertile bulls. Similarly, *in vitro* development of resultant zygotes from High-fertile bulls had significantly higher cleavage and blastocysts development rate than those classified as Low-fertile bulls. Potential application of this technique on water buffalo bull farm management and semen collection and analysis for buffalo breeding programs are discussed.

Keywords: Buffalo sperm, bull fertility, fourier harmonic analysis, harmonic amplitudes, sperm perimeter

INTRODUCTION

In the production of genetically superior animals, artificial insemination is the most frequent reproductive biotechnology that is used and bull fertility is very important in ensuring the success. Even when the artificial insemination was carried out by considering all these factors; perfect time of estrus, correct semen handling and thawing, correct insemination site in the reproductive tract, failure of pregnancy can still occur due to fertility problem in a bull. Not all semen quality analyses such as motility, livability, percent normal, and sperm concentration can ensure bull fertility because these analyses are subjective and does not measure the fertility (Ostermeier et al., 2000). Subfertility among bulls is caused by fertility defects exist in sperm (Saacke et al., 1988). Subfertility is less noticeable than infertility. It's a diminish reproductive performance that cost livestock raisers big lost each year. In male, subfertility among bulls is a serious problem in artificial insemination and *in vitro* fertilization programs. It is caused by compensable and non-compensable sperm defects (Saacke et al., 1994; Evenson, 1999).

Compensable sperm defect is the inability of sperm cells to reach the egg for insemination caused by defects such as immotile sperm, sperm with tail defects, and immature sperm (Saacke et al., 1994). This kind of defects can be augmented by increasing the sperm concentration or semen dose during insemination. The non-compensable sperm defect on the other hand, is a defect that inhibits the sperm to fertilize an egg and or if fertilization occurred, cannot support the development of an embryo full-term (Eid et al., 1994; Ostermeier et al., 2001b). It is the failure of motile sperm to sustain fertilization and pregnancy (Eid et al., 1994) as shown in Figure 1. High and low fertility bulls had the same pronuclear formation however, a delay on the development was observed among zygotes sired by low fertile bulls with significantly lower developmental competence, a defect that even though we increase the sperm concentration or dose during artificial insemination, successful fertilization or pregnancy does not occur. This kind of sperm defect was determined as caused by DNA fragmentation in the sperm cell (Ostermeier et al., 2001b) and causes tremendous loss on livestock breeding efficiency (Evenson, 1999). So, the challenge is to find a semen quality analysis that can determine the non-compensable sperm defect in order to point out which buffalo bulls are not good for use as semen donor for collection and processing. This is also to be able to recommend the needed management interventions for genetically superior bulls predicted to be sub-fertile.

The DNA fragmentation in the sperm cell was determined by Fourier Harmonic Analysis or FHA. It was found that by staining the sperm cells with Hoechst stain, a DNA-dye, the shape of the DNA of the sperm cells can be analyzed and the fragmentation can be observed. On the other hand, the YOYO-1 is a biological stain used to identify live and dead sperm cells. In FHA, the bull semen is stained with Hoechst or propidium iodide and/or YOYO-1 to magnify the DNA of the sperm and identify those that are alive that are used for the analysis. Previous studies in boars, bull, and stallion showed that this analysis is efficient in predicting the fertility of a bull (Ostermeier et al., 2001b; Willenburg, 2012). Below are important points and breakthroughs in carrying out the FHA in predicting bull fertility that can be used as reference to improve the buffalo breeding.

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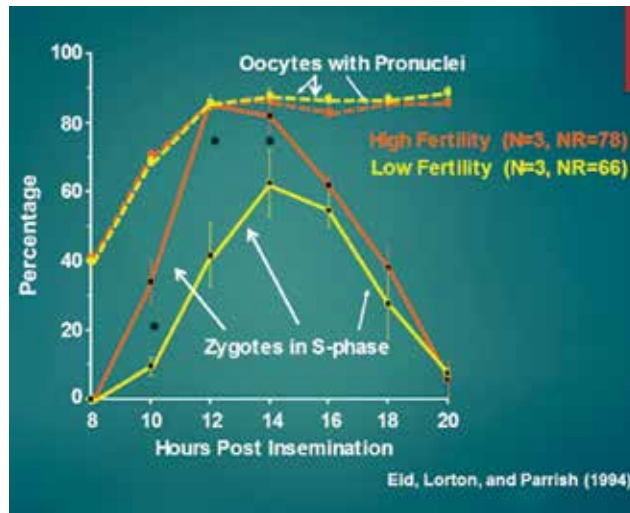


Figure 1. Result of in vitro fertilization using semen from high and low fertile bulls.

FOURIER HARMONIC ANALYSIS

Fourier Harmonic Analysis or FHA (Ostermeier et al., 2001a) is a sperm fertility analysis that involves a staining technique and a combination of image analysis using fluorescence microscopy and computer software. It is a mathematical tool that describes the sperm head shape using measures of length, width, and area (Figure 2, Parrish et al., 2014) predicting fertility of sperm samples based on the intensity of DNA staining of the samples. This was developed based on the surprising observation that brighter DNA staining of sperm heads has a positive correlation with decreased fertility rates (Parrish et al., 1998). It was known (Ballachey et al., 1987) that the sperm head is 90% DNA and the shape of the sperm head is based on the structure of the DNA. Any change in the structure of the chromatin of the sperm cell is reflected by a change in sperm shape (Evenson et al., 1980; 1994). In FHA, the curvature of sperm perimeters is explained by Fourier functions, which contain multivariate shape measures expressed as harmonic amplitudes (Figure 3, Parrish et al., 2014). Harmonic amplitude 0 (HA0) is the overall size of the sperm where HA1 through 5 are component integers of HA0 that help to characterize HA0. Harmonic amplitude 1 describes the anterior portion of the nucleus, as this value increases so does the overall roundness of the cell. Elongation and width of the sperm cell nucleus is characterized with HA2 and 4, whereas the posterior half of the nucleus is represented by HA3 and 5. Fourier harmonic analysis of shape is a potential approach to evaluating perimeter curvature (Ostermeier et al., 2001a).

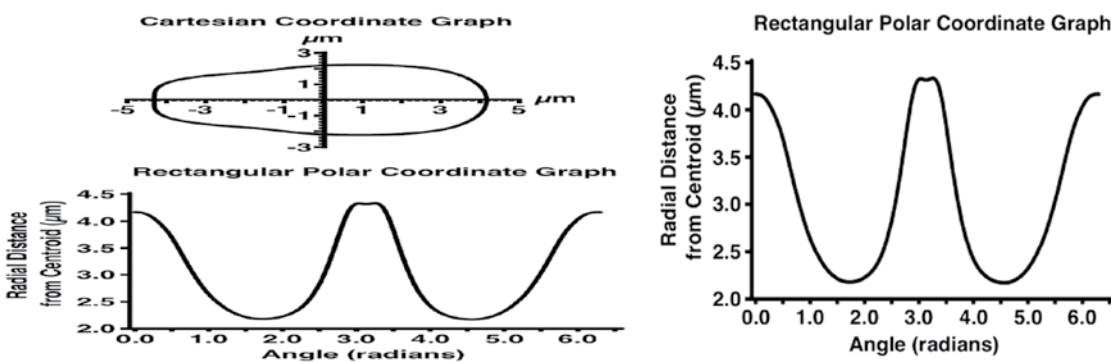


Figure 2. The average shape of sperm from 5 bulls. Sperm nuclei were by image analysis of epifluorescent images. Sperm were stained with Hoechst 33342. The shape is derived from averaging the perimeter coordinates of 100 sperm/bull. The Cartesian coordinate graph is in microns (μm). The same average sperm is shown in the rectangular polar coordinate graph with the y-axis the radial distance in μm from the centroid to each perimeter point at specific angles in radians. The value for 0 radians is the point crossing the positive x-axis of the Cartesian coordinate graph. The angle then increases by rotating counter clockwise around the sperm perimeter (Parrish et al., 2014)

The procedure of FHA can be summarized as: a sample of sperm from a bull is obtained, stained with a fluorescent DNA-binding dye, collecting the image of the stained sample, determining the edge of the nucleus



of the sperm within the stained sample, measuring the intensity of the DNA-binding dye within the area defined by the edge of the nucleus, using statistical analysis perimeter coordinates are converted from Cartesian to polar coordinates and Fourier functions constructed and harmonic amplitudes (HA) of the functions determined, and finally comparing the average intensity per unit area to an average intensity per unit area for high-fertile sperm and low-fertile sperm to determine if the sample has high or low fertility (Parrish, 2015).

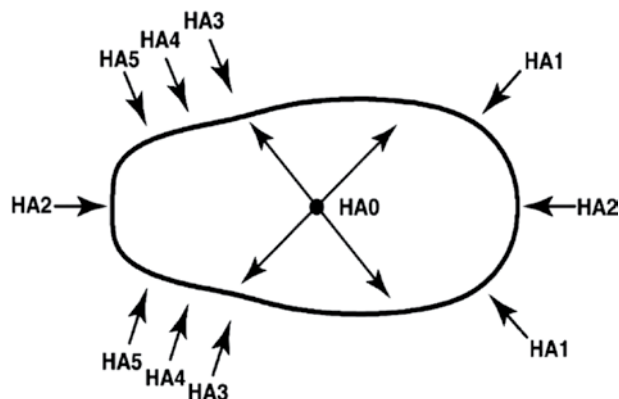


Figure 3. Harmonic Amplitudes (HA) on sperm nuclear shape in the bovine. The HA0 represents an increase in overall size of the sperm. The HA1 impacts the pinching of the anterior head but also movement of the centroid either more anterior or posterior. The HA2 increases with increasing length of the sperm nuclei but at the same time it also decreases the width. The HA3-5 impacts the pinching in the posterior head and to a lesser extent the anterior head. The impacts of specific HA on the sperm nuclear shape is mathematically precise but general descriptions are more complex than indicated. Further, specific impact of HA may differ among species (Parrish et al., 2014).

FHA in Cattle

The first study to examine bull fertility based on sperm nuclear shape as determined by FHA analysis utilized frozen-thawed semen from 59 bulls collected at a major US bull stud in late spring (Parrish et al., 1998). The estimated relative conception rate (ERCR, >100 breedings/bull) was the fertility data used and bulls were classified as good fertility or of poor fertility. Low fertility was defined as those bulls that were <1 SD below the mean for the population of bulls evaluated. The remaining bulls were classified as acceptable or good fertility. There were 2 dyes used for staining sperm, Hoechst 33342 and YOYO-1, with YOYO-1 used to identify the dead sperms. Analysis was thus possible on live, dead or all sperm. Differences between the low and good fertility group bulls were greater for live sperm, so this is what is described. Bulls in the low fertility group had increased variance for HA0, HA2, HA4 and HA5 ($P < 0.05$) but no difference in mean HA 0-5 values ($P > 0.05$). In this experiment, low fertility bulls produced sperm nuclei with more variation in shape than high fertility bulls (Parrish et al., 2014).

To assess the effect of sperm orientation on the accuracy of FHA, Ostermeier et al. (2001a) stained the sperm cells with propidium iodide and digital images were collected followed by describing the perimeters of individual sperm nuclei using Fourier functions. Fourier functions showed that harmonic amplitudes are independent of sperm orientation during digitization. They found that 6 harmonic amplitudes of a sperm are adequate to describe sperm nuclear shape. By cluster analysis, 20 different sperm groups were generated with sperms within groups had similar morphologies and unique shape characteristics distinguished statistically. It was also found that harmonic amplitudes 0 to 5 can be used to distinguish previously reported abnormalities such as tapered, pyriform, macrocephalic, and microcephalic, as well as gradations in between. Furthermore, differences were detected among bull harmonic amplitude centroids ($P < 0.05$), indicating that bulls with different fertility differ in mean sperm nuclear shape.

To get a more accurate estimate of bull fertility, a second trial (Ostermeier et al., 2001b) was conducted in which 6 bulls were evaluated that each had over 5000 first service insemination records. There were 3 high fertility bulls with a mean \pm SE non-return rate of $78 \pm 2\%$ and 3 low fertility bulls with a non-return rate of $69 \pm 1\%$. A discriminate analysis utilizing the mean HA 0-5 as components of the model found a significant canonical correlation of 0.55 ($P < 0.05$) with HA 2, 4 and 5 the most important for differentiating the 2 fertility groups. If the discriminate analysis simply was asked to place the 6 bulls into 2 groups based on shape, it correctly placed all the bulls into the right fertility group and had a canonical correlation of 0.74 ($P < 0.05$) for bulls within a group. The FHA approach yields predictors of bull fertility that are related to shape or its dispersion within an ejaculate.

An important question is about the variation among bulls and collections within bulls. So in the studies by Parrish et al. (1998), 3 collections on 11 bulls within a 2-week interval was examined, partitioned the variance



components, and determined the contribution of different components to the overall variation in sperm nuclear shape. They found that 84-98% of the variation was among bulls while 1-9% variation was found within collections within a bull. There were effects of both variations within and among bull ($P < 0.05$) but most of the variation is due to differences among bulls. Parrish indicated that assuming no environmental impacts occur to a bull; this means that a single ejaculate is sufficient to evaluate sperm nuclear shape of a bull at a particular point in time.

With those results, FHA approach appeared to be promising and Parrish et al. (2006) conducted a much larger study with 210 bulls representing most of the Holstein bulls in the US for which semen are being commercially collected. They used frozen semen in egg yolk tris, egg yolk citrate or milk based extenders. They based the fertility data on the estimated relative conception rate (ERCR) value which is a rolling 3-year average of data collected and normalized to have a population mean of 0. There was a mean \pm SEM of 2,449 \pm 254 breedings/bull ranging from a high of 21,931 to a low of 50. For the bulls in the study, mean \pm SD ERCR value was 0.05 \pm 1.82. Low fertility bulls ($n=32$) were defined as those with ERCR values < 1 SD below the mean or < -1.77 . Bull's with ERCR values above the cutoff were defined as having acceptable fertility ($n=178$). The study was designed to determine if the FHA approach could identify bulls classified as having low fertility. The mean, variance, skewness and kurtosis of HA0-HA5 was determined from 1 collection day for each bull and the results are presented in Table 1. The low fertility bulls had increased values for HA2, HA4 and decreased values for HA0 ($P < 0.05$). The variations in HA0-5 between the bull fertility groups were only present for variance of HA1 and HA4 and Kurtosis of HA0. The MANOVA analysis found the overall mean shape of sperm nuclei differed between the bull fertility groups ($P < 0.01$) but not the dispersion measures, variance, kurtosis or skewness of the HAs ($P > 0.05$). Parrish et al. (2014) noted that the improvements to algorithms used to identify the edge of the sperm during image analysis has resulted to the ability to identify mean shapes being different among bull fertility groups.

Table 1. Harmonic Amplitude (HA) 0-5 within bull fertility group in cattle (Parrish et al., 2014)

Criteria	Low ^b (Mean \pm SEM ^a)	Acceptable/High (Mean \pm SEM ^a)
HA0	3.111 \pm 0.0018	3.155 \pm 0.007*
HA1	0.119 \pm 0.004	0.117 \pm 0.001
HA2	1.111 \pm 1.013	1.079 \pm 0.005*
HA3	0.120 \pm 0.003	0.126 \pm 0.001
HA4	0.236 \pm 0.009	0.215 \pm 0.002**
HA5	0.090 \pm 0.004	0.086 \pm 0.001

^a Values are in microns and the SEM reflects variation among bulls within a group.

^b MANOVA found the cull group to be different from the acceptable group, $P < 0.05$.

*, ** Means within a row differed, $P < 0.05$ or $P < 0.01$, respectively.

In a final comparison to in vivo fertility, bulls from a progeny test program differed by 1 SD below the mean (low) and 1 SD above the mean (high) were selected. Thawed semen was stained with only Hoechst 33342 and all sperm present were evaluated for FHA of HA0-5 and variation of these harmonics within an ejaculate. Results shown (Table 2) that both HA0 and HA1 were impacted by bull fertility status, $P < 0.05$.

Table 2. Harmonic amplitude (HA) 0-5^a and associated variances^b of bulls of differing fertility (Parrish et al., 2014)^a.

Criteria	Mean \pm SEM (n=4)	
	Low(N=54)	High (N=53)
Fertility Group ^b	-4.7 \pm 0.3	4.1 \pm 0.1
HA0	2.927 \pm 0.010	2.975 \pm 0.009**
HA1	0.143 \pm 0.003	0.134 \pm 0.003*
HA2	1.082 \pm 0.010	1.090 \pm 0.007
HA3	0.129 \pm 0.002	0.124 \pm 0.002
HA4	0.233 \pm 0.006	0.230 \pm 0.004
HA5	0.097 \pm 0.003	0.093 \pm 0.003
Var HA0	3.219 \pm 0.138	2.691 \pm 0.189*
Var HA1	187.9 \pm 11.00	134.2 \pm 10.54***
Var HA2	26.99 \pm 1.744	16.97 \pm 1.760***
Var HA3	0.546 \pm 0.028	0.444 \pm 0.044
Var HA4	0.131 \pm 0.007	0.105 \pm 0.009*
Var HA5	0.036 \pm 0.002	0.028 \pm 0.001***

^a Sperm were only stained with Hoechst 33342 and no other stains. The HA data represents all sperm in the ejaculate, live and dead.

^b Data and bulls were from progeny testing at Alta Genetics Inc. and 108 testing herds spread across the US. Fertility values represent conception rates with pregnancy confirmed along with DNA parentage of offspring verified. There was a mean \pm SEM breeding of 2368 \pm 137 in the high and low fertility group respectively. The population fertility of bulls from Alta Genetics Inc. is adjusted to 0.

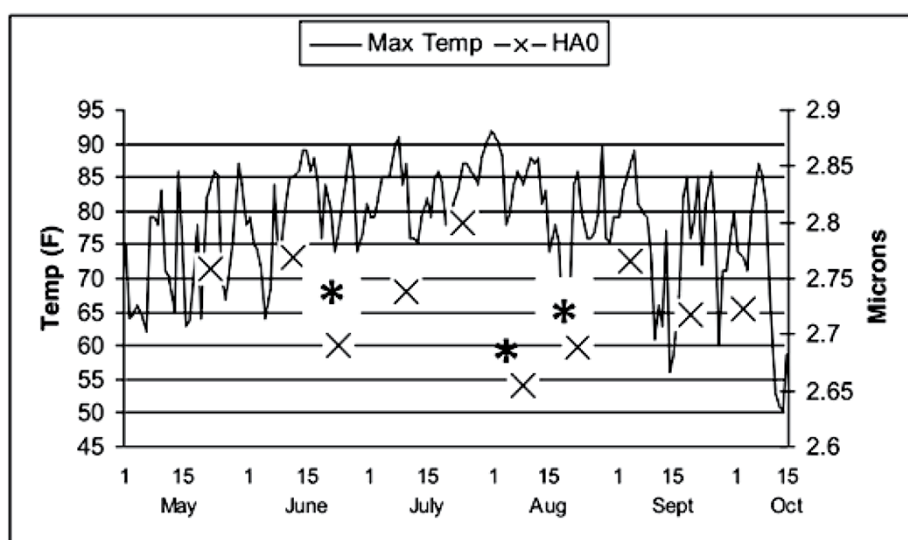


While skewness and kurtosis were not impacted by bull fertility group, $p > 0.05$, the variance was higher in low fertility bulls for HA0-3, and HA4-5, $P < 0.05$. The MANOVA evaluation found both shape as defined by HA0-5 and variation as defined by the variance of HA0-5 were different for the bull fertility groups, $P < 0.01$. The results confirmed that fertility groups have different sperm shapes with an increased variation in the sperm shape within low fertility bulls (Parrish et al., 2014).

FHA in Swine

In the boar, the FHA was used to identify low fertility boars and those semen samples impacted by effect of environmental changes. It has been reported (Cameron and Blackshaw, 1980) and well-known that the swine species are susceptible to seasonal variation regardless of controlling for temperature and light. Kunavongkrit et al. (1989) have shown that high ambient temperatures decrease reproductive efficiency and temperatures above 30°C can affect spermatogenesis. Further studies by Kunavongkrit and Prateep (1995) showed that high temperature cause testicular degeneration resulting in a lower sperm concentration and a decrease in semen volume that also increase in sperm abnormalities (McNitt and First, 1970) and the time interval for a boar to mount a collection dummy or sow (Claus and Weiler, 1985).

In a study described by Willenburg (2012) using 8 boars of proven fertility from a commercial stud and FHA was performed biweekly as a diagnostic tool to identify changes in sperm nuclear shape during the summer i.e. May until the middle of October in southern Wisconsin. Harmonic amplitude 0-5 were analyzed along with temperature, humidity, semen volume, concentration, motility, viability and gross morphology (abnormal heads, tails, proximal and distal droplets). The results showed (Figure 4) differences in sperm nuclear size (HA0) three times during the summer ($P < 0.001$) that corresponded to an increase in temperature and humidity approximately 1 month prior. A notable decrease on the average sperm size (HA0) was also observed with a size of 2.655 ± 0.041 in August compared to an HA0 of 2.758 ± 0.025 microns in May when there was not a significant increase in temperature and humidity. Harmonic amplitude 4 was also significantly lower on July compared to the first measurement in May (0.175 ± 0.013 vs 0.190 ± 0.012 microns). Interestingly, the effects on HA4 were observed approximately 3 weeks after the increase in temperature and humidity. There were also differences in sperm concentration, motility, viability, normal sperm and distal droplets evident approximately 5 weeks after increases in temperature and humidity ($P < 0.05$). The data shows the potential use of FHA as diagnostic tool to identify boars that have been affected by summer stress. Identification via harmonic amplitudes can occur 1-2 weeks prior to the increase in sperm abnormalities, which would potentially allow producers to maintain a high level of fertility throughout the breeding herd without any significant profit loss.



^Values for the average sperm nuclear size are indicated via symbol "X" with their respective values on the secondary Y axis.

* Indicates significance ($P < 0.01$) for June 23rd, August 8th and August 22nd.

Figure 4. The relationship between daily maximum temperature and average sperm nuclear size^e (HA0) for boars (N=8) from May until October (Willenburg, 2012)



To examine the impact of scrotal insulation on spermatogenesis, Parrish et al. (2012) performed testicular morphometry on control or insulated bulls and boars. They found reduced numbers of Sertoli cells ($p < 0.05$) following scrotal insulation of bulls and loss of primary spermatocytes ($P < 0.05$) in the boar. Testicular gene expression experiments following scrotal insulation in the bull found decreased expression of TIMP2. The disruption to the seminiferous epithelium due to scrotal insulation may explain resulting changes to sperm nuclear shape and reduced fertility among bulls and boars during summer season.

Also in 2012, Parrish et al. examined the ability of FHA analysis to differentiate between sperm from low and adequate fertility boars. Fertility was determined from at least 100 single sire mating per boar and farrowing rates determined. There were 26 boars in the study. Low fertility boars ($n=2$) were those with farrowing rates $< 60\%$ and adequate fertility boars ($n=24$) having fertility $\geq 60\%$. This equates to fertility below or above 1 sd below the mean. The average fertility was $44.7\% \pm 3.3$ for the low fertility group and $72.2\% \pm 0.6$ for the adequate fertility group. Extended semen was shipped to the lab at 17°C and evaluated with the same procedure as for bulls to determine the live sperm nuclear shape. They observed no difference in the percentage of motile sperm, percentage viable sperm, HA0, HA1, HA3 or HA5 between the two boar fertility groups ($P > 0.05$). However, similar to the bull, the mean \pm sem HA2 and HA4 decreased ($P < 0.05$) from the low to adequate fertility groups, 1.110 ± 0.020 vs. 1.010 ± 0.009 and 0.224 ± 0.011 vs. 0.168 ± 0.00 , respectively. In the bull and boar, sperm are slightly longer and more tapered in the lower fertility males. Additionally, in the bull the average size, HA0, is decreased in the sperm from lower fertility males. These results showed that HA2 and HA4 are indicators of differences on the fertility of bulls and boars and these are the parameters affected by environmental changes.

To assess the accuracy of FHA in predicting fertility in swine, Parrish et al. (2012) used boars with a minimum of 50 single sire mating and separated them into two fertility groups (Acceptable and Unacceptable) based on three standard deviations from the mean for total born (TB) and adjusted farrowing rate (AFR). The average TB and AFR from all the boars was 12.93 ± 0.98 (8.40 to 14.90) and $91.43 \pm 8.50\%$ (55.30 to 100.00), respectively. The boars in the acceptable fertility group ($n = 92$) had an average TB = 13.02 ± 0.78 whereas the unacceptable fertility group ($n = 2$) had a TB = 8.75 ± 0.50 . The boars ($n = 91$) in the AFR acceptable group had an average AFR of 92.48 ± 6.22 compared to 59.63 ± 7.25 for the unacceptable boars ($n = 3$). The results (Table 3) show that fertility groups differed in sperm nuclear shape ($P < 0.05$) and only HA1, HA2 and HA4 were different between the groups for TB and HA2 and 4 were different for AFR ($P < 0.05$). This supports the FHA fertility data from the bull (Ostermeier et al., 2001b) where HA 2 and 4 were different between acceptable and unacceptable fertility groups showing the similarities of low fertility animals regardless of species or genetics. Unacceptable boars have longer and slimmer shape than the average sperm shape of the acceptable boars. Finally, a model was constructed to predict the fertility group membership of a boar. A boar can be classified in one of four different categories (true positive (TP, classifying an acceptable boar correctly), true negative (TN, classifying an unacceptable boar correctly), false positive (FP, classifying an unacceptable boar incorrectly) and false negative (FN, classifying an acceptable boar incorrectly)) based on the model created. The TB model with the fewest parameters and highest TP + TN value included HA 1, 2 and 4 with a TP + TN value of 1.93. The AFR model consisted of HA2 and HA4 with a TP + TN value of 1.90. The TB model correctly classified 94% of the boars, misclassifying 6 of the acceptable boars, whereas the AFR model correctly identified 90% of the boars misclassifying 9 of the acceptable boars. These works of Parrish et al. (2012) showed the accuracy of FHA in predicting the fertility classification of boars.

Table 3. Mean and standard error of the mean (SEM) for harmonic amplitudes (HA) measured in micrometers (mM) from the boars with acceptable and unacceptable fertility for both total born and adjusted farrowing rate models (Willenburg, 2012)

(μM)	Total Born ¹			Adjusted Farrowing Rate ²		
	Acceptable	Unacceptable	P Value	Acceptable	Unacceptable	P Value
HA0	2.970±0.060	3.007±0.049	0.415	2.970±0.060	2.992±0.045	0.564
HA1	0.075±0.011	0.059±0.001	0.003	0.075±0.010	0.078±0.017	0.536
HA2	1.162±0.052	1.039±0.048	0.002	1.162±0.053	1.079±0.071	0.009
HA3	0.046±0.011	0.034±0.007	0.151	0.045±0.011	0.050±0.009	0.407
HA4	0.229±0.037	0.157±0.026	0.008	0.229±0.037	0.180±0.039	0.026
HA5	0.033±0.006	0.027±0.002	0.211	0.033±0.006	0.030±0.003	0.470

¹The boars had a minimum of 50 single sire matings. Boars in the unacceptable group ($n=2$) were 3 standard deviations below the mean of the boars in the acceptable fertility group ($n=92$).

²The boars had a minimum of 50 single sire matings. Boars in the unacceptable group ($n=3$) were 3 standard deviations below the mean of the boars in the acceptable fertility group ($n=91$).



FHA in Water Buffalo

In water buffalo, a collaborative research project was carried out by the Philippine Carabao Center (PCC) and the University of Wisconsin-Madison (Hufana-Duran and Parrish et al., 2014) through a funding support by the Department of Agriculture Biotechnology Program (DA-Biotech 14004). FHA model for water buffalo was developed using 31 buffalo bulls (existing and non-existing) with fertility record out of AI. Using the FHA Model developed for water buffalo, 44 bulls that are being used as donors of semen in PCC Bull farm were subjected to FHA. FHA resulted to the classification of the 44 bulls into 12 as High fertile and 10 as Low fertile bulls with the rest categorized as Mid-fertile group. Frozen semen from five each of the bulls categorized as High Fertile and Low Fertile groups were subjected for in vitro fertilization assay individually to assess the efficiency of FHA in classifying the bulls. On the average, results showed that oocytes sired by frozen-thawed semen of bulls classified by FHA as High-fertile had significantly higher ($P<0.05$) male and female pro-nuclear formation compared to bulls classified as Low-fertile group (Table 4, Hufana-Duran et al., 2017). Similarly, cleavage and blastocysts development of in vitro matured and in vitro fertilized oocytes with frozen-thawed semen from High-fertile bulls classified by FHA are significantly higher than the Low-fertile bulls (Figure 5, Daag et al., 2017). However, assessment of the individual bull within each group also showed significant difference ($P<0.05$) on male and female pronuclear formation, cleavage rate, and blastocysts development rates. These results suggest that the number of animals used to develop the Model for water buffalo is limited, thus, accuracy of classification is still low. This supports the study on boars (Parrish et al., 2012) misclassifying 4 to 10% of the boars studied. Increasing the number of animals with accurate fertility data in designing the Model would increase the accuracy of the FHA.

With the above results, FHA is considered as an efficient tool in analyzing the water buffalo bull fertility and thus, provided a means to identify the bulls that can be best used as donors of semen for improved success rate of artificial insemination and in vitro fertilization programs.

Table 4. Male pronuclear formation and cleavage rate of in vitro matured and fertilized oocytes (Hufana-Duran et al., 2017)

Bull Category	No. of Oocytes**	Ave. Penetration rate, %±SEM	No. of Oocytes	Cleavage Rate, %±SEM
High Fertile, (N=5)	699	83.5±0.2 ^a	514	73.5±0.9 ^a
Low Fertile, (N=5)	704	71.01±0.9 ^b	425	60.1± 3.0 ^b

*Frozen semen from water buffalo bulls subjected to FHA for fertility classification by Parrish et al. of University of Wisconsin-Madison, USA.

** Water buffalo oocytes from slaughter-house derived ovaries.

^{a,b}Values in the same column with different superscripts are significantly different, <0.05

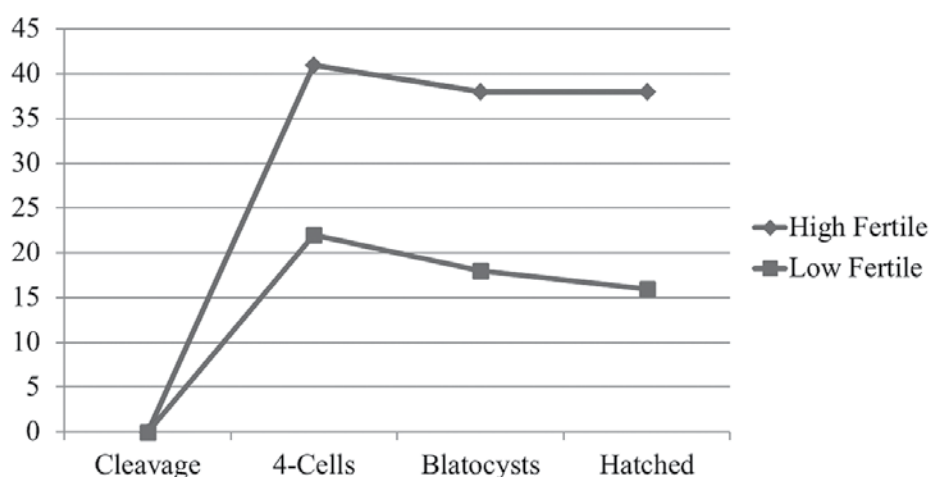


Figure 5. Developmental competence of in vitro matured oocytes in vitro fertilized with frozen-thawed semen from high and low fertile bulls classified by Fourier harmonic analysis. More zygotes sired by high fertile bulls developed to the 4-cells stage 36 hours post IVF. Blastocysts development and hatching rates were also significantly higher ($P<0.05$) in zygotes from high fertile than low fertile group (Daag et al., 2017).



POTENTIALS FOR IMPROVED BUFFALO BREEDING

The fertility of the bull and quality of cryopreserved semen contribute to the outcome of AI as much as the cow factor (Jodar et al., 2013). With the increasing importance of buffalo as milk-producing animal and source of income from rural farming families, the practice of artificial insemination (AI) with frozen semen has become a prerequisite to enhance reproduction and promote improved milk productivity. However, insemination of females using semen of low-fertilizing capacity accounts for a significant loss to the dairy industry (Lucy, 2001; Lopez-Gatius, 2003). Improvement on the effectiveness of AI programme needs tests that can precisely identify the fertilizing potential of cryopreserved spermatozoa.

With the above scientific evidences on the developments and application of FHA in predicting the fertility of cattle, boars, and water buffalo bull, it is apparent that adoption of this technology in screening the semen donor bulls for artificial insemination and in vitro fertilization would guarantee a more efficient bull management and semen collection and processing activities. Water buffalo breeding is a challenging task considering the inherent problems manifested by this animal species. Any failure to impregnate a buffalo cow would mean economic loss in the production system. Ensuring that all parameters involve in AI especially the bull fertility is important to guarantee success of the breeding service.

CONCLUSION AND RECOMMENDATIONS

Fourier harmonic analysis emerged as an effective tool in classifying and predicting bull fertility. It is a sperm-DNA analysis that uses Hoechst or propidium iodide, and YOYO-1 stains and fluorescence microscopy to capture sperm image, Image J software to automatically capture the curvature of sperm nuclear shape, computer software to determine the sizes, and statistical analysis to determine individual bull sperm differences. Accuracy and repeatability is high provided the analysis is done following the Model designed for each species. Species differences exist that a Model is developed for each species. Using it as tool in classifying water buffalo bulls as high fertile or low fertile would help improve the bull management system, improves efficiency of bull contribution in a breeding service, and helps improve the overall reproduction potential in buffalo breeding program.

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EFFECTS OF DIETARY SUPPLEMENTATION OF FEED CONTAINING OMEGA-3 FATTY ACIDS ON THE FREEZE ABILITY OF NILI-RAVI BUFFALO (*BUBALUS BUBALIS*) BULL SEMEN

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ABSTRACT

Deep freezing of semen enables its long term storage and transportation to far off places. However, the spermatozoa are damaged during the process of freezing and thawing due to oxidative stress, which adversely affects their fertilizing ability. Animals are unable to synthesize unsaturated fatty acids due to lack of fatty acids desaturase enzyme. Therefore, dietary supplementation of these fatty acids can improve the post-thaw quality of bull semen through minimizing the effects of oxidative stress. In the present study, the effects of dietary supplementation of linseed oil as a source of Omega-3 fatty acids on the post thaw quality of Nili-Ravi buffalo bull semen were investigated. For this purpose, 12 adult Nili-Ravi buffalo bulls were randomly assigned to three equal groups, A, B and C. Bulls in group-C were kept as control, while those in groups A and B were fed ration supplemented with linseed oil @125 ml and 250 ml/day, respectively for 12 weeks. From each bull, two ejaculates were collected with the help of artificial vagina at day 0 (day before start of feeding experimental rations), then 5th, 6th, 7th, 8th, 9th, 10th, 11th and 12th week of the experiment. These ejaculates were evaluated and qualifying ejaculates were processed for deep freezing. At least after 24 hours of storage in liquid nitrogen, three semen straws of each bull were thawed and evaluated manually for percent motility of spermatozoa, percent live spermatozoa, plasma membrane integrity, acrosome integrity, livability and absolute indices of livability. Statistical analysis of data revealed that supplementing ration with 125 ml (group A) and 250 ml (group B) of linseed oil resulted in significantly ($P<0.05$) higher post-thaw motile spermatozoa ($60.00\pm 0.91\%$ for each group) than control ($45.00\pm 0.58\%$) at the 12th week of treatment. Moreover, bulls of groups A and B showed significantly ($P<0.05$) higher number of live spermatozoa (72.00 ± 1.15 and $72.00\pm 2.04\%$) than that in control bulls ($64.00\pm 2.38\%$). However, differences in post-thaw motile and number of live spermatozoa between groups A and B were non-significant at the 12th week of treatment. Post thaw plasma membrane integrity and acrosome integrity of spermatozoa did not differ among bulls of the three groups. Spermatozoa of bulls fed linseed oil 125 ml showed significantly ($P<0.05$) higher duration of livability (14.4 ± 2.04 hours) than that in bulls fed linseed oil 250 ml (8.68 ± 1.15 hours) and in bulls kept as control (6.89 ± 1.73 hours) at the 12th week of treatment. Similarly, spermatozoa of bulls of group A showed significantly ($P<0.05$) higher absolute index of livability (272.50 ± 12.14) than that in bulls of group B (217.87 ± 9.11) and C (176.17 ± 12.14) at the 12th week of treatment; difference between the latter two groups was also significant ($P<0.05$). In conclusion, supplementation of feed with linseed oil (having Omega-3 fatty acids) improved post-thaw semen quality of Nili-Ravi buffalo bulls. Moreover, low (125 ml) and high (250 ml) levels of linseed oil showed almost similar results in terms of most parameters of post-thaw semen quality.

Keywords: Buffalo bull, semen, linseed oil, semen parameters



AGE RELATED CHANGES IN BODY WEIGHT, ORCHIDOMETRY AND TESTICULAR DYNAMICS IN AZIKHELI BUFFALO BULLS RAISED IN SWAT KHYBERPUKHTUNKHWA, PAKISTAN

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ABSTRACT

The study was conducted on Azikheli male buffalo, an indigenous dairy buffalo of Pakitan, in its home tract of Khwazakhela, District Swat, Pakistan, under traditional farming conditions to assess 1) non-invasive readily measurable traits such as age, body weight and testicular biometrics including scrotal circumference (SC), testicular weight (TW), average testicular length (AvgL), average testicular width (AvgW) and average testicular volume (AvgV); 2) histomorphological dynamics in testes and 3) correlation between different testicular parameters. A total of 80 Azikheli buffalo bulls of various age groups viz. G1 (<1.5 year), G2 (1.5-2.5 year), G3 (2.5 to 3.5 year), G4 (>4.5year), each comprised of 20 bulls per group were studied. Each group G1, G2, G3 and G4 had BW of 268.29±24.29 kg, 354.2±33.37 kg, 369.57±8.35 kg, 600.88±28.29 kg, SC of 15.47±3.82 cm, 28.70±1.61 cm, 31.2±2.28 cm, 35.02±1.38 cm, TW of 120.57±4.33 gm, 249.9±14.09 gm, 319.66±5.68 gm, 512±5.33 gm, AvgL of 4.69±0.46 cm, 6.27±.31 cm, 8.41±.34 cm, 9.32±.04 cm, AvgW of 2.35±0.37 cm, 3.09±.28 cm, 4.18±.04 cm, 4.87±.22 cm and AvgV of 63.14±16.52 cm³, 143.8±12.06 cm³, 1.4±3.97 cm³, 509.88±4.13 cm³, respectively. The tubule diameter and epithelial height increased with age, varied significantly among groups and was found to be different than those observed in Nili Ravi, Murrah and Swamp buffalo bulls at the same ages. Significant positive correlations (P<0.01) between body weights, scrotal circumference, age and testicular biometrics were observed. The results of this study proved that Azikheli buffalo bulls had superior reproductive characteristics and morphometric parameters compared with other breeds of buffaloes.

Keywords: Azikheli buffalo, orchidometry, histometry; testes

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EVALUATION OF SOYA LECITHIN BASED EXTENDER AS A SUBSTITUTE OF TRIS-EGG YOLK EXTENDER FOR THE CRYOPRESERVATION OF NILI-RAVI BUFFALO BULL SEMEN

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ABSTRACT

Cryopreservation of semen is used world-wide for its long-term storage and transportation to far-off places. Egg-yolk is commonly used to protect spermatozoa from adverse effects of cold shock during cryopreservation. Chemically defined media, free from products of animal origin, are gaining popularity for their use as cryoprotectants for deep freezing of semen. Therefore, the present study was undertaken to evaluate soya lecithin based extender as a substitute of tris-egg yolk for deep freezing of Nili-Ravi buffalo bull semen. In this study, ejaculates were collected from three adult buffalo bulls twice weekly for four weeks, using artificial vagina method. Ejaculates collected from three bulls on each collection day were pooled and diluted at 37°C in tris-egg yolk (control) or soya lecithin based ((Bioxcell) extender (treatment). Extended semen was cooled to 5°C, equilibrated for 2, 4 or 6 hours, filled in 0.5 ml straws, frozen and stored in liquid nitrogen for at least 24 hours. After thawing semen straws at 37°C for 30s, they were evaluated for post-thaw sperm quality parameters. Results revealed that sperm motility after freezing was lower ($P<0.05$) in soya lecithin based extender ($33.00\pm 1.64\%$) than tris-egg yolk extender ($40.84\pm 1.91\%$). Similarly, values for livability (4.98 ± 0.23 versus 5.69 ± 0.29 hrs) and livability index of spermatozoa (102.65 ± 8.63 versus 141.09 ± 13.10) were lower ($P<0.05$) for the former than the latter extender. Similar trends were recorded for percentages of live and morphologically normal spermatozoa. Moreover, all these quality parameters of frozen semen were better ($P<0.05$) for 6 hours of equilibration compared to 2 and 4 hours for both extenders. In conclusion, soya lecithin based extender may not be used as a substitute of egg-yolk for deep freezing of semen from Nili-Ravi buffalo bulls. Moreover, an equilibration period of 6 hours showed better results in terms of post-thaw semen quality parameters than 2 or 4 hours

Keywords: Soya lecithin, semen extender, tris-egg yolk extender

INTRODUCTION

Buffalo is the major dairy animal in many countries of the world, including Pakistan. Artificial insemination is the modern technique for rapid genetic improvement in the productive and reproductive performance of livestock, including buffalo, through discriminating the superior germ plasm of elite bulls. The maximum benefits from this technique can be achieved if the semen is stored for long time and transported to far-off places without damaging its fertilizing ability.

Cryopreservation of semen in liquid nitrogen has proven to be the best approach for long term storage of semen. It facilitates the regular supply of superior genetic material, as well as helps in building gene bank to conserve endangered species (Watson and Holt, 2001). However, deep freezing of semen causes harmful effects on sperm through the formation of intra-cellular ice crystals and oxidative stress, which damages sperm cell and can also affect the genome related organelle of the sperm (Isachenko, 2003). Although remarkable improvements have been made in the freezing protocols of semen from different species, quality of frozen semen in terms of sperm motility, viability and fertility is still poor when compared with that of the liquid semen (Kakar *et al.*, 2012). According to Gravance *et al.* (1998), frozen-thawed bull sperm exhibited reduced size of the head when compared with unfrozen sperm, perhaps pondering alternation to the protecting membrane structure.

For successful cryopreservation of semen, the ideal semen extender should have an energy source, anti-cold shock agents, compounds for maintaining osmotic pressure and pH and antibiotics (Vishwanath and Shannon, 2000). Different cryoprotectants have been used in the extender in order to protect spermatozoa from damaging effects of cold shock during deep freezing. Some of these include egg yolk and skimmed milk, alone or in combination with glycerol. However, these cryoprotectants are considered as a source of contamination of semen by microorganisms, which can damage the fertilizing ability of sperm. Egg yolk also has high viscosity and contains particulate debris, which can interfere with the microscopic examination of the sample and also reduce sperm fertility.

Different commercially available soya lecithin based preparations, such as Andromeda (MINITUB GMBH), Biociphos-plus (IMV, France) and Bioxcell (IMV, France) have been tried for cryopreservation of semen collected from different species, with varying results. These preparations are claimed to maintain post-thaw quality of semen and also yield reasonable fertility rates (Sansone *et al.*, 2001). The low density lipoprotein fractions are present in

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soya lecithin, which protect the sperm phospholipids integrity during deep freezing and thawing process (Moussa *et al.*, 2002).

This study was planned to investigate the comparative effectiveness of tris base egg yolk and soya lecithin (Bioxcell, IMV, France) extenders for the cryopreservation of semen collected from Nili-Ravi bulls. Attempts were also made to monitor the effects of different equilibration periods on the quality of semen following cryopreservation using these two extenders.

MATERIALS AND METHODS

Experimental Bulls and Semen Collection

In this study, three adult and clinically healthy buffalo bulls of the Nili-Ravi breed, donating semen of acceptable quality, were used. These bulls were maintained under standard farm conditions and ambient climate, with proper arrangements for protection against severe weather. They were offered good quality green fodder at the rate of 10% of body weight. Each bull was also offered 3-5 kg concentrate feed. Bulls had free access to clean drinking water, and physical exercise was also practiced for about one hour daily.

Semen Collection and Preliminary Assessment

Semen from each bull was collected twice weekly for four weeks by using artificial vagina (AV) method. One false mount was allowed to each bull for proper sexual stimulation before semen collection. In total, 24 samples were collected from three bulls during four weeks, with eight samples from each bull.

Immediately after collection, semen ejaculates were placed at 37°C in water bath and examined for colour, volume, sperm motility and sperm cell concentration. Colour and volume were recorded directly from the semen collection vials. For estimation of sperm motility, a drop of semen and 2.9% sodium citrate were placed on the warm slide (37°C), covered with a cover slip and examined under microscope. Spermatozoa progressively moving in straight line were taken as motile, while those oscillating at a point or moving in different directions or no moving at all were taken as immotile. A digital photometer was used at 546nm wavelength for sperm cell concentrations. Ejaculates with thick creamy colour and showing at least 70% progressively motile spermatozoa were selected for further processing.

Extension and freezing of Semen

The semen samples were diluted with tris-citrate-fructose-egg yolk-glycerol (control) and soya lecithin based (Bioxcell) extender (treatment) at 37°C. Then samples were filled in 0.5 ml French straws, cooled to 4°C in a cooling cabinet and kept at that temperature for 2, 4 or 6 hours for equilibration. After equilibration, the semen straws were frozen by fast method and stored in liquid nitrogen.

Post-thaw Examination of Semen

Semen straws were stored in liquid nitrogen for least 24 hours. Then straws were picked from liquid nitrogen, thawed at 37°C for 30 seconds in water bath and examined for sperm motility, livability, livability index, live sperm percentage and sperm morphology. For the assessment of sperm motility, a drop of thawed semen was examined under microscope. For determination of livability, sperm motility was observed at hourly intervals till the death of last sperm. Absolute livability index was computed as described by Melovenof (1962). Eosin-nigrosin staining was used for recording percentage of live sperm and those with various abnormalities of the head, mid-piece and the tail.

Statistical Analysis

Mean values (\pm SE) were calculated for different post-thaw semen quality parameters for various treatments. The magnitude of variation in these parameters among different groups was ascertained through two-way analysis of variance. Duncan's Multiple Range test was applied for comparison among different means, where necessary. Pearson's correlation coefficients among various parameters were also computed.

RESULTS

Comparison Between Extenders

The effects of tris-egg yolk and soy lecithin based extenders on post-thaw semen quality parameters of buffalo bulls are given in Table 1. It shows that the mean sperm motility in tris-egg yolk based extender ($40.84 \pm 1.91\%$) was significantly higher ($P < 0.05$) than $33.00 \pm 1.64\%$ recorded for the soy-lecithin based extender. Similarly, the tris-egg yolk based extender exhibited higher ($P < 0.05$) mean value of livability of spermatozoa compared to soy-lecithin

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based extender. A similar trend was seen for the livability index of spermatozoa and percentage of live sperm. However, significantly higher ($P<0.05$) percentage of sperm with abnormal heads was recorded in semen samples frozen in soy lecithin extender than those frozen in tris-egg yolk extender. A similar trend was seen for the sperm with mid-piece or tail abnormalities (Table 1).

Table 1. Effects of two extenders on semen quality parameters (Values are means \pm SE)

Parameters	Extenders	
	Tris-egg yolk (control)	Soy lecithin (Treated)
Sperm motility (%)	40.84 \pm 1.91A	33.00 \pm 1.64B
Sperm livability (hrs)	5.69 \pm 0.29A	4.98 \pm 0.23B
Livability index	141.09 \pm 13.10A	102.65 \pm 8.63B
Sperm head abnormalities (%)	6.87 \pm 0.60B	8.60 \pm 0.66A
Mid piece abnormalities (%)	4.79 \pm 0.63B	6.30 \pm 0.64A
Tail abnormalities (%)	9.13 \pm 0.82B	9.99 \pm 0.75A
Live spermatozoa (%)	53.72 \pm 1.88A	49.64 \pm 1.59B

A,B: Values with different letters for each parameter differ significantly ($P<0.05$).

Comparison of Equilibration Periods

Table 2 shows the effects of equilibration periods of 2, 4 and 6 hours on mean values (\pm SE) for various parameters of semen quality of buffalo bulls. Sperm motility was highest for equilibration period of 6 hrs, while it was lowest for 2 hrs equilibration period. Statistical analysis revealed that the effects of different equilibration periods on sperm motility was significant ($P<0.05$). Further analysis by DMR showed that post-thaw sperm motility differed significantly among all three equilibration periods.

A similar trend was recorded for the livability, livability index of spermatozoa and percentage of live sperm; the mean values for all these parameters differed significantly among three equilibration periods ($P<0.05$), being highest for 6hrs and lowest for 2 hrs equilibration. However, when sperm with abnormal head, mid-piece or tail were considered, the highest mean values were noted for 2 hrs, while the lowest values were for 6 hrs of equilibration ($P<0.05$).

Table 2. Effects of three equilibration periods on semen quality parameters (Values are means \pm SE)

Semen quality parameters	Equilibration period (hrs)		
	2	4	6
Sperm motility (%)	31.09 \pm 1.58C	36.64 \pm 1.72B	43.02 \pm 2.17A
Sperm livability (hrs)	4.45 \pm 0.15C	5.40 \pm 0.20B	6.15 \pm 0.24 A
Absolute index of livability	86.81 \pm 5.59C	119.74 \pm 8.99B	159.07 \pm 13.3A
Sperm head abnormalities (%)	9.94 \pm 0.47A	7.66 \pm 0.35B	5.61 \pm 0.37C
Mid piece abnormalities (%)	7.63 \pm 0.49A	5.62 \pm 0.30B	3.40 \pm 0.37C
Tail abnormalities (%)	12.32 \pm 0.27A	9.36 \pm 0.30B	7.00 \pm 0.31C
Live spermatozoa (%)	45.69 \pm 0.83C	52.05 \pm 1.00B	57.32 \pm 1.36A

A,B,C: Values with different letters for each parameter differ significantly ($P<0.05$).

Correlation Matrix

Data on correlation coefficients among different parameters of semen quality (Table 3) revealed that sperm motility, livability, livability index and percentage of live sperm were significantly ($P<0.01$) and positively correlated with one another, while all these parameters showed significant negative correlation with morphologically abnormal sperm (head, mid-piece and tail). Moreover, significant positive correlations were seen among sperm with different morphological abnormalities.

Table 3. Pearson's correlation coefficients among various semen quality parameters

Sperm motility	Livability	Livability index	Abnormal head	Abnormal mid piece	Abnormal tail	Live sperm (%)



Sperm motility	1					
Livability	0.707**					
Absolute livability index	0.856**	0.837**				
Abnormal head	-0.812**	-0.741**	-0.858**			
Abnormal mid piece	-0.789**	-0.705**	-0.832**	0.871**		
Abnormal tail	-0.723**	-0.700**	-0.781**	0.829**	0.826**	
Livesperm (%)	0.759**	0.725**	0.832**	-0.809**	-0.762**	-0.745**

**Significant (P<0.01).

DISCUSSION

Egg yolk is commonly used as a cryoprotective agent in extenders currently in practice for the cryopreservation of semen from different animal species. Low density lipoproteins present in the egg yolk protect spermatozoa from adverse effects of cold shock (Shahverdi *et al.*, 2014). However, because of sanitary risks with the use of egg yolk, scientists are trying to replace it with some other cryoprotectant of non-animal origin. Therefore, one objective of this study was to investigate whether egg yolk based extenders can be replaced by the soya lecithin based extender (Bioxcell) for the deep freezing of Nili-Ravi buffalo bull semen. Results of the present study revealed that post-thaw quality (in terms of sperm motility, livability, livability index, percentages of live and morphologically normal sperm) of semen samples collected from buffalo bulls and frozen using soya lecithin was significantly lower compared to the quality of ejaculates frozen in egg yolk based extender. These results are supported by those of Van-Wagtendonk-de Leeuw *et al.* (2000), who reported significantly higher post-thaw sperm motility for tress-egg yolk extender (41.9±0.5%) than soya lecithin based extender (39.2±0.5%) following cryopreservation of bovine semen. Similarly, in their study on ram semen, Sharafi *et al.* (2009) found significantly lower post-thaw percentage of live sperm in soya lecithin extender compared to tris-egg yolk extender (47.4±2.88 vs. 49.1±3.4%). However, Akhter *et al.* (2010) could not observe any significant difference in post-thaw sperm motility following cryopreservation of semen in tris-egg yolk and soya lecithin (Bioxcell) extenders. Thus, the results of the present and previous studies suggest that soy lecithin based extender may not be used as a substitute of egg yolk based extenders for the cryopreservation of the buffalo bull semen.

Previous studies have demonstrated that seminal plasma of male goats contains semen specific lipase enzymes, which may interact with egg yolk lipids, resulting in toxic effects on the sperm. When egg yolk is used in the semen extenders, removal of seminal plasma (washing) through centrifugation is usually recommended to avoid harmful effects of lipase enzymes (Dorado *et al.*, 2009; Dorado *et al.*, 2010). However, washing of sperm is a cumbersome and time consuming process. Alternately, use of the soy lecithin based extender (Bioxcell) may be used as a substitute of egg yolk for deep freezing of the buck semen (Roof *et al.*, 2012).

Besides composition of the extender, quality of frozen semen has also been shown to be affected by equilibration period, during which sperm adapt themselves to the extender before undergoing freezing. Therefore, the second objective of this study was to compare quality of Nili-Ravi buffalo bull semen subjected to three equilibration periods (2 hrs, 4 hrs and 6 hrs) before freezing. Results revealed that the quality of frozen semen was improved as the equilibration period was increased from 2 to 6 hrs for both experimental extenders. These results are supported by those of Rickenbacher *et al.* (2009), who tried equilibration times of 1.5, 3.0, 4.5, 6.0 and 24.0 hrs and recorded best results in terms of semen quality for 6 and 24 hrs. Similarly, Shahverdi *et al.* (2014), employed equilibration periods of 2, 4, 8 and 16 hrs for freezing buffalo bull semen and observed low quality of semen (in terms of progressive motility, plasma membrane integrity, and intact epical ridge of sperm) with 2 hrs compared to other periods. However, these workers did not observe any difference in sperm DNA integrity among different periods. According to Eriani *et al.* (2017), efficacy of equilibration period can vary with the nature and amount of cryoprotectant used in the extender. These workers observed best results for 3 hrs equilibration when the extender contained 60 mM lactose with 7% glycerol, while equilibration time of 4 hrs yielded best results when extender had 120 mM lactose with 7% glycerol as cryoprotectants.

This indicates that increasing the exposure time of spermatozoa to the cryoprotectants before freezing minimizes the harmful effects of cold shock. According to Anzara *et al.* (2011), long equilibration period allows a cryoprotectant to interact with the sperm plasma membrane, enabling the sperm to withstand effects of low temperatures. The longer equilibration time can also enhance the efficiency of a busy semen unit, where large



number of ejaculated are collected each day for processing (Shahverdi *et al.*, 2014). Under such conditions, after collection and preliminary processing, ejaculates can be left for equilibration and freezing can be carried out the next morning.

Based on the results of the present study, it can be concluded that soya lecithin based extender may not be used as a substitute of egg-yolk for cryopreservation of Nili-Ravi buffalo bull semen. Moreover, an equilibration period of 6 hours showed better results in terms of post-thaw semen quality parameters than 2 or 4 hours.

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CLINICAL OUTCOME OF CORRECTION OF UTERINE TORSION IN PLURIPAROUS BUFFALOES AT FIELD LEVEL

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ABSTRACT

Total 30 buffaloes field cases of uterine torsion were studied for the outcome of treatment initially treated by veterinarian and non-veterinarian. For this study animals were divided into 3 groups. Group 1 comprised fresh cases and correction with Schaffer's method was done by veterinarian within 36 hrs of onset of sign of torsion. Group 2 comprised those cases which were tried to distort within 36 to 72 hrs by veterinarian but failed and cesarean section was done. Group 3 comprised of all neglected cases of uterine torsion where torsion was initially treated by non-veterinarian unsuccessfully and later attended by veterinarian after 36 hrs of onset of signs of torsion. In Group 3 either correction by Schaffer's method or cesarean was done. The overall dam survival rates in the three respective groups were 100%, 100%, and 45.5%, respectively. The overall fetal survival rates in three respective groups were 100%, 0%, and 0%, respectively. The higher incidence of uterine torsion was recorded of right sided (96.66%). In Group 3, vaginal evisceration of small intestine, uterine rupture, severe laceration of vaginal wall, unable to diagnose side of torsion and incorrect method of rolling were recorded in 9%, 4.5%, 22.7%, 100% and 100% of cases, respectively. In conclusion, quick approach, right diagnosis and early execution of right type of treatment by veterinarian for torsion helped in better survival rate to dam and fetus. The Schaffer's method was the best method in fresh and unspoiled cases of uterine torsion, and the cesarean section was the best if rolling failed to correct torsion.

Keywords: Uterine torsion, pluriparous buffaloes, uterine rupture, Schaffer's method

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BREED IMPROVEMENT THROUGH INTENSIVE LOCAL SELECTION OF INDIGENOUS BUFFALOES FOR FOOD AND NUTRITIONAL SECURITY AND LIVELIHOOD IMPROVEMENT IN MID-HILLS OF NEPAL

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ABSTRACT

Buffalo is the first choice animal in Nepal. Buffalo farming is the integral part of agricultural system especially in Mid-hills and Terai regions. Though the percentage of improved breed of buffaloes is increasing year after year, there are still more than 65% local buffaloes which are very valuable breeds that have been rearing by the farmers especially in rural areas of Mid-hills. Among the local buffaloes, Lime and Parkote are still popular for their production parameters and inherent capacity to withstand in the harsh environmental conditions and low profile of feeding regime. Directorate of Livestock Production (DoLP) under the Department of Livestock Services (DLS), Nepal has been giving high priority for proper conservation, promotion and utilization of indigenous buffaloes. However, very limited research works have been done in these buffaloes in the farmers' field conditions. In this context, 35 Lime and Parkote buffaloes from Northern Arghakhanchi were purposively selected for their production performance analysis. The research work at village conditions was carried out from September 2016 to August 2017. The findings of this research work revealed that the average lactation milk yield of Lime was 1615±205 liter and that of Parkote was 1800±215 liter. Other production parameters for Lime and Parkote buffaloes, e.g., age at first service, lactation period, dry period, average daily milk production, calving interval, calf weaning period, economics of production for two breeds have been studied in details. There was no significant difference ($P>0.05$) for these parameters between these two breeds. The study also revealed that there is high potentiality of breed improvement through intensive local selection among the local breeds of buffaloes for food and nutritional security as well as for the livelihood improvement of the farmers in Nepal.

Keywords: Breed improvement, buffalo, lime and parkote, local selection, production performance

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BUFFALO MASTITIS IN DAIRY PRODUCTION: TECHNOLOGICAL ADVANCES IN UNDERSTANDING OF MASTITIS

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ABSTRACT

The high-yielding Murrah buffalo is the Holstein-Friesian of the buffalo world. The Indian dairy sector is growing very fast and undergoing major changes to meet the higher demand of milk and milk products with the pressure of quality control. Our target is to achieve the goal of about 180 million ton milk production per annum by the year of 2020-2021. India stands at the first position in milk production but still have many challenges to improve “milk quality” as per Food Safety and Standards Authority of India (FSSAI). The production of buffalo milk in the Asian-Pacific region exceeds 45 million tons annually of which over 30 million tonnes are produced in India alone. Mastitis is one of the major causes of reduced milk quality, which need special attention of scientists and veterinarians to control at an early stage by an early diagnosis and treatment. Mastitis is a costliest disease of dairy animals and it denotes as inflammation of mammary gland which adversely affects the mammary parenchyma and milk quality. However, prevalence of mastitis in buffaloes is comparatively low (27.36% to 45.00%) than the cows (29.34% to 78.54%). Buffaloes possess a powerful defense mechanism against mastitis than cows due to their tight teat sphincter (Smooth muscles), more keratin content in teat canal and long narrow teat canal, which can be expected to effectively prevent micro-organisms from invading the udder. We infected mammary epithelial cells (MECs) with *S. aureus* and *E. coli* and treated with guanine riboswitch ligand analog and also studied expression of various pro-inflammatory cytokines and anti-inflammatory cytokines in infected MECs. Riboswitch ligand showed as potential antibacterial activity against both bacteria. In conclusion, it is time to understand mastitis at molecular level with technological advances and find out most suitable antibiotic alternatives. Blind antibiotic therapy in mastitis often results in the presence of antibiotic residues in milk rendering it unsuitable for human consumption or further processing.

Keywords: Buffalo, mastitis, mammary epithelial cells, treatment

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EVALUATION OF MASTITIS RELATED MEASURES & THEIR APPLICATIONS TO CLASSIFY BUFFALO MILK IN CHITWAN, NEPAL

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ABSTRACT

A study was performed to evaluate the epidemiological aspects of buffalo mastitis in the District Chitwan, Nepal for characterizing the California mastitis test (CMT), somatic cell count (SCC), electrical conductivity (EC) values, and bacteriological analysis for defining buffalo milk. The maximum number (16%) of clinical cases of mastitis were observed in the month of July and lowest in the month of April (1.6 %). when the temperature and humidity increased indicating that there is need for better care of lactating buffaloes during this month. On a quarter basis, 16% of the foremilk samples in buffaloes were diagnosed as having subclinical mastitis and 11% were diagnosed as having clinical mastitis. The results of CMT scores and SCC showed the evidence that subclinical and clinical mastitic milk were having CMT positive scores (+1~+3) with $\geq 200 \times 10^3$ cells/ml. The mean pH of clinically normal buffalo milk was 6.75 (range 6.39 to 7.08) and subclinical mastitic and clinical mastitic milk was 6.85 (range 6.37 to 7.10) and 6.88 (range 6.41 to 7.20), respectively. Analysis of EC value in the milk revealed the presence of mastitis in buffaloes and the cut-off values was 3.7 mS/cm. The coagulase negative Staphylococcus (CNS) such as *S. albus* and *S. epidermidis* were the predominant organisms associated with subclinical mastitis, and CNS and Coliforms in clinical mastitis. Minor pathogens such as environmental Streptococci and Coliforms were often isolated from the clinically normal mammary gland. This information suggests that environmental mastitis was prevalent in buffaloes of Chitwan District. In this study, 9.5% of the quarters were having bacterial count (BC) more than 250 cfu/ml. The proposed criteria for normal milk are absence of clinical signs, CMT negative, $SCC < 200 \times 10^3$ /ml, $EC < 3.7$ mS/cm and < 250 cfu/ml bacteria. The parameters for defining subclinically mastitic milk are absence of clinical signs, CMT positive, $SCC \geq 200 \times 10^3$ /ml, $EC > 3.7$ mS/cm and > 250 cfu/ml bacteria. Similarly, Clinical mastitic milk was defined as milk having presence of clinical signs, CMT positive, $SCC \geq 200 \times 10^3$ /ml, $EC > 3.7$ mS/cm and bacterial count > 250 cfu/ml.

Keywords: Somatic cell count, electrical conductivity, bacterial count, buffalo milk

INTRODUCTION

The economic losses of mastitis due to severe drop of milk production, potential health risks for other animals and human beings, increased cost of treatment and culling processes are tremendous (Dhakal and Thapa, 2002). In addition to economic losses to the farmers, an effective control of mastitis is also important from the consumer's and processor's point of view, because of the milk from affected animals may harbor organisms potentially pathogenic to humans and the processing of such milk results in substandard fermented products (Bilal et al., 2004). Milk and milk products play vital role in human nutrition but they are also an ideal medium for growth and transmission of various pathogens.

Buffalo milk contains less water, more total solids, more fat, slightly more lactose, and more protein than cow's milk. It seems thicker than cow's milk because it contains more than 16% of the total solids compared with 12 to 14% per cow's milk. A high somatic cell count (SCC) affects milk quality and cheese making resulting in a reduction in cheese yield and quality (Piccini et al., 2006). Higher SCC in buffalo milk causes a cheese softer and more brittle, which may be associated with an increase in moisture content. With a high SCC, buffalo milk has an alkaline pH and therefore, the renneting time increases many folds. High SCC does not support growth of starter organisms thereby making it unfit for preparing fermented products (Schultz, 1977).

The mastitis related measures such as California Mastitis Test (CMT), SCC, electrical conductivity (EC) and bacterial findings are needed for defining the buffalo milk. The requirement to define mastitis is to use at least two parameters describing the causative agents and the second parameter describing the inflammation. Studies on the occurrence of mastitis in buffaloes have been based on different tests and parameters, however, with unclear criteria for defining normal and mastitic milk, the results are difficult to interpret in buffaloes. So far normal threshold value of somatic cells and the cut-off value of EC in buffalo milk have not been evaluated.

The present study was designed to ascertain the epidemiological and bacteriological analyses of buffalo mastitis in Chitwan District of Nepal, characterize normal, subclinical and clinical mastitic milk using the possible parameters expressing hygienic quality of buffalo milk; In addition, the study was performed to define the threshold

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values of the CMT, SCC, EC and bacteriological counts in the milk, and to develop the diagnostic criteria for classification of milk from buffaloes

MATERIALS AND METHODS

Isolation of Bacteria and Bacterial Count

One hundred μ l of milk from each quarter was streaked onto MacConkey and 5% ovine blood agar plates for bacterial culture and isolation. The colonies were counted after 48 hours of incubation at 37°C. Pure colonies from the respective plates were identified on the basis of Gram's stain, morphological findings, colony characteristics and biochemical tests (Cowan and Steel, 1993).

California Mastitis Test (CMT)

The CMT method was carried out according to the method as described by Schalm and Noorlander, (1957).

Somatic cell count (SCC)

The SCC and differential leucocyte counts were determined by Prescott and Breed method as described by Schalm et al. (1971). Milk was mixed thoroughly before testing. Ten μ l of milk from each quarter were spread over 1 cm² marked square area on a glass slide. The milk film was left at room temperature until dry. The slide was then fixed in methanol for 5 minutes, dried, held in the Newman - Lampert stain for 2 minutes, dried at room temperature, and then washed in tap water 3 times and distilled water 2 times before a final drying at room temperature (Dhakal, 2006).

Somatic cells were counted under 1,000 \times magnification using oil immersion. The total number of fields counted was 50. The working factor (WF) was $400,000/50 = 8000$. The SCC /ml = $8000 \times$ the number of cells counted (Dhakal, 2006).

Electrical Conductivity (EC)

A manual digital mastitis detector (Milk checker N4 Oriental Instruments Ltd, Tokyo, Japan) was used for measurement of electrical conductivity in milk. EC in milk was expressed as millisiemens per centimeter (mS/cm) at 25°C. Absolute EC score was defined as the reading obtained directly from the EC meter and differential EC score was the difference between highest and lowest absolute EC score for the 4 quarters of each animal (Musser et al., 1998).

Statistical Analysis

CMT scores and mean SCC were analyzed using the Student's *t* – test. Inter-quarter comparisons of clinically normal and subclinical mastitic buffaloes were analysed using χ^2 tests.

RESULTS

Comparison of Clinical Mastitis

The maximum number of cases of clinical mastitis was observed in the month of July (16%) followed by June (12.2%) and August (13.5%). Results showed that 37.3% of buffaloes had clinical mastitis during the summer season followed by the autumn season (31.7%). The month from June to August have the highest rainfall in Chitwan District, when the average temperature was 30°C (Figure 1).

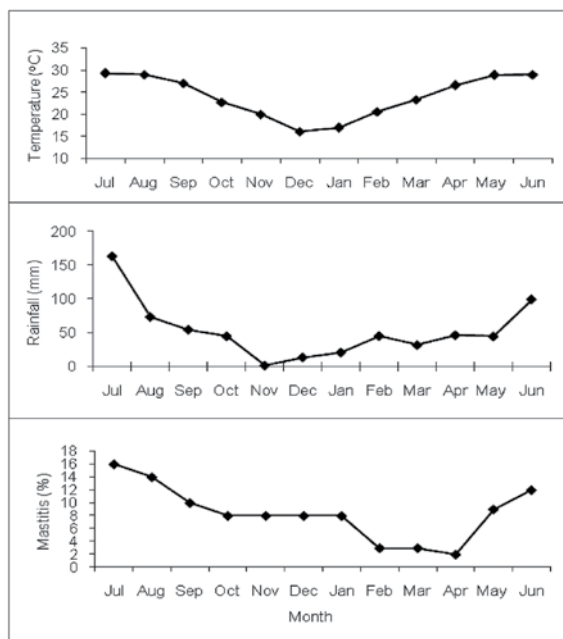


Figure 1. Comparison of clinical mastitis in buffaloes with mean temperature and rainfall for 4 years. Data for temperature and rainfall were from Bharatpur Municipality, Chitwan District, Nepal

In clinical mastitis, CNS (36%) and Coliforms (11%) were the most commonly isolated pathogens (Figure 2).

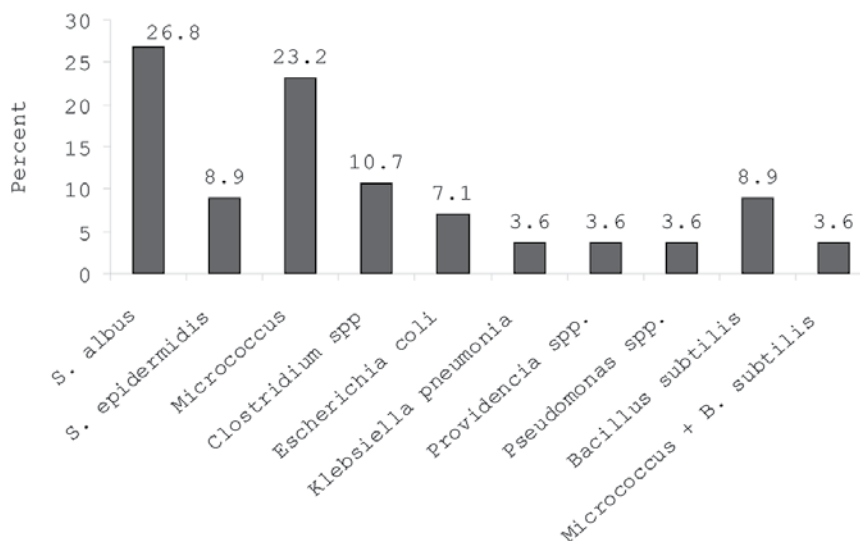


Figure 2. Occurrence of microorganism in 56 isolates from the quarter milk of buffaloes with clinical mastitis

CMT and pH

Out of 188 quarter samples tested, 9% had CMT positive scores in normal buffaloes (Figure 3). CMT scores in the foremilk revealed 6% of the trace reactions in the quarter milk samples. Nearly 85% quarters had CMT negative scores.

Mean values of pH were highly significant ($P < 0.01$) for CMT scores 2+ and 3+ groups compared with normal groups (Figure 3). The normal pH of buffalo milk was $\text{pH } 6.75 \pm 0.14$ at 25°C ranging from 6.39 to 7.08. The pH of subclinical mastitic and clinical mastitic milk was 6.85 ± 0.14 and 6.88 ± 0.24 , respectively.

The SCC in subclinical and clinical mastitis were significantly higher ($P < 0.01$) than normal milk. Mean SCC in normal, subclinical and clinical mastitic milk were 176 (range 30 to $800 \times 10^3/\text{ml}$), 958 (300 to $6,500 \times 10^3/\text{ml}$) and $6806 \times 10^3/\text{ml}$ (250 to $27,950 \times 10^3/\text{ml}$), respectively (Figure 4).

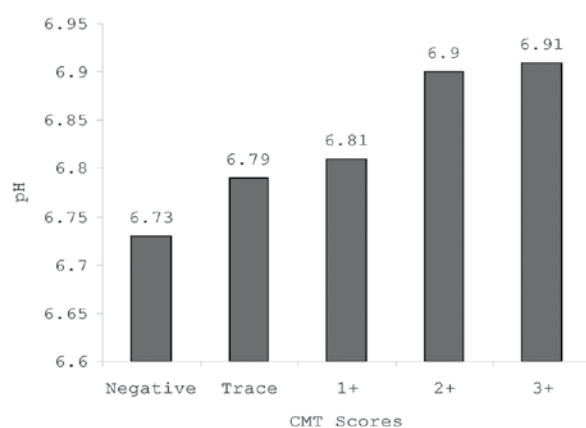


Figure 3. Percentage distribution and relationship of CMT scores and mean pH of milk in 188 clinically normal buffaloes

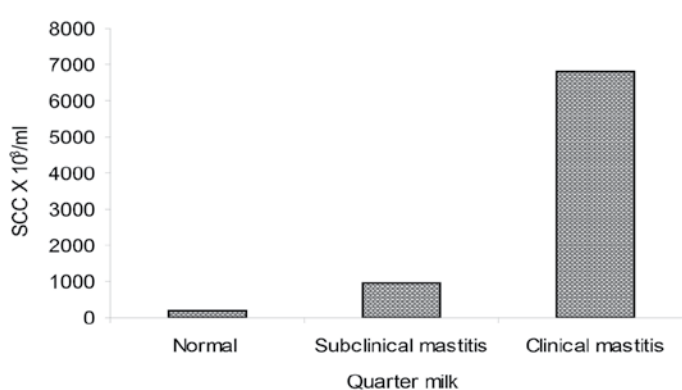


Figure 4. Comparison of mean SCC × 10³/ml of milk in normal, subclinical and clinical mastitis

Relationship between EC and SCC

As shown in Table 1, ROC analysis revealed that the best trade-off between sensitivity and specificity for diagnosing mastitis in buffalo from EC score occurred at the cut-off value of 3.7 (3.9 for clinical). The cut-off score 2.1 was required for 100% sensitivity, and 5.2 was required for 100% specificity in all cases, which would severely affect diagnostic accuracy (specificity and sensitivity) of the test.

Table 1. Sensitivity, specificity, likelihood ratios, and predictive values of EC test for mastitis detection

Category of mastitic milk	Cut-off score	Sensitivity (95% C.I.)	Specificity (95% C.I.)	+ LR	- LR	PPV	NPV
Sub clinical	3.7	73.3 (44.9-92.0)	59.2 (50.6-67.3)	1.8	0.5	16	95.5
Clinical	3.9	85.7 (63.6-96.8)	75.4 (67.4-82.2)	3.5	0.2	34	97.3

C.I. = Confidence Interval, + LR, - LR = Positive Likelihood Ratio, Negative Likelihood Ratio, PPV, NPV = Positive Predictive Value, Negative Predictive Value

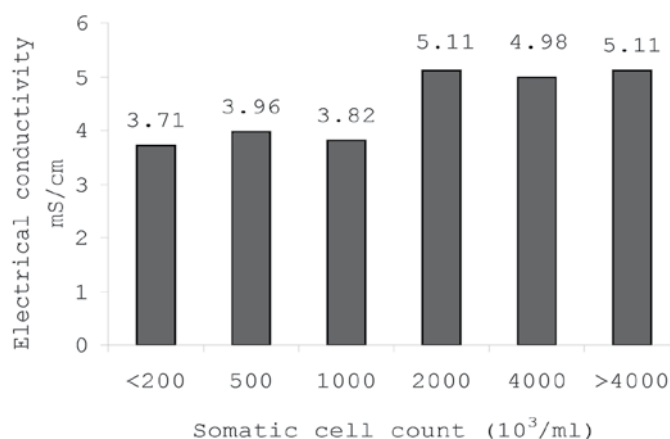


Figure 7. Relationship between SCC and EC in 188 quarter milk of buffaloes

In normal milk, EC values and SCC/ml are positively correlated ($r=0.05$). The milk electrical conductivity was found higher when SCC/ml of milk was increased (Figure 5). There was a relationship between individual quarter milk conductivity and occurrence of mastitis.

Classification of Buffalo Milk

Normal milk was defined as having $SCC \leq 200 \times 10^3/ml$, CMT negative, bacterial count <250 cfu/ml and the absence of clinical signs (Table 2).

Table 2. Classification of normal, subclinical, and clinical mastitic milk using 5 parameters

Parameters Classification	SCC/ml	CMT	EC mS/cm	BC* cfu/ml	Clinical signs
Normal	< 200,000	Negative	3.7	< 250	Absent
Subclinical	> 200,000	Positive	> 3.7	> 250	Absent
Clinical	> 200,000	Positive	> 3.7	> 250	Present

*BC = Bacterial count

DISCUSSION

Bacteriological Analysis

The highest incidence of clinical mastitis was found in the summer season in Chitwan District, Nepal and the most frequently isolated bacteria were CNS and Coliforms. This finding was consistent with that of Moroni et al. (2006) who reported that CNS were the most common pathogen (66% of the positive samples) isolated in dairy buffalo milk in northern Italy. The greatest number of clinical cases of mastitis was observed in the month of July. The reason for the high incidence of clinical mastitis during this month may be associated with not only heat stress but poor hygienic conditions found in buffaloes during high temperature and humidity in the summer season. In addition, this period is conducive for proliferation and easy access of pathogenic organism into the mammary gland of buffaloes for precipitating the disease (Taraphder et al., 2006).

Minor pathogens and Coliforms are often isolated from the skin of the udder due to contamination with soil and feces. The challenges in buffalo farming include poor hygiene in the environment, contaminated housing, inadequate milking hygiene, hand milking of wet teats, organic bedding materials (chopped straw), calving and milking in the same shed, and calf suckling.

Coagulase positive *Staphylococcus* spp. were primary pathogen or major pathogen where as CNS and *Micrococcus* were secondary pathogen or minor pathogens. *Staphylococci* are the most problematic and significant mastitis pathogens. Faulty milking encourages the transfer of bacteria into the teat cistern (Dhakal, 1997). *Staphylococci* typically colonize the broken skin and can enter the udder through abrasions of the teat end increases the risk of *Staphylococci* colonization at the teat end and subsequent transfer into the udder. Characteristic findings in buffalo mastitis in Chitwan, Nepal revealed that clinical mastitis was influenced by season, parity, and stage of lactation.



CMT, pH and SCC

It is generally accepted that mastitis causes a rise in milk pH. This is primarily due to leakage of blood bicarbonate into milk following damage to mammary epithelium. The increasing pH decreases significantly the activity of enzymes used to clot the milk, thus having implications in the manufacturing potential of milk for dairy products. The average pH of normal buffalo milk was 6.75, and was similar to that reported by Silva et al. (1995) in Sri Lanka showing 6.5 (6.1 to 7.0). There was a positive relationship between milk pH and EC in normal, subclinical and clinical mastitic milk. Lactose is the main determinant for pH and is the most important osmotic component of the milk. If lactose concentration falls in the udder during mastitis then the sodium and chloride level increases ten times to maintain the osmotic pressure of the milk (Kitchen, 1981).

In clinically affected buffaloes, 45% of the quarters were found CMT positive. This indicates that the greater number of somatic cells was migrated in the milk. In this study, 27% of the quarters having CMT positive scores were found bacteriologically negative indicating nonspecific clinical mastitis, i.e. latent mastitis where SCC value was also increased.

The mean SCC in different categories of CMT in buffalo milk revealed that a negative score for normal milk had $134 \times 10^3/\text{ml}$. In the present study, 9.57% of the quarters had CMT positive scores in healthy buffaloes, which is similar to the finding of Praveen et al. (2001) i.e. 6.86 to 10.34%. In contrary to the present findings, Patil et al. (2005) reported a higher prevalence rate of CMT positive quarters, i.e. 28.3%. Such variations in CMT result may be due to varied managemental and hygienic practices in different farms under village conditions. Somatic cell counting has become one of the recommended tests in bulk milk analysis as an indicator of mastitis because a major factor responsible for a high SCC is udder infection due to pathogenic organisms.

The study of SCC at the buffalo farms in Chitwan and Hissar gave an upper limit of $151 \times 10^3/\text{ml}$ in the buffalo milk being based on the mean \pm 2SD of a total of SCC (Dhakal, 2006). Total SCC includes leucocytes (such as neutrophils, lymphocytes and macrophages) and epithelial cells. Therefore, the upper limit was defined to be $200 \times 10^3/\text{ml}$ for practical diagnostic purpose. In contrast to the present finding, lower average SCC such as $136 \times 10^3/\text{ml}$ in July to August, $108 \times 10^3/\text{ml}$ in May to June and $76 \times 10^3/\text{ml}$ in December to January were reported in Indian Murrah buffaloes (Singh and Ludri, 2001). However, in Sri Lanka, it is reported that total SCC in normal buffalo milk varied from 50×10^3 to $375 \times 10^3/\text{ml}$ of milk (Silva et al., 1994). Possible reasons for higher SCC in normal buffaloes may be due to various factors such as compositional changes of the milk, environmental stress and the quarter udder capacity of buffaloes, as found in dairy cows (Moroni et al., 2006). This threshold level of $200 \times 10^3/\text{ml}$ of milk for the SCC may be the standard value for detection of subclinical mastitis in buffaloes in the future.

The SCC in the quarter milk of clinically normal buffaloes in Rampur, Nepal and surrounding area was $176 \pm 132 \times 10^3/\text{ml}$. The SCC reported for normal buffaloes in Brazil was $> 200 \times 10^3/\text{ml}$, i.e. $(63.6 \pm 185.7 \times 10^3 \text{ cells}/\text{ml})$ (Munoj et al., 2006). The SCC of healthy buffalo milk was $100 \times 10^3/\text{ml}$ (76 to $135 \times 10^3/\text{ml}$) in India (Singh and Ludri, 2001). It is known that type of breed, husbandry, age, parity, injury of the udder and stage of lactation affects the SCC in milk (Singh and Ludri, 2001). The SCC of normal milk of Murrah buffalo in Chitwan, Nepal and Hissar, India was $< 200 \times 10^3 \text{ cells}/\text{ml}$ (Dhakal, 2006).

EC Values

EC values in normal, subclinical and clinical mastitic milk were found to be 3.76 mS/cm, 3.93 mS/cm, and 5.34 mS/cm, respectively. The best trade-off between sensitivity and specificity for diagnosing mastitis in buffalo from EC score was found at the cut-off value of 3.7 mS/cm.

Mastitis can lead to damage of mammary epithelium, which increases the release of electrolytes such as sodium and chloride. Increases in the sodium and chloride content of mastitic milk has also been correlated with an increase in EC (Vallejo and Carreira, 1985). The EC value in clinically normal buffalo was 3.72 mS/cm. Silva et al. (1995) detected the EC in buffalo milk and reported a slightly higher EC value of 3.86 mS/cm in normal milk and 4.46 mS/cm in subclinical mastitic milk. In contrary to this, Thomas et al. (2004) reported the variation of EC in normal milk of cows from 4 to 5.5, and the variation of the quarters within the same cow with normal milk was 0.5 mS/cm.

The EC score in buffalo milk was comparatively lower than that of cow milk. This may be associated with higher fat content (7.5%) in buffalo milk composition as suggested previously, i.e. milk fat concentration increases, EC decreases (Nielen et al., 1992). Fat is a poor conductor and fat globules form a physical hindrance to migrating ions and reduce the volume of conducting medium. Increases in the sodium and chloride content of mastitic milk have also been correlated with an increase in EC (Wagner and Stull, 1978).

The EC values increased in association with increasing SCC, bacteria in the milk and flakes or clots in the milk of buffaloes. Milk infected with coliforms has a significantly higher ($P < 0.05$) EC than that of CNS. Milner et al. (1996) reported that the presence of bacteria, changes in SCC and clots in milk are the common factors resulting in changes in EC of milk. The correlation between SCC and EC was $r = 0.53$ in buffalo mastitic milk. Similar value



($r=0.4$) in cows was reported by Sheldrake et al. (Sheldrake et al. 1983). Although an increase in the EC of milk at the quarter level or animal level indicates subclinical and clinical mastitis, the correlation between EC and SCC was not very high (Hogeveen et al., 1998). Since there is little effect from the EC in quarters free of infection (Sheldrake et al., 1983), EC may have an advantage of defining infection status. Despite the effect of several factors on the EC of milk, only intramammary infection is likely to influence EC of individual quarters (Schultz, 1986).

Acceptable levels of sensitivity and specificity of tests will depend upon the purpose of the test, the setting of testing (e.g. for general population or for a specific subgroup at risk for the condition), the prevalence of the condition in the group being tested, alternative methods of assessment, and costs and benefits of testing. The cut-off score found by ROC curve analysis is the value corresponding with the highest accuracy. However, when a test is used for the purpose of screening, a cut-off value with a higher sensitivity may be selected; whereas, when a test is used to confirm a disease, a higher specificity may be required (Hanley and Mc Neil, 1983). Changing the cut-offs between positive and negative test results will affect the test characteristics (Musser et al., 1998.)

To the best of our knowledge, this is the first report describing cut-off value of EC in buffalo milk. Based on the samples studied, the specificity of the EC test was rather low at the cut-off value for highest accuracy, especially for subclinical mastitis. The false positives are less serious than false negatives in terms of permanent udder damage and economic losses. Therefore, if the animals are to be screened on the basis of EC scores of milk false positive animals should be ruled out with further tests. Both positive likelihood ratio (LR) and negative LR are significantly different from one, which confirms the relationship of EC and mastitis. Since the area under the ROC curve is significantly different from 0.5 (with $p < 0.001$, but not for subclinical mastitis), there is a higher probability that a randomly selected mastitic animal will have greater EC value than a randomly selected normal animal.

Classification of Buffalo Milk

Subclinical mastitis was defined as milk having $SCC \geq 200 \times 10^3/ml$, CMT positive, bacterial count > 250 cfu/ml and the absence of clinical signs. Clinical mastitis was defined as milk having $SCC \geq 200 \times 10^3/ml$, CMT positive, bacterial count > 250 cfu/ml and the presence of clinical signs.

This study categorize buffalo milk into normal, subclinical and clinical mastitis using 5 parameters. In normal milk, the SCC was $< 200 \times 10^3/ml$ and pathogenic bacteria were absent. In subclinical mastitis, SCC/ml was $\geq 200 \times 10^3/ml$ and bacteria were cultured from the samples. In clinical mastitis, SCC/ml was $\geq 200 \times 10^3/ml$, BC > 250 cfu/ml and presence of clinical signs.

On quarter basis, 16% and 11% of the foremilk samples in buffaloes were having subclinical and clinical mastitis respectively using five parameters, i.e. SCC, CMT, EC, bacterial count and clinical signs. The prevalence of subclinical mastitis in Murrah buffaloes was 16%. However, Costa et al. (2000) reported higher prevalence (18.8%) of subclinical mastitis in the lactating buffaloes in the state of Sao Paulo, Brazil. A lower prevalence of subclinical mastitis (11.7%) was reported in Iraq (Rahman et al., 1983). The prevalence of clinical mastitis in this study was 11%. However, slightly higher incidence of clinical mastitis (12.5%) in Murrah buffaloes was reported by Taraphder et al. (2006) in India.

This study provides useful and practical information concerning the classification of buffalo milk, and can contribute to the effective control of mastitis and further mastitis research in buffalo.

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MORPHOMETRY OF UDDER, TEAT, AND MILK VEIN IN NILI RAVI BUFFALO (*Bubalis bubalis*) AND IT'S RELATIONSHIP WITH MILK YIELD

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ABSTRACT

Morphometry of the udder is considered as an important tool in selection of dairy buffaloes locally, therefore, present study was designed to document differences of morphometric parameters of udder between two groups of Nili Ravi Buffaloes. Forty healthy buffalos in second or above lactation were divided into MP-1 (milk production 4-6.9 liters/day) and MP-2 (milk production 7-9.9 liters/day) groups. Morphometry of the udder, teat and milk vein was performed before milking (BM) and after milking (AM). Statistical differences at two time points were analyzed using paired t-test whereas amongst the groups they were compared using unpaired t-test. Differences were considered significant at $P < 0.05$. Results revealed that within MP-2 group TL of right-fore teat was higher at AM (8.17 ± 1.15) compared to BM (7.47 ± 1.01). Within MP-1 group, external teat diameter (ETD) decreased at AM in right-fore, left-fore and left-hind teats (21.01 ± 1.76 , 22.65 ± 1.68 and 28.16 ± 3.27 , respectively) compared to BM (26.33 ± 1.55 , 27.07 ± 1.43 and 35.85 ± 3.41 , respectively) whereas ETD did not vary between the groups. Teat cistern diameter (TCD) of the right-fore teat increased at AM (21.01 ± 1.48) as compared to BM (17.99 ± 1.36), and vice versa for left-fore teat. In case of MP-1 group, milk vein diameter, as measured with vernier calipers, of both left and right side was higher at BM (25.54 ± 1.80 and 29.19 ± 1.92 respectively) compared to AM (21.56 ± 1.36 and 22.55 ± 2.22 , respectively). Similarly in case of MP-2 group, increased diameter of milk vein was observed at BM on both left and right side (30.16 ± 1.23 and 29.80 ± 1.63 respectively) compared to AM (24.81 ± 2.08 and 23.04 ± 2.44 , respectively). The MVD, when recorded ultrasonographically, was higher before milking on the left side in MP-2 group (30.16 ± 1.23) compared with MP-1 group (25.54 ± 1.80). The MVD (measured ultrasonographically), depth of udder and udder horizontal circumference did not differ ($P > 0.05$) between or within the MP-1 and MP-2 groups. In conclusion, the differences of milk production were only partially supported by differences of morphometric variables observed in the current study.

Keywords: Morphometry, Nili Ravi, udder, milk yield

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UDDER MORPHOMETRIC OBSERVATIONS IN MURRAH AND NILI-RAVI BUFFALO BREEDS

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ABSTRACT

The present study compared the udder morphometric traits between Murrah (n=47) and Nili-Ravi (n=33) breeds of buffalo. The udder morphometric parameters included were: udder shape (bowl, round, goaty and stepped), udder conformation (pendulous and non-pendulous), udder length (UL), udder width (UW), udder depth (UD) and distance between different pair of teats (front, rear, left-sided and right-sided). Overall, bowl-shaped udders were highly prevalent (55.5%) followed by round (38.3%) and around 6% were goaty and stepped udders. Breed wise, no significant difference ($P>0.05$) was found in the occurrence of udder shapes, although Murrah breed has more bowl (64.6%) and Nili-Ravi has more round (45.4%) shaped udders. Non-pendulous udders were more frequent (92.6%) with no breed association ($P>0.05$). A significant effect of parity on the occurrence of pendulous udders was observed ($P<0.01$). The mean \pm SE values of UL, UW and UD were 70.7 ± 0.8 , 50.2 ± 0.87 and 12.6 ± 0.38 cm, respectively. Only UD varied between breeds; values being higher in Nili-Ravi (13.5 ± 0.58 cm) as compared with Murrah (12.0 ± 0.48 cm) buffaloes. An increase in mean UL, UW and UD was observed as the parity increased from first to third ($P<0.05$). The average distance (cm) between the front teats, rear teats, left-sided teats and right-sided teats were 12.7 ± 0.34 , 8.6 ± 0.25 , 7.3 ± 0.21 and 7.3 ± 0.19 , respectively. Rear teats were 1.85 times closely placed as compared with front teats ($P<0.01$). Among breeds, Nili-Ravi buffaloes were having significantly lower ($P<0.05$) distance between the rear teats than that in Murrah buffaloes (7.9 ± 0.36 vs. 9.1 ± 0.33 cm). The distances between other teat pairs did not differ significantly with respect to breeds. From 2nd parity onwards, although non-significant, but a general increasing trend in distances between different teat pairs was observed. Results of this study indicated the occurrence of some udder morphometric variations according to breed and parity.

Keywords: Buffalo, Murrah, Nili-Ravi, udder morphometry, parity

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LATENT BACTERIAL INFECTIONS IN BUFFALOES: EARLIER DIAGNOSIS THROUGH ACUTE PHASE PROTEINS (APPS)

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ABSTRACT

Diseases those are transmitted from animals to humans are known as zoonotic diseases. Brucellosis and tuberculosis are important zoonotic diseases. Brucellosis is a disease, which causes severe economic losses due to late term abortion, weak and stillborn calves, the causative agent of the infection is a gram negative bacterium of the genus *Brucella*. Tuberculosis is another serious problem in bovines. It is mainly caused by *Mycobacterium bovis*. Both have a very long incubation period due to intracellular nature of the organisms and hence difficult to diagnose at early stage. Now-a day, acute phase proteins (APPs) are considered to be an important tool for diagnosis of such diseases. The objective of the present study was to investigate the role of APPs in disease diagnosis. Serum antibodies against *Brucella* were detected by Rose Bengal Plate Test and for tuberculosis by Enzyme-linked immunosorbent assay (ELISA). The serum amyloid A (SAA) and heptoglobin (Hp) were measured by a solid phase sandwich ELISA in both brucellosis and tuberculosis positive animals. Equal numbers of apparently healthy animals declared negative on the basis of screening tests were also subjected to these acute phase proteins analysis. The results of the present study indicated that positive animals (both for brucellosis and tuberculosis) have a markedly higher concentration of SAA and Hp as compared to healthy animals. Hence, it has been concluded from this study that these acute phase proteins can be used to diagnose these two diseases at very early stage.

Keywords: Buffaloes, brucellosis, tuberculosis, APPs, diagnosis

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ADAPTING TECHNOLOGIES TO IMPROVE BUFFALO HEALTH AND PRODUCTIVITY

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ABSTRACT

Technologies are developing rapidly to improve dairy cattle health and productivity. Technologies such as point of care pathogen detection assays or milk based pregnancy detection assays allow for more precise management of health and performance. Many of these technologies are easily adaptable to buffalo and would facilitate improved health and better performance. An example is diagnostic assays to detect pregnancy associated glycoproteins or PAGS. PAGS are present in blood and milk of cattle when they are pregnant. With the simple collection of a blood or milk sample, pregnancy status can be determined. This allows for pregnancy determination when skilled/trained personnel are not available to determine pregnancy status by palpation or ultrasound. Adaption and/or validation of PAG assays in buffalo would provide an immediate tool for farmers to help monitor and manage reproductive programs in buffalo. Adapting technologies can improve buffalo health, productivity, farm economics and ultimately food security.

Keywords: Pathogen detection, diagnostic assays, reproduction, farm economics, food security

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SEROLOGICAL AND MOLECULAR STUDIES OF BRUCELLOSIS IN BUFFALOES OF PUNJAB, PAKISTAN

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ABSTRACT

Brucellosis is highly contagious and zoonotic disease that causes huge economic losses to the farmers. Limited data are available on the molecular investigation of brucellosis in Pakistan and virtually it is considered that *Brucella abortus* is the sole cause of brucellosis in buffaloes. Keeping in view the fact, blood samples (n=360) were collected from different farms of buffaloes with the history of abortions from different regions of Punjab province. Initially samples were screened for the presence of *Brucella* antibodies through Rose Bengal Plate Test (RBPT). RBPT positive samples were subjected to c-ELISA. Detection of *Brucella* genome was carried out through conventional PCR by detection of highly conserved bcs-31 gene. Afterwards, to identify the causative organism species, specific primers for *Brucella abortus* and *Brucella melitensis* were used. Overall 38.88% (n=140, 95% CI 33.82-44.14) of buffaloes were seropositive with RBPT and 34.44% (n=124, 95% CI 29.54-39.60) were seropositive with cELISA. Prevalence of brucellosis was significantly varied in animals with different geographical source, sex, age and health status. In molecular analysis, out of 124 samples analyzed through conventional PCR, 11.29% (n=14) samples were positive for *Brucella* genus. The sequences (Accession # KX618690, KX618691) were aligned with reported sequences in NCBI Gene Bank which revealed 100% sequence homology with bcs-31 gene of *Brucella*. Samples (n=124) were further analyzed for species detection i.e., *Brucella abortus* and *Brucella melitensis* which were found positive in 10 (8.06%) and 1 (0.80%) samples, respectively. Three PCR positive samples could not be speciated as there could be another specie involved. This is the first report of detection of *Brucella melitensis* in buffaloes in Pakistan. The study concludes huge epidemiologic impression of the disease and threat to public health. The study also provides basis for designing diagnostic improvements and effective control strategies.

Keywords: Buffaloes, brucellosis, RBPT, c-ELISA, PCR, Pakistan

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HEMATO-BIOCHEMICAL ALTERATIONS IN TUBERCULIN REACTOR WATER BUFFALOES IN PAKISTAN

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ABSTRACT

Cross sectional study was conducted; 321 water buffaloes were screened positive out of a population of 2526 buffaloes owned by the government livestock farms in Punjab province and private farmers in and around three major cities viz Faisalabad, Okara and Lahore through comparative intradermal tuberculin test CIDT for bovine tuberculosis (BTB). Blood samples collected were subjected to hemato-biochemical parameters in tuberculin reactors and none reactors animals for ascertaining the alterations. Results revealed overall significant ($P<0.05$) decrease in total erythrocyte count (TEC), packed cell volume (PCV) and hemoglobin concentration (Hb. conc.) in positive CIDT reactors animals compared with negative. Similarly significant ($P<0.05$) decrease in TLC, neutrophil was observed compared with CIDT negative animals. Significant ($P<0.05$) increase in lymphocyte, monocyte was observed in CIDT positive animals compared with negative animals. Similarly significant ($P<0.05$) increase in gamma globulins and fibrinogen concentrations in CIDT positive animals was observed. While non-significant difference was observed in total bilirubin, creatinine, calcium, zinc and copper concentrations. Correlation coefficient for various parameters was significantly stronger among majority of studied parameters. The significant decrease or increase in the parameters studied can be used as diagnostic markers along with conventional diagnostic tools for the confirmation of subclinical form of bovine tuberculosis (BTB) in water buffaloes.

Keywords: Hematology, BTB, bovine tuberculosis, CIDT, tuberculin

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CLINICO-HAEMATOLOGICAL AND OXIDATIVE STATUS OF NILI RAVI BUFFALOES INFECTED WITH TRYPANOSOMIASIS

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ABSTRACT

Haemoparasitic diseases like trypanosomiasis have adverse influence on the health and working capability of infected animals. Monitoring and identification of blood born parasitic infections in dairy animals is of vital importance to get the optimum production. In this study blood samples were collected from Nili Ravi buffaloes (n=390) kept at different villages of district Lodhran, Punjab province for Trypanosomiasis examination. Blood samples were also evaluated for red blood cell counts, total and differential leukocyte count, hematocrit, hemoglobin, total proteins and different serum parameters such as aspartate transaminase (AST), lipid peroxidation (LPO), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glucose, phosphorous, potassium, calcium and sodium. Overall prevalence of 4.61% (18/390) on the basis of microscopic smear examination, 8.46% (33/390) with pack cell volume, 11.02% (43/390) with formol gel test and 16.15% (63/390) with PCR was recorded. Infected buffaloes showed different clinical signs including high fever ($105^{\circ}\text{F}\pm 1.0^{\circ}\text{F}$), edema of face and legs, hyperemic mucosa of eyes, lacrimation, bulging eyes, pale mucus membranes and frequent urination. Microscopic examination of blood films showed morphologically different parasites. Statistical analysis did not indicate association of infection on the basis of age and sex of buffaloes. Results revealed significantly lower values of red blood cell counts, hemoglobin, hematocrit and total proteins, while increased values of total white blood cells, monocyte, lymphocyte, neutrophils and eosinophils in infected animals. The results showed significant increase in serum lipid per oxidation product (malondialdehyde) level, glucose, sodium and all the enzymes were significantly increased in trypanosomiasis positive buffaloes as compared to normal buffaloes. The results of this study suggest lower hematological and higher serum values associated with oxidative stress might have been induced by parasite resulting anemia in buffaloes.

Keywords: Buffalo, trypanosomiasis, hematology, serum biochemistry, oxidative status

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PATHOLOGICAL INVESTIGATION OF FOOT AND MOUTH DISEASE IN BUFFALOES (*Bubalus Bubalis*) IN SOUTHERN AREAS OF PUNJAB PROVINCE, PAKISTAN

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ABSTRACT

Foot and mouth disease (FMD) is highly endemic in Pakistan which causes heavy economic losses to livestock holders in terms of decrease milk production, high morbidity and mortality in cattle, buffaloes, sheep and goats. In present study we examined the clinico-pathological lesions during an outbreak of naturally occurring FMD in buffaloes. The current study was ascertained from November, 2016 to March, 2017 at three districts (Bahawalpur, Lodhran and Vehari) of Southern Punjab. The morbidity (31.48%) and mortality (4.77%) was recorded during the current outbreak. The morbid animals were lethargic, depressed and exhibited marked lameness, profuse salivation, myositis of tongue, vesicular fluid and epithelial sloughing. Vesicular fluid and epithelial sloughing collected from clinically sick animals were positive for FMDV predominantly O strain and Asia I through ELISA technique. Grossly, in infected and dead animals, the mandibular lymph nodes were swollen and hemorrhagic. The mucosa of abomasum was severely congested, ulcerated and showed hyperemic edges with centrally yellow necrotic areas. Severe purulent inflammation of jejunum, petechial hemorrhages over base of heart were also observed. Histologically degenerative changes in keratinocytes in stratum spinosum, marked acanthosis and intracellular accumulation of eosinophilic acellular transudate forming characteristic vesicles and bullae were observed in epidermis. The mandibular lymph nodes exhibited prominent capillaries engorged with erythrocytes, necrosis and dendritic cells with engulfed necrotic cells. Hemosiderin accumulation was also observed. Severe ulceration, congestion of mucosa of abomasum, fusion and stunting of intestinal villi along with infiltration of inflammatory cells were characteristic lesions in infected animals. From the findings of our study it can be suggested that clinical signs, necropsy lesions and histopathological changes are valuable and useful tools for the diagnosis of foot and mouth disease in *Bubalus bubalis*.

Keywords: FMD, *Bubalus bubalis*, Clinical Signs, Pathology

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IN VITRO AND IN VIVO EVALUATION OF TRADITIONAL MEDICINAL PLANTS AGAINST *Rhipicephalus microplus* (Asian cattle tick)

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ABSTRACT

The current study was focused on evaluation of anti-tick activities of a herbal formulation (HF) based on leaves of *Azadirachta indica* and *Nicotiana tabacum*, flowers of *Calotropis procera* and seeds of *Trachyspermum ammi*. HF demonstrated anti-tick activity *in vivo* against *Rhipicephalus microplus* (Asian cattle tick) by inhibiting the egg laying, larval mortality and reduced tick intensity/infestation on animals. A dose dependent anti-tick effect was observed in all the tests carried out in this study. Egg laying was significantly lower in ticks exposed to different concentrations of HF compared with those exposed to distilled water used as control. Likewise, there was increasing mortality of *in vitro* cultured tick larvae with increasing concentrations of HF as opposed to no mortality in the control. There was a significant reduction ($P < 0.05$) in the number of ticks exposed to 45% HF compared with control. Enhancement of revenue generation of farmers through adopting of HF in integrated tick management practices is anticipated. Limitations of the study were low number of animals used in the *in vivo* studies. Large scale controlled studies are, however, recommended for standardization of the doses and applications of the product. Studies on fraction based activity of formulation will be useful in identifying the active principles leading to development of a refined product with better anti-tick efficacy.

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TICK BORNE DISEASES EXPLOITING THE BLACK GOLD (BUFFALO) OF PAKISTAN

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ABSTRACT

Haemo-parasitic diseases are important diseases of livestock transmitted by ticks, which adversely affect the productive performance of animals. This study was designed with the objective to determine the frequency of haemo-parasitic diseases in central zone of Khyber Pakhtunkhwa along with associated risk factors and hematological profile. For this purpose total of 250 blood samples were collected from three districts in anti-coagulant added vacutainer. Microscopic results revealed overall occurrence of haemo-parasites as 49.6% in which anaplasmosis was recorded as 36%, whereas theileriosis was 13.6%. Haemo-parasites occurrence with respect to different predisposing factors i.e. season, location, age and gender was studied. Frequency of haemo-parasites was significantly ($P < 0.05$) higher in summer season as compare to winter. Similarly frequency was higher in Peshawar (74.1%) as compare to other two districts and the difference was statistically significant ($P < 0.05$). Age is also considered as an important risk factor for the occurrence of haemo-parasitic diseases and adult were found more susceptible in the current study. Frequency was higher in female as compare to male but the case was not significant ($P > 0.05$). Infected animals in the study area showed significant ($P < 0.05$) variation in hematological parameters and anemia was categorized as microcytic hypochromic on the basis of erythrocytic indices. Hot and humid weather favors the development and growth of ticks which may be the possible reason for the higher incidence of haemo-parasites in summer season. Hyperactive free radicals i.e. superoxide are produced in the parasitic diseases which may be the reason for anemia.

Keywords: Haemo-parasitic diseases, anaplasmosis, theileriosis, hematology, microcytic hypochromic anemia

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IMPACT OF CLIMATE CHANGE ON THE EPIDEMIOLOGY OF TICK-BORNE HAEMOPARASITES IN AZAKHELI BUFFALO IN DISTRICT SWAT, PAKISTAN

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ABSTRACT

Haemoparasite infections are among the most economically important large ruminant diseases in Pakistan. The current cross sectional survey was conducted to determine the impact of temporal changes on the epidemiology of haemoparasites in Azakheli (Indigenous local breed) buffalo in randomly selected villages of district Swat in 2014-15. Giemsa-stained blood smears were prepared from the sampled animals from January 2014 to January 2015 were examined for the presence of tick borne haemoparasites (i.e. *Anaplasma sp*; *Babesia bigemina*; *Borrelia sp*; *Eperythrozoon sp*; *Theileria mutans*, and *Theileria velifera*). Total of 127 different age group animals were selected and followed for at least one year as the inclusion criteria for selection. Each month samples were taken from these animals. Monthly and cumulative incidence was determined for different tick borne parasite species. *Anaplasma* specie was the most common parasite in buffalo with 100% incidence in young calves by the five months of age, and *Theileria* specie in adult Azakheli buffaloes of more than one year of age. The relative prevalence of all the parasites identified in during the study varied with the temporal changes in this region, as well as the actual infection rate. Seasonal trends in the incidence of the highly prevalence haemoparasites were evident. The highest incidence and cumulative prevalence was recorded in the months with higher temperature and humidity respectively, with elevated infection rate. It is envisaged that the current study results will stimulate future in depth research to determine epidemiology of haemoparasites in large ruminants by using more sensitive molecular techniques in the country and will help to design control strategies in future.

Keywords: Haemoparasites, azakheli, giemsa-stain, prevalence, cumulative incidence, Pakistan.

INTRODUCTION

Hemoglobinuria caused by an intracellular blood pathogen is responsible for extensive losses of health and lives in bovine, particularly the exotic animals and their crosses (Singh *et al.* 2012). However, the distribution of anaplasmosis is dependent upon the availability of the definitive host and the carrying vector (Ogden *et al.* 2002). Anaplasmosis has a wide distribution over six continents, and the high animal losses can be attributed to this malady (Kocan *et al.* 2010). Members of the *Anaplasma* genus are obligate intracellular pathogenic microbes which are biologically transmitted by ticks, while the mechanical mean of transmission is through blood sucking insects, fomites contaminated by infected body fluid such as blood, as well as transplacental transmission (Aubry and Geale 2011). Anaplasmosis is highly prevalent hemo-rickettsial bovine disease in Pakistan with its prevalence ranging from 4 to 75.5% (Khan *et al.* 2004). *A. marginale* produces clinical anaplasmosis in large ruminants which can be presented as mild to severe fatal disease causing considerable losses to the livestock sector (Kocan *et al.* 2004).

Microscopy of stained blood smears can reveal the *A. marginale* at levels of 10⁶ erythrocyte infected per milliliter (Gale *et al.* 1996). Similar studies were followed for other species diagnosis and identifications. Microscopy of stained smear can be used suitably for the diagnosis of anaplasmosis in acutely ill animal but show negative results in asymptomatic and carrier animals (Carelli *et al.* 2007). The first reason is fewer infected erythrocytes in asymptomatic carriers which could be skipped in microscopic examination; secondly differentiation between *A. centrale* and *A. marginale* is not possible through microscopy. Additionally it needs sound experience to differentiate between various members of the genus *Anaplasma*, structures like Howell-Jolly bodies, Heinz bodies or staining artifacts (Ge *et al.* 1995).

Domestic ruminants in Pakistan according to literature may be infected by a very higher number of different kinds vector borne eukaryotic and prokaryotic hemoparasites. Previous studies have shown that temporal changes over a specified area might have great influence on the infestation and prevalence rate of tick borne hemoparasites. Previous studies conducted in current study area (Khan *et al.*, 2014) reported impact of climatic factors fluctuation a great deal of impact on ticks population and infestation. Considering the importance of local breeds as the main source of dairy products for the local population in the Khyber Pakhtunkhwa a longitudinal survey for a period of one year was conducted to evaluate the impact of climate change on the prevalence of hemoparasites in Azakheli buffalo (local native breed).

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MATERIALS AND METHODS

Sample sites and Animal Species

A longitudinal survey was conducted, where blood samples were collected every month from a total of 127 pure bred and cross bred Azakheli buffaloes irrespective of age and gender. Sampling was performed for a period of one year from Jan 2014 to Jan 2015. Two clusters were selected based on the geography and topology of the area i.e. Hilly and Plane clusters.

Blood Sample Collection, Preparation, and Examination

Blood was taken from jugular vein of the selected animal in EDTA containing vacutainer tubes as an anticoagulant. Samples were transported using ice packs to the main laboratory where the samples were processed. A thin smears were prepared from every blood samples, air dried, fixed, stained on Giemsa stain and rinsed into two changes of distilled water buffering to pH 7.2. Procedure for identification of hemoparasites was performed following (Bell-Sakyi et al., 2004).

Statistical Analysis

Data was analyzed using SPSS software (version 20.00). Chi-square analysis was conducted to test the association of different climatic factors on the prevalence of Hemoparasites different species and seasonal and temporal trends of the prevalence of different hemoparasite species in the sampled population.

RESULTS

Tick-borne haemoparasites of different genera's were observed in Azakheli buffalo population of Khyber Pakhtunkhwa. Based on the morphological characteristics and their epidemiological considerations different species were identified (Figure 1 and Table 1). *Theileria* species were identified as *T. mutans* (pleomorphic, large, mainly oval piroplasms, non-pathogenic) and *T. velifera* (smaller, veiled piroplasms, non-pathogenic). It was not possible to confidently identify the *Anaplasma*, *Borrelia* and *Eperythrozoon* to species level in the current study.

Temporal distribution of the results has been given in Figure 1. Temperature and humidity was recorded per month and average values were recorded and analyzed to test the impact of change in these parameters on the prevalence of haemoparasites in buffalo population. The commonest parasites were *T. mutans* and with 100% of surviving animals infected with both species by the end of the study period. *Anaplasma* and *theileria* species were in general prevalence was recorded higher as compared to the other species. Comparison of the prevalence of the detected species at different levels is given in Table 1. The prevalence of different species were compared based on gender, breed, age, and based on cluster. Clusters were made based on the difference in the climate and topology of the soil. The results showed that young calves were more found significantly infested by all kinds of the haemoparasites species identified here. While local pure breed animals were found more resistant to the haemoparasites than the cross bred Azakheli buffaloes. While prevalence was significantly higher in plane areas as the average temperature and humidity was recorded higher in that region as compared to the hilly areas.

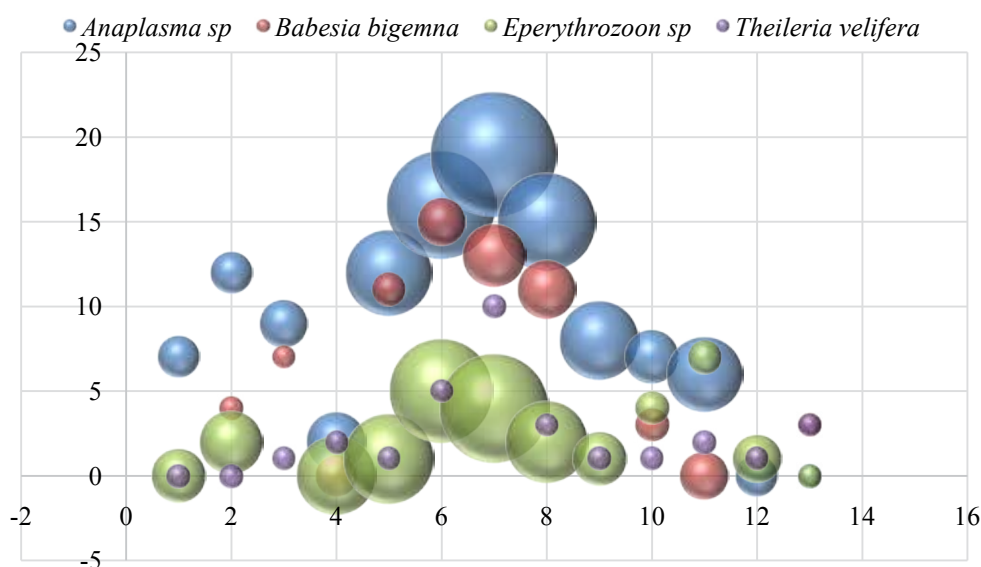


Figure 1. Temporal trend of the Hemoparasites detected in the survey from Jan 2014 to Jan 2015 in Khyber Pakhtunkhwa in Azakheli Buffalo.



Table 1. Description of number of species identified in one year survey from Jan 2014-Jan 2015 in district Khyber Pakhtunkhwa

Hemoparasites detected Jan2014-15	Age of animal		Gender of animal		Defined cluster		Breed of animal	
	<1.5 Y	>1.5 Y	Male	Female	*Hilly	**Plane	^Pure	***Crossed
<i>Anaplasma</i> Sp	61	52	19	94	33	80	43	70
<i>Theileria</i> Sp (general)	49	76	13	112	28	97	51	74
<i>Babesia bigemna</i>	31	49	7	73	29	51	21	59
<i>Borelia</i> Sp	11	21	3	29	8	24	17	15
<i>Eperythrozoon</i> Sp	12	18	6	24	11	19	13	17
<i>Theileria mutans</i>	15	18	8	95	61	42	48	55

^ Pure bred of Azakheli buffalo
 *Animals sampled from hilly areas
 ** animals sampled from plane areas
 ***Cross bred Azakheli buffalo

DISCUSSION

The present study was conducted to collect the basic epidemiological data regarding the hemoparasites and impact of climate change on their prevalence. The findings of the current study confirm the occurrence and impact of local climate change on the prevalence of haemoparasites in local native buffalo breeds of Khyber Pakhtunkhwa. Several studies have conducted in different regions of Pakistan reporting different species of the haemoparasites those are vector borne causing hemoglobinuria. Climatic data was obtained from the local meteorological department regarding daily temperature and humidity. All the epidemiological questionnaires data was entered into the Epi-Data software 3.1 (www.epidata.dk/download.php). Data were validated via crosschecking the computerized records with all the original hard copies of complete data. Analyzing data the mean and average values of these factors were tested to evaluate their association with the presence of haemoparasites. Identification to species level of tick-borne haemoparasites in blood smears from field animals should be carried out with caution. In the present study this was only attempted with *Theileria* and *Babesia*, as the authors had insufficient facilities and expertise in differentiating species of *Anaplasma*, *Borrelia* and *Eperythrozoon* by light microscopy. In addition to morphology, epidemiological factors such as presence of appropriate tick vectors, occurrence of clinical disease and incidence can be considered.

The present study reports the presence of range of tick borne haemoparasites in native local breed of buffalo in Khyber Pakhtunkhwa. These results are agreement with (Assoku, 1979). Identification to genus level in thin blood smears, this method used here and apparently by Macfie (1915), is relatively a straightforward and it permits identification of all intra-erythrocytic *Anaplasma*, *Theileria*, and *Babesia* organisms at the parasitaemias of 0.01% or more. The approach used by Assoku (1979), of examining a thick blood smears and after that only preparing a thin smears when the haemoparasites were detected in thick smears, would favor the detection of the large organisms i.e. *Babesia* and *Borrelia*. A higher percentage of haemoparasite was recorded in the months in which the temperature and humidity level increased respectively. It might be due the increase population and activity of the tick population which promotes the transmission and spread of haemoparasites. These findings were in line with (Friedhoff, 1997). Prevalence of haemoparasites was significantly higher in cross bred native breeds as compared to pure bred Azakheli buffalo. It might be due to the adaptiveness of the local breeds in the local environment. Cross bred animals in the study area were found mostly infested with ticks continuously all the year. Farmers of this region were also found less aware of the control management of ticks. Most of the farmers did not use any acaricidal drugs for tick's control. In the current study the tick infested animals showed high prevalence (24.34%) than tick free animals (12.04%) the findings are in line with (Ashraf et al. 2013, Atif 2015, Sajid, 2014) who reports increasing pattern in the disease incidence with abundance in the tick population. In this study the animals previously had tick infestation history were showing more prevalence than those not previously exposed these findings are in line with the findings of (Zhang et al. 2014, Leiby and Gill 2004) relating the higher prevalence with direct or indirect contact to ticks.

It can be concluded that tick borne haemoparasites are prevalent in the study area infecting mostly cross bred animals in the months with high temperature and humidity. Strategic control planes must be adopted. Farmers must be encouraged to improve pure breeds. Farmers should be also educated regarding management of ticks control and biosecurity.



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A HOLISTIC VETERINARY MEDICINE MODEL FOR THE BUFFALO INDUSTRY IN THAILAND

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ABSTRACT

The principles of holistic medicine have been in existence since ancient times. Its insights and approaches, however, are not commonly applied to the veterinary treatment of farm animals. The holistic model of medicine seeks to treat the whole person or animal – including all of the factors that influence their health and wellbeing. A holistic approach to veterinary medicine, rather than focusing solely on the immediate symptoms of animal disease, looks at the whole context in which disease arises and thus - in addition to any necessary medicinal treatments – seeks to ameliorate and/or prevent disease by changing or improving contributing causal factors such as: genetics, nutrition, living conditions and stress levels of the animals involved. In the past, veterinary medicine was rarely or only minimally applied to buffalo in Thailand. Buffalo were raised primarily as domestic farm animals and beasts of burden – they grazed and reproduced on their own. Sick animals were usually culled rather than treated. Buffalo farming was not an agribusiness in the modern sense. While this is still the case in many rural areas in Thailand – organized buffalo breeder, milk and meat farming has begun in the country. This type of intensive industrial farming presents unique challenges and has created a strong demand for veterinary services to protect and maximize their livestock investments. While traditional veterinary interventions remain relevant – they are not enough in this new context. Proactive, preventive medicine is not only possible – it is economically necessary. The insights from holistic veterinary medicine need to be integrated with modern farm management to prevent the conditions that lead to disease and the need for medical intervention. To accomplish this, farmers themselves must be educated and trained by their veterinarians to cost effectively address their animals needs within the context of their specific type of buffalo farm.

Keywords: Holistic, veterinary medicine, buffalo, Thailand

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MILK YIELD RESPONSE OF BYPASS PROTEIN DURING DRY SEASON ON SMALLHOLDER DAIRY ANIMALS IN THE HILLS OF NEPAL

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ABSTRACT

Protein need for small holder dairy animals in dry season is very limited in the hills of Nepal. Considering this problem one on-station and one on-farm trials were conducted in 2013 and 2014 in Nepal respectively. In on-station trial (2013) 15 milking buffaloes were randomly assigned to 5 dietary treatments (0, 0.5, 1, 1.5 and 2 kg de-oiled soybean meals/day) and in on-farm trial (2014), 20 milking buffaloes were assigned to 0.5 kg de-oiled soybean meal/day for two months dry period. From on-station trial, significantly higher (42 %) milk production was observed from 0.5 kg and 1 kg de-oiled soybean meal/day feeding groups. From the on-farm trials in village dairy buffaloes 20 percent milk production increment was observed compared with the normal farmer's feeding practices (de-oiled soybean meals unfed condition). It was concluded that, in addition to normal diet, 0.5 to 1 kg de-oiled soybean meal (depending up on the body weight of animal and feeding situation) per day feeding as bypass protein sources could be beneficial during dry period where green fodder is scarce to maintain milk production from dairy animals.

Keywords: Bypass protein, milk yield, buffaloes, small holder

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USE OF FEEDING SUPPORT TOOL FOR ENHANCING DAIRY ANIMAL PRODUCTIVITY

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ABSTRACT

The dairy animal feeding system in Nepal is mostly based on the availability of feeding resources and the existing knowledge of farmers rather than based on nutrient requirements for various functions and production. Balanced Energy, Protein, Calcium, Phosphorus and other nutrients need to be provided in order to produce milk as per their genetic potential. Performance and health of dairy animals are greatly compromised due to imbalanced or inadequate feeding. This paper highlights the existing dairy animal feeding system, process to balance least cost ration for dairy animals and its prospects for adoption.

Keywords: Feeding support tool, nutrient requirements, dairy animals

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HUSBANDRY PRACTICE AND REPRODUCTIVE PERFORMANCE OF BUFFALOES IN CHITWAN AND KASKI DISTRICTS OF NEPAL

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ABSTRACT

The present study aimed to document husbandry practice and reproductive performance in farmer's level using questionnaire survey of total 120 respondents in two districts of Nepal during August 2017 that included 77 buffaloes in Kaski and 65 in Chitwan. The data were analyzed by using MS- Excel and Open Epi software. Nearly half of the buffaloes in Kaski and 83% buffaloes in Chitwan were Murrah cross breed. Two-thirds of total respondents kept their buffaloes in 24-hour tie-stall. Majority of buffalo care takers were female (64%). Significantly ($P < 0.01$) higher number of households in Chitwan (71.7%) sold their surplus milk than those in Kaski (28.3%). Feeding cultivated fodder, commercially formulated feed and mineral mixture was practiced by 51.5%, 19% and 16.6% households, respectively. About three-fourth of respondents de-wormed their buffaloes at six month interval while 6% never de-wormed. The major reason for culling buffalo was management problem (51.5%) followed by anoestrous (20.8%) and senility (10.8%). Age of first conception (2.83 ± 0.58 and 2.93 ± 0.71 years), lactation length (9.81 ± 2.38 and 9.87 ± 3.04 months), calving to first estrous interval (3.15 ± 2.33 and 3.48 ± 3.60 months), number of breeding per conception (1.41 ± 0.66 and 1.24 ± 0.51 times) were similar in both local and Murrah cross buffaloes. However, peak milk yield (lit/day) was significantly ($P < 0.01$) higher in Murrah cross (8.68 ± 3.08) than in local breed (5.90 ± 1.69). Majority of buffaloes were bred during autumn season in both Kaski (56%) and Chitwan (45%) followed by winter, summer and spring. In conclusion, buffalo farming was more commercialized in Chitwan than in Kaski. The proportion of farmer feeding cultivated fodder, mineral supplement and commercially formulated feed to their buffaloes indicates the buffaloes in both districts were kept under low nutrition. Although the reproductive performance was similar between two breeds, the Murrah cross breed buffaloes can be suggested because of its high milk yield and good adaptability in both districts.

Keywords: Buffalo, husbandry, reproduction, Kaski, Chitwan

INTRODUCTION

Buffalo is a one of the major livestock species of Nepalese farming system for milk and meat production. They are mainly raised by small holder farmers across the all physiographic agro-ecological zones of the country. Contribution of buffaloes is the highest as compared to other livestock species as it solely contribute 57.4 % of total meat production and 67.7 % of milk production of Nepal (IBS leaflet, 2017). Around 97 % of world's buffaloes are found in Asia in which Nepal ranks 4th position in terms of world buffalo population (Ranjhan and Qureshi, 2006). Buffaloes are distributed throughout the country and there are 5.17 million head buffalo in Nepal (IBS leaflet, 2017). Buffalo is considered as socio-economically important livestock species in Nepal as it contribute 53 % of livestock share in national GDP reported by Shrestha (2005). The most of buffalo in Nepal are River buffalo (H. Wahid and Y Roshina, 2011) and swamp buffalo are limited in wild form. More than 85% of Nepalese buffalo are indigenous (Poudel et al., 2015). Of total buffalo local buffalo including Parkote and Lime are predominated in hilly region and primarily used for household purpose however the cross and pure breed of Murrha buffaloes are reared in Terai region, mostly by commercial farmer throughout the country. Three indigenous breeds of buffaloes in country which has been identified and characterized so are Lime, Parkote and Gaddi (Neopane et al., 2007). This study was carried out to document the husbandry practice and reproductive performance of buffaloes reared by farmers of Chitwan and Kaski districts of Nepal.

MATERIALS AND METHODS

Research Area

This study was carried out at three major buffalo pocket areas of Kaski district namely Malepatan, Dhodbeshi and Nagadada and from three buffalo pocket areas of Chitwan district namely Fulbari, Kalyanpur and Mangalpur. The site were selected on the basis of information provided by DLSO Kaski and Chitwan.

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Sampling Procedure, Sampling Size, Data Collection and Analysis

The households having at least one buffalo were selected for survey. Sixty respondents from each district were interviewed with pretested questionnaire during August 2017. There were 77 buffaloes from Kaski and 65 from Chitwan. The data were entered and analyzed by using MS- Excel and Open Epi software.

RESULTS AND DISCUSSION

Husbandry Practice

Housing System

Out of 60 households surveyed in each district 47 households (78.34%) in Kaski and 34 households (43.33%) in Chitwan did not graze their animals. It means they reared their buffaloes in 24 hours tie-stall system. In total, nearly one third of farmer (32.4 %) adopted practice of grazing their animals and 67.5% of total respondents kept their buffaloes in 24-hour tie-stall. There is wide variation in system of buffalo raising through the different regions of world and it is determined by several interacting factors that include climate, location, cropping system, type of operation i.e. small or large farm, subsistence or commercial etc (Perera, 2011).

Major Holder and Purpose of Rearing

Majority of buffalo care takers were female (64%). This result was in accordance with study of Poudel et al. (2015). Significantly ($P < 0.01$) higher number of households in Chitwan (71.7%) sold their surplus milk than those in Kaski (28.3%). It can be speculated that buffalo farming in Chitwan is more commercialized than in Kaski.

Major Contributing Feeds/Fodder for Buffalo Feeding

Finding revealed that natural bushes, cultivated tree fodder, native grass, rice straw and other crop residues were major supplements of fodders for buffalo feeding in study site with slight seasonal variation. Feeding cultivated fodder, commercially formulated feeds and mineral mixture was practiced by 51.5%, 19% and 16.6% households, respectively. Generally these animals did not receive mineral mixture supplementation in their basal diet. Farmers in the study were not adopting the practice of mineral mixture feeding, cultivation of fodder and use of commercially formulated feed; as a result the animals might have been influenced by nutritional deficiency or imbalance.

De-worming frequency

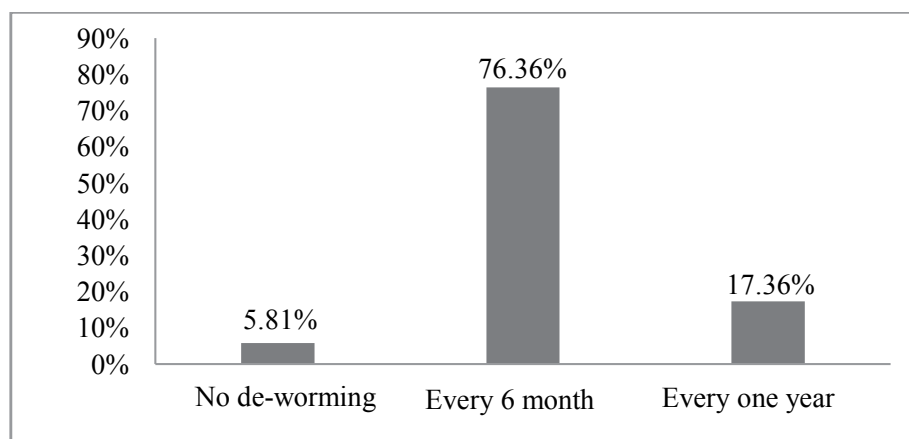


Figure 1. Showed that about three-fourth of respondents de-wormed their buffaloes at six month interval while nearly 6% never de-wormed

Culling Criteria of Buffalo

Table 1. Culling criteria of buffalo

Culling Criteria	Kaski (n=60 household)	Chitwan (n=60 household)	Overall (n=120 household)
Managmental	45.09 %	58.45 %	51.46 %
Anoestrous	28.39 %	13.36 %	20.75 %
Senility	10.02 %	11.69 %	10.79 %
Repeat breeding	8.35 %	5.01 %	6.64 %
Prolapse	1.67 %	11.69 %	6.64 %
Other	6.68 %		3.32 %

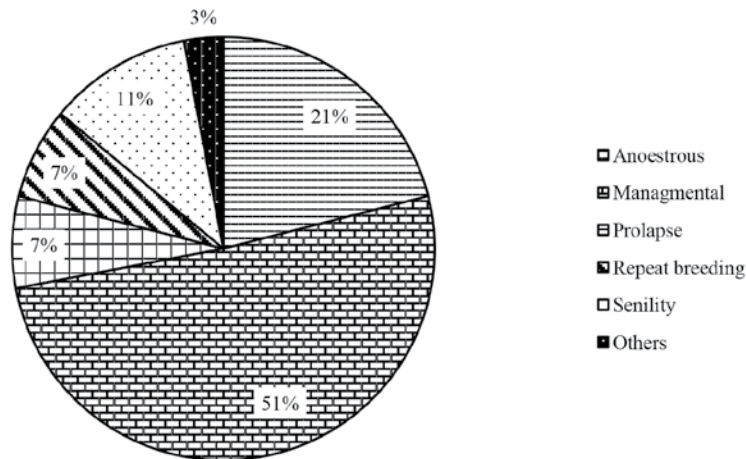


Figure 2. Culling criteria for buffalo (n= 120)

The Table 1 and Figure 2 summarize reason for culling buffalo in study site. The major reason for culling buffalo in both districts were management problem (51.46%) which includes lack of manpower, low milk production and unavailability of fodder for feeding animal etc. Besides that, anoestrous (21%) was second reason to cull the buffalo followed by senility (11%), prolapse (7%), repeat breeding (7%) and other (3%).

Reproductive and Productive Performance

Breed Distribution

Table 2. Breed distribution in two district

Breed /district	Chitwan (n=65)	Kaski (n=77)	Total (n=142)
Local	11 (16.33 %)	42 (54.54 %)	53 (37.33%)
Murrha Cross	54 (83.7 %)	35 (45.46%)	89 (62.67%)

Table 2 summarizes the distribution of two major breed of buffalo in two districts. Kaski district was predominated by local breed (54.54%) while Chitwan was predominated by Murrha cross breed (83.7%). Overall data represent Murrha cross (62.67%) were the predominated over local (37.33%). Similar finding was reported by Poudel et al. (2015). However, previous study explained that more than 65% of the total buffalo in Nepal were local breed (Neopane et al., 2007).

Reproductive Parameter

Major reproductive performance of local and Murrhacross and local breed of buffalo has been shown in Table 3.

Table 3. Comparison of reproductive parameters between local and Murrha breed

Parameter/ breed	Local breed	Murrha cross
Age of 1 st conception (years)	2.83±0.58	2.93±0.71
Calving to 1 st estrous interval (month)	3.15±2.33	3.48± 3.60
Calving interval (month)	14.75 ± 3.57	14 ± 3.44
No of breeding per conception	1.41± 0.66	1.24± 0.51

The data represent no significant difference in age of 1st conception, calving to 1st estrous interval, calving interval and no. of breeding per conception in between two breed. Study by Shrestha et al. (2005) in western hill of Nepal showed similar result. The difference in age at first calving, lactation length among different buffalo genotypes was not significant. The age at first conception, calving to 1st estrous intervals and calving interval in local buffaloes were 3.56 years, 6.6 months , 20 months, respectively (Shrestha et al., 2005).

According to Regional Agricultural Research Station Lumle, the average calving interval of local buffalo was 17.73 months. Buffaloes calve for first time at 3-5 years of age. Most buffaloes conceived at 2-3 years of age (Wahid and Roshina, 2011). Postpartum anoestrous remains the major problem contributing to long calving intervals. Similar finding on age of first conception (3.29±0.45 year), calving interval (15.01±1.17 month) were reported; but service per conception was higher (2.57±0.45 times) in previous studies (Sachan et al., 2015) than present finding. Age at first conception (AFC) was 2.75±0.34 years (Meena et al., 2016). The mean number of



services per conception was 2.20 (Dhaka et al., 2017).

Variation in reproductive parameter observed by different studies might be due to influence of genotype, nutrition, management, and climate of the study area.

Peak Milk Yield and Lactation Length

Peak milk yield (lit/day) was significantly ($P < 0.01$) higher in Murrah cross (8.68 ± 3.08) than in local breed (5.90 ± 1.69). The average peak yield of buffalo in the field condition was estimated to be 8.56 ± 0.85 litre/day and (Meena et al., 2016) and 8.1 ± 1.9 lit./day (Sachan et al., 2015). In present study there was no significant difference in lactation length of Murrah cross (9.81 ± 2.38 month) and local buffalo (9.87 ± 3.04 months). The lactation length reported by different studies were 9.78 ± 0.91 months (Sachan et al., 2015), 8 months (Dhaka et al., 2017), 9.2 ± 0.46 months (Meena et al., 2016).

Seasonal Influence on Reproductive Performance

Buffalo are capable of breeding throughout the year but in many countries seasonal pattern of ovarian cycle occurs. Present study showed that the majority of buffaloes were bred during autumn season in both Kaski (56%) and Chitwan (45%). Overall performance showed that autumn season (48.6%) was most common season for breeding followed by winter (26.05%), summer (15.5%) and spring (9.9%).

Birth Sex Ratio

Results of present study reflected that the sex ratio of the buffalo calves was found 1.25 (75:60, male:female).

CONCLUSION

Based on the findings of present study, it is concluded that buffalo farming was more commercialized in Chitwan than in Kaski district. Quite low proportion of farmers feeding cultivated fodder, mineral supplements and commercially formulated feed to their buffaloes indicates that the buffaloes in both districts were kept under low nutrition. Although the reproductive performance was similar between two breeds, the Murrah cross breed buffaloes can be suggested because of its high milk yield and good adaptability in both districts. Lack of manpower at household level being the main criteria for culling buffaloes necessitates for commercial buffalo production in coming days.

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CAN MUSIC INFLUENCE MILKING BEHAVIOR AND YIELD IN BUFFALO COWS? : A REVIEW

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ABSTRACT

Music is thought to have both analgesic and anxiolytic properties. The music therapist usually uses music either from flute or piano or music from any other source that pleases the mind. Various documentations of the effects of music therapy on the physiology and psychology of humans have been done. The effect of music on physiology and behavior has been a matter of study on various animal models too. Many of these studies claim that even animals cannot remain unaffected by the music. The potential benefits that music provided to animal in different studies give evidence that music can be a means to improve the milking behavior as well as milk yield of farm animals like buffalo cows. The authors review the current researches on whether music influence milking behavior and yield of buffaloes and discuss its usefulness for increasing milk production. The available literatures indicate that the music can influence the milking behavior and milk yield as well.

Keywords: Music, effect, human, buffalo cows, physiology, behavior, yield

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EFFECT OF PARITY ON LACTATIONAL EFFICIENCY OF MURRAH BUFFALOES (*Bubalus bubalis* L.) IN CENTRAL NEPAL

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ABSTRACT

Buffalo (*Bubalus bubalis* L.) is the major milk contributing dairy species in Nepal enhanced with multiple utilities. It occupies 1st rank in case of milk production. Murrah buffaloes that have important phenological and adaptation trait to thrive in adverse climatic condition were the subject of present research. Milk production trait is closely related with the parity of animal. With the aim of determining effect of parity on different traits related to lactational efficiency, Kalika Municipality, Padampur of Chitwan District was purposively selected and field assessment was conducted from April to August, 2017. Primary data were collected through direct interview with 37 buffalo rearing farmers using closed ended semi-structured questionnaire. Various traits related to lactational efficiency including colostrum period, days to reach peak milk yield, lactation length and dry period were taken under consideration. One way analysis of variance was performed to assess the effect of parity on the traits considered using GenStat4. Findings of present study revealed that overall mean of colostrum period, days to reach peak milk yield, lactation length and dry period were 5.2±1.8 days, 23.3±8.8 days, 344.0±102.0 days, 110.9±61.4 days, respectively which were obtained with ranges 2-11 days, 10-45 days, 90-600 days, and 30-300 days, respectively. Meanwhile, parity had no significant effect on major lactational traits considered under this study. However, buffaloes with mid parity (3rd-4th) were superior in terms of colostrum period, days to reach peak milk yield and dry period. Thus, it is concluded that parity of buffaloes was not an important source of variation with respect to major lactational traits. However, further research should be carried out considering larger sample size and wider agro-ecological domain is recommended for the validation of present findings.

Keywords: Production traits, Murrah, parity, Chitwan

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STATUS OF BUFFALO (*Bubalus bubalis*) PRODUCTION IN NEPAL: A REVIEW

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ABSTRACT

Livestock is the principle component in agrarian country, Nepal; contributing 11% in national GDP, 26.8% in AGDP. Buffalo is one the most important livestock species with its multiple utilities and ranks in first position in terms of milk and meat production. Altogether 5 breeds including 3 indigenous namely Lime, Parkote, and Gaddi, and 2 pure Murrah and its crosses are existed in Nepal. Current statistics revealed that the population of buffalo is 5.16 million at an annual increasing rate of over 0.58%. Meanwhile, total milk and meat production of buffalo is just over 1.2 million tones and 175 thousand tons contributing 67.7% and 57.7% of total milk and meat production. Data from 2005 to 2014 showed that the overall ghee and hides production of buffalo is an average value of 157600 tons and 426 hg/An. respectively. The average import and export quantity from 2009 to 2013 was 33731 and 24968 heads and similarly, value was found to be 1080 and 1504 1000US\$, respectively. Buffalo milk is ranked second in the world in production, contributing towards nearly 13% of the world's milk production. Buffalo milk contains vitamin B6 and casein content nearly an order of magnitude larger than that of cow milk. Thus, buffalo is known as "living bank" which has great socio-culture value as manure, religious functions, tourist attraction and urgent financial need.

Keywords: Buffalo, production, milk, meat, import

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EFFECT OF MEDICATED AND NON-MEDICATED UREA MOLASSES MULTI-NUTRIENT BLOCK (UMMB) ON MILK PRODUCTION, MILK COMPOSITION AND GASTRO-INTESTINAL PARASITES IN BUFFALO

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ABSTRACT

Livestock plays important role in household incomes especially on rural area of Nepal. During dry winter season, animal feed is in shortage by 36% in Nepal. Crop residue mainly rice straw, corn stover, wheat straw, millet straw, and other similar types of residues are used to feed these ruminant. These feed resources are very poor in nutrient contents. However, these feedstuffs can be improved in quality and utilized by supplementing with certain feed supplements such as urea molasses mineral block (UMMB). Objective of research was to find out the effect of UMMB supplement on milk yield, SNF%, fat%, lactose%, protein% and effect of *Azadirachta indica* (Neem) medicated UMMB supplementary feeding against nematode parasite performance in dairy buffaloes. The experimental trial was conducted in the livestock farm of Agriculture and Forestry University (AFU) and “Dhakal Dairy farm” from February, 2015 to April, 2015. 45 lactating crossbred buffaloes were selected for the experiment. Each animal were ear tagged for identification of animal. The research was conducted by using randomized complete block design in which three different treatments namely, T₁ = T₂ + powder of Neem leaves (i.e. normal feeding + 500g UMMB + neem powder), T₂ = T₁ + 500 g UMMB/day (normal feeding + UMMB), T₃ = on normal feeding (control). Based on milk production 45 lactating buffaloes were divided into 15 blocks. 3 treatments allocated in each block (RCBD). Mean milk yield (kg) and mean milk lactose of buffalo supplemented diet with UMMB is significantly effectiveness (P<0.05) was observed. Accordingly, higher milk production was recorded in diet supplement with UMMB (503.44 kg) treatment group. During 90 days of experimental period, there was an increment in milk production by 8.54% in medicated UMMB fed animal as compared to control group, which statistically similar to non-medicated UMMB supplemented group, where increment was by 8.53% as compared to control group. Dietary supplementation with UMMB of dairy buffaloes with medicated and non-medicated UMMB in two definite group increased milk yield and lactose significantly. In our study, medicated UMMB blocks resulted in substantial reductions in fecal worm egg counts and increased milk yield in dairy buffaloes. The result indicated that the use of UMMB as a strategic feed supplement will be economically beneficial.

Keywords: Buffalo, milk, UMMB

INTRODUCTION

Cattle and buffalo have significant contribution on national milk production. But these ruminants demand more feed due to their larger body size. During winter season, animal feed is in shortage by 36% in Nepal (Pande, 1997). Crop residue mainly rice straw, corn stover, wheat straw, millet straw, and other similar types of residues are used to feed these ruminant. These feed resources are very poor in nutrient contents which are even not able to supply the nutrients required for maintaining the animals. However, these feedstuffs can be improved in quality and utilized by supplementing with certain feed supplements such as urea molasses mineral block (UMMB). UMMB blocks can be prepared in desired size (generally of 2 kg in brick size) and offered for licking by the animals which provide them energy and protein for the rumen microbes. It has become important to mechanize its production, improve the composition, and increase the production to cover wide areas of distribution to benefit many dairy farmers in the country (Avilla 2006).

UMMB contains urea, molasses, rice bran, and binding agents. Different ingredients used in UMMB have major roles in ruminant feeding such as (1) Urea: provides small amount of extra nitrogen required for utilization of the dry matter. Nitrogen must be sufficient to maintain the ammonia level in the rumen, at a constant and sufficient amount for better development of the rumen microbes leading to better degradation of the cellulosic components. (2) Molasses: a good source of energy, being a concentrated plant juice, provides a range of minerals (except phosphorus) and a complete mixture of vitamins. It should have more than 800 Brix. (3) Minerals: they correct the macro and trace minerals deficiency in animal diet (4) Cement or quick limes: it is used as binding agent. The use of UMMB feed as supplement is to improve on the nutrient contents and straw utilization. The blocks are easy to handle, transport, and commercially feasible to manufacture (Avilla 2006).

Sharma et al. (1971) reported that, goat treated with ethanol extract of Neem (*Azadirachta indica*) leaves

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at the dose rate of 100 mg/kg body weight showed 81% efficacy against nematode on 9th day. Neem has got good anthelmintic property because Neem leaf powder contains Azadirachtin. In toxicological test, the Environmental Protection Agency (1985) was unable to establish the LD₅₀ for major component found in Neem, the side effect or symptoms couldn't be recorded even in highest possible dosage. As a result no residual or withdrawal period is necessary following treatment with Neem.

Feeding of UMMB to cattle usually results in increasing in feed intake by 25-30% and its digestibility (Aarts et al., 1990; Upreti, 2008). It is because small farmers have limited feed resources, which are generally low in essential nutrients and thus unable to support efficient rumen fermentation. Low-ammonia concentration in rumen fluid is a major limiting factor for achieving optimum microbial growth and digestibility. A solidified UMMB provides critical nutrients namely nitrogen as urea, readily available energy as molasses, and minerals for efficient microbial fermentation in the rumen. UMMB supplementation in animal feed increases microbial protein in the animal body which saves the expensive concentrates. It has also been documented that feeding UMMB increases or maintains body condition, and production of ruminant animals during winter on straw based diet. Therefore, UMMB is nearly a complete diet for ruminants as it contains most of nutrients. Supplementation of deficient nutrients in the form of urea-molasses multi nutrient block has been shown to be very effective in ruminant production system (Khanum et al, 2006). The main aim of the study is to know the the effect of medicated and non-medicated UMMB against gastrointestinal parasites and on milk production and composition in buffalo.

MATERIALS AND METHODS

Experimental Site and Time Period: The experimental trial was conducted in the livestock farm of Agriculture and Forestry University, and “Dhakal Dairy farm” Chitwan, Nepal from February, 2015 to April, 2015.



Animals and Management

45 lactating crossbred buffaloes were selected for the experiment. Each animal were ear tagged for identification of animal. All the animals were normal management condition in the farm. Fresh drinking waters were given to the animals throught out the experimental period. Rice straw was offered *ad libitum*. The animals were taken to the grazing as usual like other animals.

Experimental Design

The research was conducted using randomized complete block design (RCBD) in three different treatment groups.

T₁ = T₂ + powder of Neem leaves (i.e. normal feeding + 500g UMMB + neem powder)

T₂ = T₁ + 500 g UMMB/day (normal feeding + UMMB)

T₃ = on normal feeding (control)

Based on milk production 45 lactating buffaloes were divided into 15 blocks. 3 treatments allocated in each block in Randomized complete block design (RCBD).

Table 1. Randomized complete block design (RCBD)

B ₁ T ₁	B ₂ T ₂	B ₃ T ₃	B ₄ T ₁	B ₅ T ₁	B ₆ T ₂	B ₇ T ₃	B ₈ T ₁	B ₉ T ₂	B ₁₀ T ₃	B ₁₁ T ₁	B ₁₂ T ₁	B ₁₃ T ₂	B ₁₄ T ₃	B ₁₅ T ₂
B ₂ T ₂	B ₂ T ₃	B ₃ T ₂	B ₄ T ₃	B ₅ T ₂	B ₆ T ₃	B ₇ T ₂	B ₈ T ₃	B ₉ T ₃	B ₁₀ T ₂	B ₁₁ T ₃	B ₁₂ T ₂	B ₁₃ T ₃	B ₁₄ T ₂	B ₁₅ T ₃
B ₁ T ₃	B ₂ T ₁	B ₃ T ₁	B ₄ T ₂	B ₅ T ₃	B ₆ T ₁	B ₇ T ₁	B ₈ T ₂	B ₉ T ₁	B ₁₀ T ₁	B ₁₁ T ₂	B ₁₂ T ₃	B ₁₃ T ₁	B ₁₄ T ₁	B ₁₅ T ₁

Note: Experimental layout in RCBD with three treatment replicated 15 times each., B = Block, T= Treatment

- The observation was taken in 30 days interval at 0, 30th and 60th days by using EPG technique for egg count
- Daily milk yield will be recorded from January to March, 2015.



- Chemical composition of milk (Total solid, SNF, protein, lactose, conductivity, fat %) will be recorded fortnightly.
- Proximate composition of UMMB block was tested.

UMMB preparation and block composition

The preparation of UMMB has of four stages: Preparation of feed ingredients; as per the formula presented below.

- Mixing the feed ingredients: Small amounts were mixed first and added to the bulk and then thoroughly mixed.
- Molding: Mixed materials were packed in metal container (capacity of 2 kg) and pressed with blocker for 3 minutes.
- Drying: Blocks were dried under shed for 7 days.

The ingredients composition of 2 formulated UMMB blocks, as formulated by Animal Nutrition Division, NARC, whereas analytical result is presented in Table 2.

Table 2. Block composition

Ingredients	Percentage
Urea	10%
Molasses	32%
Rice bran	36%
Minerals	6%
Common salt	5%
Cement	5%
PowderNeem leaves*	300mg/kg body wt

*only in medicated UMMB

Table 3. Nutrient analysis of UMMB blocks

	Medicated UMMB	Non-Medicated UMMB
Dry Matter	92%	92.8%
Ether Extract	3.4%	2.9%
Crude Fiber	9.2%	8.1%
Crude Protein	21.74%	19.90%
Ash (minerals)	7.8%	8.2%

RESULTS

Milk Production

Mean milk yield (kg) of buffalo supplemented diet with or without UMMB is presented in Table 4. Significantly effectiveness ($P < 0.05$) of UMMB was observed on milk production of buffalo supplemented fed with UMMB.

Accordingly, higher milk production was recorded in diet supplement with UMMB medicated (503.44 kg) treatment group. During 90 days of experimental period, there was increment in milk production by 8.54% in medicated UMMB fed animal as compared to control group, which is statistically similar to non medicated UMMB supplemented group, where increment was by 8.53 % as compared to control group. However, lower milk production was recorded in buffaloes without UMMB supplement (control group).

Table 4. Mean milk yield (kg) of buffalo supplements diet with Medicated UMMB , UMMB and control group

Treatment	Total 90 days(kg)	Daily(kg)	% increment
Medicated UMMB	503.44	5.59A	8.54
UMMB	503.40	5.59A	8.53
Control	463.80	5.15B	
F-Value	39.48	39.48	
Prob	< 0.01	< 0.01	
CV %	2.88	2.88	
Lsd	10.55	0.12	

Means in column with different superscript differ significantly by L.S.D($P < 0.05$)

**Milk Lactose**

Mean milk lactose of buffalo supplemented diet with or without UMMB is presented in Table 5. Significantly effectiveness ($P < 0.05$) of UMMB was observed on milk lactose of buffalo supplemented diet with UMMB.

Higher milk lactose was recorded in diet supplement with UMMB medicated (4.92) treatment group. During 90 days of experimental period, there was increment in milk lactose by 4.46% in medicated UMMB fed animal as compared to control group, which is statistically similar to non-medicated UMMB supplemented group, where increment was by 4.88% as compared to control group. However, lower milk lactose was recorded in control group without UMMB supplement group.

Table 5. Mean milk lactose of buffalo supplements diet with medicated UMMB, UMMB and control group

Treatment	Lactose	Increment (%)
Medicated UMMB	4.92A	4.46
UMMB	4.94A	4.88
Control	4.71B	
F-Value	4.42	
Prob	< 0.05	
CV %	4.93	
LSD	0.18	

Means in column with different superscript differ significantly by L.S.D ($P < 0.05$)

Milk Protein

Mean milk protein of buffalo supplemented diet with or without UMMB is presented in Table 6. Significantly effectiveness of UMMB was not observed on milk protein of buffalo supplemented diet with UMMB. However, higher milk protein was recorded in diet supplement with medicated UMMB (4.11) treatment group. During 90 days of experimental period, there was increment in milk lactose by 0.24% in medicated UMMB fed animal as compared to control groups. However, lower milk lactose was recorded in group with UMMB supplemented group, which is lower by 0.49% as compared to control group.

Table 6. Mean milk protein of buffalo supplements diet with medicated-UMMB , UMMB and control group

Treatment	Protein	Increment (%)
Medicated UMMB	4.11	0.24
Control	4.10	
F-Value	0.04	
Prob	> 0.05	
CV %	8.17	
LSD	-	

Milk SNF

Mean milk SNF of buffalo supplement diet with or without UMMB is presented in Table 7. Significantly effectiveness of UMMB was not observed on milk SNF of buffalo supplemented diet with UMMB.

Higher milk SNF was recorded in diet supplement with UMMB Medicated (9.92) treatment group. During 90 days of experimental period, there was increment in milk SNF by 3.12% in medicated UMMB fed animal as compared to control group, which is statistically similar to non-medicated UMMB supplemented group, where increment was by 2.08% as compared to control group. However, lower milk SNF was recorded in control group.

Table 7. Mean milk SNF of buffalo supplements diet with medicated-UMMB, UMMB and control group

Treatment	SNF	Increment (%)
Medicated UMMB	9.92	3.12
UMMB	9.82	2.08
Control	9.62	
F-Value	2.19	
Prob	> 0.05	
CV %	4.07	
LSD	-	



Milk Fat

Mean milk fat of buffalo Supplement diet with or without UMMB is presented in Table 8. Significantly effectiveness of UMMB was not observed on Milk SNF of buffalo supplemented diet with UMMB.

Higher milk fat was recorded in diet supplement with non-medicated UMMB (8.42) treatment group. During 90 days of experimental period, there was increment in milk fat by 8.23% in non-medicated UMMB fed animal as compared to control group, which is statistically similar to medicated UMMB supplemented group, where increment was by 3.73% as compared to control group. However, lower milk fat was recorded in control group.

Table 8. Mean milk fat of buffalo supplements diet with medicated-UMMB, UMMB and control group control group

Treatment	Fat	% increment
Medicated UMMB	8.07	3.73
UMMB	8.42	8.23
Control	7.78	
F-Value	1.10	
Prob	> 0.05	
CV %	14.62	
Lsd	-	

Egg per Gram

Table 9. Egg per Gram (EPG) of buffalo supplements diet with Medicated UMMB, UMMB and control group

Treatment	Day 0	Day 30	Day 60	Day 90
T1	853.3 A	633.3 B	640.0 B	600.0 B
T2	840.0 A	793.3 AB	800.0 AB	806.7 A
T3	860.0 A	853.3 A	886.7 A	933.3 A
F-value	0.02	3.03	3.66	7.51
Probability	>0.05	>0.05	<0.05	<0.01
CV%	31.22	33.29	32.66	30.49
LSD	198.7	189.2	189.5	177.9

Means in column with different superscript differ significantly by L.S.D (P<0.05)

Mean egg per gram (EPG) of buffalo supplemented diet medicated-UMMB, UMMB and without UMMB is presented in Table 9. Before start of the experimental trial the mean EPG count was non-significantly different with each other. After one month of the trial, EPG count of buffalo fed with medicated-UMMB was significantly (P<0.05) different with control group. Similar result was also observed after 60 days. However after 90 days of experimental trial, the EPG count of buffalo fed with medicated UMMB was remarkably (P<0.01) different with EPG count of buffalo fed with non-medicated UMMB and control group.

DISCUSSION

In this study increase in milk production and milk lactose was observed significantly. However, milk protein, SNF and fat were slightly increased but did not differed significantly. Improvement in the milk yield due to UMMB supplement to paddy straw in low yielding cattle was reported (Kunju, 1998). Similarly, Mandal et al. (2001) reported that there is positive correlation between gradually increased UMMB intake and level of milk yield. Generally, protein intakes around normal level had little effect on protein content of milk conversely, extra protein or nitrogen intake may increase NPN content in milk (Roffler and Satter, 1975). Crude protein (21.74%) was similar as 23% reported by Avilla (2006).

UMMB supplemented buffaloes recorded higher level of milk production in both treatments compared to the control groups. A higher milk production level low recorded in medicated-UMMB group. Increment in average milk yield by 8.54% was found in UMMB fed animals as compared to control group. An average increment of 8% milk yield was recorded following UMMB supplementation during postpartum (Brar and Nanda, 2002). Similarly, earlier study Makkar and Saijpal (1996) also reported 6-8% increase in milk production in cows consuming 400-500g UMMB daily. Parera and Parera (2000) reported that Nilli-Ravi buffaloes under field conditions gave 11% more milk daily when supplemented with UMMB as compared to commercial concentrate. This increment



production level is lower as reported by Avilla (2006) where he reported 21% milk increment. Similar result was reported by Upreti et al. (2010). They reported 17.7% increment in milk production. Indonesian work has shown that the potential of milk increment is 14% per lactation (Makkar, 2001).

There was lactose increment of 4.46% and 4.88% on two treatment of UMMB feeding which is statistically significant. There was increment of milk fat 3.73% and 8.23% in medicated UMMB and UMMB fed group. Study in Indonesia showed that increase in fat was 11% with UMMB supplementation (Makkar, 2001). Upreti et al. (2010) reported UMMB supplemented group had higher fat increment level of 0.68 % compared to control group. Increment in 0.5% milk fat was recorded following UMMB supplementation postpartum (Brar and Nanda, 2002).

In this study there was increment of 3.12% and 2.08% of milk SNF in medicated UMMB and UMMB fed group. Makkar (2001) reported 3% increase in SNF by UMMB treatment. Accordingly, Upreti et al. (2010) reported increment of SNF by 5%. Yield of milk fat, milk protein and milk protein percentage were unaffected by treatment (Inostroza et al., 2010) which is similar to result of this study.

CONCLUSION

Dietary supplementation with UMMB of dairy Buffalo with medicated and non-medicated UMMB in two definite groups increased milk yield and lactose significantly. In our study, medicated-UMMB blocks resulted in substantial reductions in faecal worm egg counts and increased milk yield in dairy buffaloes. Even though supplementation of UMMB had an overall positive effect for the traits studied, the response of buffaloes in two groups varies from traits to traits. All results indicated that the use of UMMB as a strategic feed supplement will be economically beneficial.

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PERFORMANCE OF MURRAH BUFFALOES: A CASE OF KASKI DISTRICT OF NEPAL

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ABSTRACT

An attempt was made to evaluate the performance of Murrah buffalo (*Bubalus bubalis*) in Kaski district from December 2015 to September 2017. This study was conducted in Lahachowk Krishi Farm, probably biggest farm of buffalo in Nepal. Data related to management, breeding, healthcare and performance of the animals were taken from the formal data recording format developed for the farm. Data were entered in Microsoft excel and analyzed by R software package version 3.2.4. Total of 209 animals were evaluated to find out its productive and reproductive performance. Different productive performance such as 305 days milk yield, total lactation yield, lactation length and dry period were found 2653±45.78 lit, 2840±51.65 lit, 335.5 ±3.93 days and 112 days respectively. Effect of parity on all traits was found significant except lactation length. Similarly, milk composition of buffalo including milk fat, SNF, protein and lactose content of the milk sample were observed 7.17±0.19, 7.59±0.1 3.42±0.05, and 3.34±0.05, respectively. Similarly, maximum daily milk yield and maximum 305 day lactation yield were found 19 lit. and 4655 lit., respectively indicating high potential for genetic improvement. Different reproductive traits such as, average age at first calving, calving interval was observed 41.27±0.58 month and 440± 2.39days, respectively. Results of study suggested that buffalo production has great scope in Nepalese condition. More number of elite farms as considered in present study may help in boosting production as well as successful running of genetic improvement program.

Keywords: Murrah, lactation, dry period, milk composition

INTRODUCTION

Livestock is an integral component of Nepalese farming system that contributes about more than 25% of the Agricultural GDP. Farmers of western hills perceive buffaloes as first rank animals among livestock species in terms of household income (Rasali, 1997). Buffaloes have versatile role in rural households and national economy and are raised for supply of animal protein, draft power, and manure. Buffaloes are the major livestock commodity in Nepal. Nepal, in terms of buffaloes head, ranks fourth in Asia only after India, Pakistan and China (Pasha and Hayat, 2012). Buffalo enterprise alone contributes 52.9% of the livestock share in the national GDP and their population is 5.1 million buffaloes (CBS, 2011 and CBS, 2014). APP has given first priority to milk and then to meat. Buffaloes are the main source of milk and meat production in Nepal have been contributing about 65% and 54% of the total annual milk and meat production respectively (DLS, 2014).

Murrah buffaloes represent an important national genetic resource of the country. Cross breeding of the indigenous breeds of buffaloes with Murrah has been the national policy of the genetic improvement program (NARC, 2017). Therefore, it becomes imperative to genetically evaluate the production traits of Murrah buffaloes as a way of increasing their productivity through selection. Lahachowk Krishi farm is one of the biggest buffalo farm in Nepal. There are more than 300 Murrah buffaloes and their crossbreeds. This type elite farm can help in successful running of genetic improvement program. So, it is indispensable to genetically evaluate the performance of Murrah buffalo in Nepal.

MATERIALS AND METHODS

Source of Data

This study was carried out in Machhapuchchhre Gaupalika, Lahachowk Kaski which is located at an altitude of 1200 meters above sea level. Latitude and longitude of the area is 28.3244° N 83.9374° E respectively. Test day milk yield of 209 animals were collected at morning and evening at monthly interval. Animals having less than 6 test day milk yield was discarded as possibility of having infectious disease and reproductive abnormalities. In addition, milk sample of 60 animals were also collected at monthly interval at morning and evening to find out different milk constituents. Collected samples were analyzed by Lacto scan milk analyzer to find out Fat%, SNF%, Protein % and Lactose % of buffalo milk. Thus collected data were entered in Microsoft Excel and analyzed by R software package.

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Feeding and Management of the Farm

Animals are kept in intensive management by tiding with stanchion chain. More than 25 labors and 2 staff are engaged directly or indirectly in the farm. Animals were provided more than 4 Kg of concentrate which were available in market. In addition animals were provided 60 kg of chopped grass which were provided four times a day. Animals were showered three times a day in summer to decrease heat stress.

Table 1. Traits studied and their definitions

Traits	Definition
305 day yield	305 days yield after the cow is calved in its single lactation
Lactation yield	Total milk production in single lactation.
Age at first calving (AFC) (Months)	Interval between date of birth to the date of animal's first calving.
Calving interval (CI) (Days)	Interval between two successive calving.
Lactation length (LL) (Days)	Interval between date of calving to date dry off.
Dry period (DP) (Days)	Interval between date of dry off to subsequent calving

RESULTS AND DISCUSSION

Production Traits

Least-squares means (\pm SE) of different production traits are presented in Table 2. Overall daily milk yield of Murrah buffalo was observed 8.96 ± 2.3 L per day. Effect of parity in daily milk yield was found highly significant ($p < 0.001$). Daily milk yield of Murrah buffalo was lower in first parity and then gradually increases in second parity remain constant till fourth parity and then gradually decreases. Similarly, overall mean of 305 lactation milk yield and lactation milk yield of buffalo was observed 2652.8 ± 591.6 L and 2839.7 ± 680.6 L respectively. Effect of parity on 305 day lactation milk yield was found highly significant ($P < 0.001$). Maximum milk yield of 2899 ± 82.8 L and 3086 ± 95 L was observed in second parity. There is slight lower milk production in 1st parity and milk production becomes maximum in 2nd parity and then slightly decreases. This might be due to increase in body capacity of buffalo due to growth in 2nd parity and consequently reduced from sixth parity is due to aging in buffalo. The overall lactation length of buffalo was observed 322.03 ± 56.09 days. Effect of parity on lactation length was found non-significant. In addition, overall mean of dry period was observed 112.02 ± 34.27 days in buffalo. Effect of parity on dry period was observed significant ($P < 0.01$). Dry period was observed lower in first parity. There is not much difference in dry period between parity 2nd and 5th parity and then slightly increases. The highest value of productive traits and lower value of reproductive traits of Murrah buffalo in present study is due to favorable agro climatic condition and high management of buffalo in this farm. Only elite animals were purchased while expanding the number of animals and lower grade animals were immediately culled to favor high productivity. Similarly, milk composition of buffalo including milk fat, SNF, protein and lactose content of the milk sample were observed 7.17 ± 0.19 , 7.59 ± 0.1 , 3.42 ± 0.05 , and 3.34 ± 0.05 , respectively indicating rich of different milk constituents.

Table 2. Effect of parity on productive performance of Murrah buffalo

Parity	No of obs.	Daily milk Yield (lit.)	305 day milk yield (lit.)	Lactation length (days)	Lactation milk yield (lit.)	Dry period (days)
First	35	7.54 ± 0.38	2367.9 ± 100	345.27 ± 9.48	2608.6 ± 115	92.74 ± 5.79
Second	51	9.95 ± 0.32	2899 ± 82.8	319.85 ± 7.85	3086 ± 95	114.59 ± 4.8
Third	47	9.43 ± 0.33	2844.0 ± 86.3	329.14 ± 8.18	3068.4 ± 99.3	108.1 ± 5.0
Fourth	33	9.59 ± 0.39	2752.6 ± 103	304.91 ± 9.76	2872.9 ± 118.5	122.55 ± 5.937
Fifth	27	8.55 ± 0.44	2496.7 ± 113.9	313.23 ± 10.79	2665.2 ± 131	115.11 ± 6.6
Sixth	7	7.21 ± 0.86	2057.1 ± 223.6	306.94 ± 21.2	2228.6 ± 257.2	126.86 ± 12.95
More than six	9	6.6 ± 0.76	1932.2 ± 197.2	307.67 ± 18.7	2022.2 ± 226.9	133.5 ± 11.42
Overall Mean	209	8.96 ± 2.3	2652.8 ± 591.6	322.03 ± 56.09	2839.7 ± 680.6	112.02 ± 34.27
Level of Sig.		***	***	NS	***	**



Table 3. Effect of parity on different milk constituents and peak milk yield

Parity	No of obs.	Fat%	Protein %	SNF %	Lactose %	Peak Milk Yield (lit.)
First	10	6.98±0.47	3.57±0.11	7.86±0.24	3.46±0.1	9.47±0.33
Second	9	7.4±0.49	3.35±0.13	7.44±0.25	3.28±0.11	11.50±0.27
Third	11	7.13±0.44	3.34±0.11	7.45±0.23	3.27±0.1	11.09±0.28
Fourth	11	6.8±0.44	3.58±0.11	7.89±0.23	3.47±0.1	10.93±0.34
Fifth	8	7.7±0.5	3.3±0.13	7.36±0.27	3.2±0.12	9.72±0.37
Sixth	6	7.75±0.6	3.11±0.15	7.0±0.3	3.05±0.14	8.4±0.7
More than six	5	6.4±0.66	3.6±0.16	8.02±0.34	3.54±0.16	8.28±0.65
Overall Mean	60	7.17±1.4	3.42±0.37	7.59±0.76	3.3±0.35	10.73±1.88
Level of Sig.		NS	NS	NS	NS	***

Correlation between Lactation Yield and Different Milk Constituent's

Correlation between lactation yield and different milk constituents is shown in Table 4. Lactation milk yield was found to be negatively correlated to all milk constituents. Lactation yield was highly negatively correlated with fat percentage by -0.32 while minor negative correlation with SNF and protein %. Protein % and SNF % and protein and lactose % showed high positive correlation.

Table 4. Correlation between different milk constituents

	Lactation Yield (lit.)	Fat %	SNF %	Protein %	Lactose %
Lactation Yield (lit.)	1				
Fat %	-0.32441	1			
SNF %	-0.09399	0.254916	1		
Protein %	-0.03612	0.285579	0.948321	1	
Lactose %	-0.16418	-0.11727	0.415057	0.478442	1

This finding is supported by finding by Sigdel *et al.* (2014). They reported significant effect of parity on daily milk yield, annual milk yield and lactation yield of Murrah buffalo in Kaski district of Nepal. Thiruvankadan *et al.* (2014) reported parity had a highly significant ($P < 0.01$) effect on Murrah buffaloes in all the traits studied in Tamil Nadu India. First lactation peak yield was significantly ($P < 0.05$) lower than the rest. The yield increased from 1st to 4th parity and from there on it started declining. Similarly, he observed that the 305-day milk yield and lactation milk yield increased up to third parity, was maintained at fourth parity, and declined thereafter. Pairwise comparison revealed that the 305-day and lactation milk yields observed in first parity differed significantly ($P < 0.05$) from other parities. Dass & Sadana (2000) reported significant effect of season and period of calving and dam's parity on annual milk yield, lactation yield and lactation length of Murrah buffaloes. Average values of milk fat, SNF, protein and lactose percentages were recorded as 7.65±0.05, 9.36±0.02, 3.81±0.02, and 4.83±0.01, respectively by Dubey *et al.* (1998).

This finding is supported by finding by Yadav *et al.* (2013) on Murrah buffalo in Hissar, India. They observed milk yield, fat %, protein % and lactose % varied from 4.3 to 9.5 kg, 7.19±0.04 to 8.63±0.07%, 3.46±0.01 to 3.56 % and 4.36 to 4.60% respectively at center institute for research on buffalo, Hissar, India. Charlini and J Sinniah (2015) observed the least square mean for dry period was 179±4.43 days for different breed population in Srilanka. Verma and Yadav (1989) observed lower dry period of 20 days for Nili-Ravi in India. Freitas *et al.* (1995) reported lower genetic correlation between milk yield and fat% (-0.15) to present finding and higher genetic correlation between milk yield and protein% was -0.37 which was higher than present finding for cattle population. Mahato (2013) observed correlation between LYD and fat%, LYD and protein%, LYD and total solid, fat% and protein%, fat% and total solid, protein% and total solid were found -0.152, -0.059, 0.940, 0.124, 0.167 and 0.089 respectively for Jersey crossbreed in western region of Nepal.

Table 5. Reproductive traits of Murrah buffalo in Lahachowk Krihi Farm

S.N	Variable	Mean± Se	Min	Max
1	Age at first calving (month)	41.31±0.56 (25)	36.4	47.23
2	Calving Interval (days)	440±3.14 (209)	385	620

Overall least square mean and standard error of different reproductive traits of Murrah buffalo are presented in Table 5. Overall mean of age at first calving of the breed was observed 41.31±0.56 month in 25 Murrah populations. In addition average calving interval of the breed was observed 440±3.14 days in the present finding. These data



suggest good reproductive health management of the Murrah population in the herd. Thiruvankadan *et al.* (2014) observed calving interval of 532.8 ± 5.5 days and dry period of 230.2 ± 4.9 days in 1550 buffalo population in India from the period between 1979 to 2000. This finding is in contrast with finding by B Christa Charlini and J Sinniah (2015) observed the least square mean for age at first calving and calving interval for different buffalo population in Sri Lanka to be 48.2 ± 0.30 months and 470 ± 4.87 days respectively. However, slightly lower mean values were reported by Prakash *et al.* (1988) (43 months) for Egyptian buffaloes, Singh and Rathi (1990) (40 months) in India for Murrah buffaloes and Naqvi and Shami (1999) (43 months) for Nili-Ravi buffaloes in Pakistan. This finding is similar to finding by Komore *et al.* (1994) (453 days) in Sri Lanka and Banik and Tomar (2003) (453 days) for Murrah buffaloes in India. However, Thiruvankadan *et al.* (2014) observed calving interval of 470 days for different buffalo population in Sri Lanka. Hussain *et al.*, (2006) in India reported that, the calving interval in farm animals is mainly determined by service period.

CONCLUSION

This study suggests that productive performance of Murrah buffalo in western Nepal is very outstanding in a farm managed properly. Different breeding program should need to start to pool the outstanding character present in the farmer's level to national gene pool. Significant effect of parity on different productive traits suggests that it is better to cull animals after six parity.

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ARNA CROSS BREED: POTENTIALITY AND SCOPE IN KOSHI TAPPU REGION NEPAL

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ABSTRACT

To find out the possibilities of Arna (*Bubalus arnae*) crossbred, its economic values and future possibilities in Terai region of Nepal, we conducted comprehensive study on prevailing situation of Arna crossbred, their possible number in the region and future scope of this breed to address the current alarming buffalo marketing demands of Nepal. We used household survey including direct field visit and indigenous buffalo grazing land to collect data by using different data generation methods such as: key informant interview (KII), check list method and face to face interview on Arna crossbred, their production system, adoptiveness and future scope in Nepalese situation. Despite the fact that these purebred Arna being one of the threatened species from Nepal, also from the Asia, there was huge potentiality of incorporating its genetic potential in the local breed mainly Murrha and Terai buffalo. The data collected in the survey revealed that it has excellent meat quality and its genotype has huge potentiality in the export market system mainly in Bangladesh for unknown reason and probably might be due to its genetic potentiality and excellent fattening as compared to our prevailing breed. Our preliminary study suggests that, although these animal has relatively poor milking potentiality in current managerial practices in the farm, it has excellent meat value (tight muscles) and large body size. It is inevitable that these threatening species might have catastrophic and a concrete role in the current buffalo fattening research for strengthening to a new decade one in the days to come.

Keywords: Arnae crossbreed, threatened species, genetic potentiality, export based marketing

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TRANSLOCATION OF WILD ASIAN BUFFALO (*Bubalis arnee*): A WAY FORWARD FOR CONSERVATION IN NEPAL

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ABSTRACT

Wild Asian buffalo (*Bubalus arnee*) is listed as an endangered on the IUCN red list of threatened species and Appendix III of CITES. In Nepal, its population is restricted only in the Koshi Tappu Wildlife Reserve (KTWR). Diseases and parasites (transmitted by domestic livestock), rapid and complete inundation of the KTWR during the monsoon and frequent changes in river courses almost every year, cross breeding and competition for food and water between wild buffalo and domestic stock are also considered as serious threats. Therefore, it is necessary to find out alternate place where the habitat is suitable for sustainable conservation in Nepal. In this regard, 15 Wild Asian buffaloes have been translocated in 30 hector enclosure on Old Padampur area of Chitwan National Park. Park authority are maintaining the habitat similar to KTWR by different way i.e. fencing to protect them from threat by different sources, cutting the grasses, maintaining water from the Chure area, constructing ponds for wallowing, and building view tower for better monitoring of them. The foraging behaviour of buffalo was studied by using Focal Animal Sampling. Scan sampling was used to determine the herding pattern. The result shows that the performance of translocated Wild Asian buffalo was satisfactory as their foraging and herding behavior is similar to their natural habitat in KTWR. As tourism is one of the major activities around the park and many local people and their livelihood is sustained by the tourism activities, from this translocation of Wild Asian Buffalo can add the attraction of tourist which will be the opportunity to economic gain and livelihood improvement through the eco-tourism. Considering the proper habitat management of wildlife, translocation can be used as an effective management and conservation practice for endangered and spatially restricted species as well as boost the livelihood through the nature based tourism.

Keywords: Wild asian buffalo, conservation, translocation, habitat, livelihood.

INTRODUCTION

Translocation in the wildlife conservation is the capture, transport and release or introduction of species, habitats or other ecological material from one location to another. Relocating animals is a common management tool used for different conservation purposes. For example, animals that have become either globally or locally extinct in the wild are reintroduced to their historical range (Perelberg et al., 2003; Richards and Short, 2003; Bar-David et al., 2005; Brightsmith et al., 2005; Seddon et al., 2007). Animals are also translocated for rescue purposes (e.g., before intentional habitat destruction: Ostro et al. (1999), Richard-Hansen et al. (2000), and Edgar et al. (2005), for solving human-wildlife conflicts (Jones and Neelson, 2003; Wambwa et al., 2001), and for humanely reducing over population at the source site (Garai and Carr, 2001). Usually translocations and reintroductions are considered successful if they result in self-sustaining populations (Fischer and Lindenmayer, 2000). However, it may take a long time to evaluate whether a population is viable, especially when dealing with long-lived animals. Therefore, other parameters are often used to assess the ability of released animals to become established in their new home. Assessing translocations using behavioral and physiological measures and comparing them with a local resident population at the release site is especially useful when dealing with long-lived animals whose survival and reproductive success may take years to assess, and when management decisions must be reached rapidly.

Wild Asian Buffalo (*Bubalus arnee*) is listed as an endangered on the IUCN Red list of threatened species and Appendix III of CITES. In Nepal, its population is restricted only in the Koshi Tappu Wildlife Reserve (KTWR). Diseases and parasites (transmitted by domestic livestock), rapid and complete inundation of the KTWR during the monsoon and frequent changes in river courses almost every year, cross breeding and competition for food and water between wild buffalo and domestic stock are also considered as serious threats. Therefore, it is necessary to find out alternate place where the habitat is suitable for sustainable conservation in Nepal. In this regard, 15 Wild Asian buffaloes have been translocated in 30 hector enclosure on Old Padampur area of Chitwan National Park, Nepal. Park Authority is maintaining the habitat similar to KTWR by different ways i.e. fencing to protect them from threat by different sources, cutting the grasses, maintaining water from the Chure area, constructing ponds for wallowing, establishing view tower for better monitoring of them.

Several researchers have recommended for the translocation of buffaloes into either Chitwan or Bardia National Park (BNP) (Heinen 1993, Aryal et al., 2011). Both are much larger protected areas in lowland Nepal

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with abundant riverine habitat ideal for buffalo, as well as upland areas not prone to monsoonal flooding. Chitwan National Park (CNP) had a population of the species at least until the 1950s (Aryal et al., 2011), and is located closer to KTWR, making it the more feasible site for translocation. Chitwan also has extensive grassland areas with abundant adjacent riverine forests, constituting ideal habitat for buffalo, that have been extensively studied for the past several decades (e.g. Lehmkuhl 1998) and a much larger buffer zone than KTWR (Heinen and Rayamajhi 2001). Thus, the justification for a translocation is strong.

This study aims to assess the performance of those translocated wild water buffalo in their new environment using behavioral study, regarding the question, which are raised against translocation of wild animals. The research tries to cover herding pattern, site selection and foraging behavior of translocated wild asian buffalo (WAB). The behavior of translocation of WAB was studied by focal animal sampling, scan sampling and in addition, the site selection of enclosure by park administration was considered by comparing with natural environment of KTWR where they have survived for centuries. The overall objective of this research is to assess habitat management, herding pattern and foraging behavior of translocated wild water buffalo through the comparison of natural habitat on KTWR.

MATERIALS AND METHODS

The study was carried out in CNP where translocations of wild Asian buffalo (WAB) have been made. Translocated WAB were not released in natural habitat; and were kept in an enclosure. To compare the behavior and to draw the result of performance, the KTWR area was also studied where WAB were found to survive naturally. Point sampling techniques were used to observe the behavior of animals on three selected days and each of them was made at the interval of 7 days. The observation was made three times a day: morning (6-9 AM); noon (12-3 PM) and evening (3-6 PM). The Focal animal sampling was done for single animal with focus and all the activities were observed minutely. Continuous sampling was carried out in whole period and changes in the activities were recorded, while with the help of Scan sampling the behavior of all the individuals in a group of animals was recorded at predetermined time intervals.

RESULTS AND DISCUSSION

Natural Habitat Compared with Managed site (translocated)

Tropical forest, landscape which is modified by big rivers, flood plain and distributed open patch of grassland, running water, riparian habitat at KTWR have served as an existing habitat for WAB (Table 1). Whereas managed site for the translocated WAB at CNP were selected by the park authority at Old Padampur which covers about 30 ha area fenced with the mesh wire. The WABs were kept in enclosure for period to make them hardening off. Fencing of enclosure provided not only for protection and saved them from predators such as tiger and other wild animals but also helped in their regular monitoring. Observation of managed site showed the following parameters that better served good habitat for WAB as found in KTWR.

Table 1. Major parameters of the KTWR and CNP

Parameter	Natural habitat	Managed Site
Food	Koshi river determined grassland such as Sacchrom, Phragmites, beaches etc.	Rapti river determined grassland such as Sacchrom, Phragmites, beaches etc.
Drinking water	Source from the Koshi river	Source from the Churiya originated river
Wallowing	Source from the Koshi river.	Artificial and Natural Pond
Riparian habitat	Combination of plant habitat communities, shrubs, grasses, water i.e Koshi river	Combination of plant habitat communities, shrubs, grasses ,water i.e Churiya river source
Successional forest	Spend most of the scorching daylight in this forest.	Managed and protected so that they can spend day time.

Herding Pattern at Natural Habitat (KTWR) Compared to Translocated Site

The number of herd and number of individuals on each herd was recorded. During the observation, the number of individuals was found to disperse for moments and associate in a group likewise herd. The herd was assured based on stability of number of individuals for long time within group. The continuous sampling through which dispersed individuals were followed for period and when they were dissolved in a stable group; the group was taken as a herd and the number of individuals was recorded. Observations showed that highest number of herd in KTWR was observed in the morning i.e. 13 and it contained highest number of individuals i.e. 51, which was followed by the evening period. The lowest number of herd as well the number of individuals in herd was found on



the day period. While observing in the CNP, translocated site showed that the highest number of herd was observed in the evening i.e. 4 and individuals in the herd were 30 which was followed by the day period. The lowest number of herd was observed in the morning i.e. 2 and individuals in the herd were 18.

The range of individuals within a herd fluctuated from 5 -20; observed during data recording in KTWR while in the CNP only 15 individuals were translocated, so only 8-12 individuals were observed within a group. The group of herd was formed differently and observations were made in terms of females with calves and adult group, however males of young were often found to be solitary. It seemed that the males, which were old and weak and were ready for mating, were solitary in nature.

Foraging Behavior at Natural Habitat (KTWR) Compared to Translocated Site

The observation showed that the highest number of individuals on grazing in KTWR was recorded i.e. 31 and the time spent was 155 minutes. From the observation, it was found that they spent more time for searching for food and grazing which was followed by resting and wallowing, whereas the lowest number of individuals was recorded on sleeping i.e. 15 and their period was also lowest. Moreover, the time spent on grazing and searching of food was observed highest in the morning followed by the evening and lowest in the daytime. The highest time spent on wallowing was observed during daytime however, it was observed in the morning and the evening as well. Sleeping, fighting and mating were observed only in the morning rather than the day and the evening time. However, foraging behavior at translocated site (CNP) showed that the highest number of individuals was recorded on grazing and more amount of time was spent on searching food i.e. 43 and 215 minutes respectively among all observations (sum of the nine observations). Grazing was followed by resting i.e. 23 and 115 minutes among all observations. In the observation, the lowest time was spent on fighting. Only four individuals among nine observations were recorded on fighting. The highest time on grazing was observed in the evening followed by the morning and the daytime i.e. 90, 75 and 50 minutes respectively. The highest time on wallowing was also observed on the daytime rather than morning and evening. Resting, sleeping and fighting were recorded on each period.

Impact of Translocation on Livelihood

Chitwan National Park is well known for the nature based and cultural tourism. From the study of 2010 to 2016, it was found that Chitwan National Park remained in the second position for tourist attractions. On average 152586.8 tourists visit Chitwan National Park per year and NRs 116,854 royalty was received by CNP from approximately 87000 tourists in the year 2016 (NTS, 2016). Tourism has played a major role on livelihood of the people around the park. Many local people and their livelihood have sustained due to the tourism activities. In the year 2016, tourist flow decreased due to massive earthquake and many other reasons. Therefore, the translocation of wild Asian buffalo can be used to attract the tourists, which will help to increase the heavy and regular movement of researchers and visitors so that an opportunity of economic enhancement and improvement of livelihood through ecotourism can be ensured.

CONCLUSION

The first consideration that the researchers gave for the translocation of animals was site selection. The site should be as similar as possible from where the animals are translocated. Many of translocations have failed in the world due to wrong site selection. The forest types, grasslands where WWB were observed for searching food and grazing on flood plains of KTWR, which is also maintained in the enclosure area of CNP were found comparable. The peculiar habit of WWB is wallowing during daytime as well as most of the time; the availability of running water for wallowing in KTWR by Saptakoshi River is also maintained in enclosure by creating artificial ponds. Thus, the research reveals that the site that has been chosen for WAB is suitable. The park administration has maintained its maximum standard for WAB.

Within the large population of WWB in the KTWR, the individuals within a herd were 4-20 in KTWR and however, within very small population of translocated site, the range was observed 5-11 in the CNP. The highest number of herd was observed in the morning and in the evening in both sites. Activities such as grazing, wallowing and fighting were observed in the same period in both sites. Thus, the research revealed that the WAB from different sites started to make herd as normally as KTWR.

The foraging behavior observed in KTWR (natural habitat) showed that highest amount of hours of time was spent on grazing and searching of food in the morning and evening period in both sites. Similarly, wallowing was also similar in daytime in both sites. Besides, numbers of fighting was high in KTWR and lowest in CNP. The mating attempt was observed in the KTWR whereas not in the CNP. Based on the observation, the research concluded that foraging behavior of translocated WAB is normal. They have started to colonize and form the group in most of the activities such as grazing, wallowing, sleeping, fighting etc.



Translocation is the conservation activities which can enormously help to conserve spatially restricted species and reduce the risk of extinction. In this regard, this translocation of Wild Asian Buffalo has immensely played a vital role in conservation.

This research shows that the translocated Wild Asian Buffalo performance was found similar to the natural. Considering the proper habitat management of wildlife, this successful translocation of spatially restricted Wild Asian Buffalo can be used as an effective management and conservation practice and it can enhance the livelihood through the nature based tourism as well.

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SEASONAL VARIATION IN FAT AND SNF CONTENTS IN THE MILK OF MURRAH BUFFALOES IN MID-WESTERN REGION OF NEPAL

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ABSTRACT

A study was performed during July, 2016 to June, 2017 for a period of one year at Baijanath Rural Municipality Ward No. 6, Lalpur village of Banke district of mid-western region of Nepal to understand the seasonal fluctuation in fat and solid not fat (SNF) contents in the milk of Murrah buffaloes. Altogether, 1806 milk samples were taken into consideration for this study during different four seasons including spring (February to April), rainy (May to July), autumn (August to October), and winter (November to January). Data for both fat and SNF were normally distributed and altogether 276 milk samples were taken for spring, 181 for rainy, 302 for autumn and 327 for winter. Data collected in this study were analyzed using the latest version of GenStat computer software where multiple comparisons of the significantly different means were performed using Duncan's Multiple Range Test (DMRT). The overall mean fat percentage of Murrah buffaloes in this study was determined $5.13 \pm 0.019\%$ ranging from 2.00% to 5.14%, whereas the overall mean SNF percentage was observed $7.68 \pm 0.022\%$ ranging from 4.47% to 9.50%. There was great seasonal influence ($P < 0.01$) on fat and SNF content in the milk of Murrah buffaloes in this study. Accordingly, Least Square mean and standard errors for fat percentage for spring, rainy, autumn, and winter season were observed $5.13 \pm 0.04\%$, $5.37 \pm 0.67\%$, $5.13 \pm 0.04\%$, and $4.98 \pm 0.02\%$, respectively. Similarly, Least Square mean and standard error for SNF were observed as $7.87 \pm 0.04\%$, $7.28 \pm 0.73\%$, $7.59 \pm 0.04\%$ and $7.82 \pm 0.03\%$, respectively for spring, rainy, autumn, winter. Thus, results of present study reflected a great variation in fat and SNF contents in Murrah buffaloes' milk where those traits were significantly influenced by the differences in season. Based on the findings of present study, it is concluded that there is great scope of improving quality and quantity of buffalo milk in terms of fat and SNF through selection. However, it is important to determine the genetic parameters of these traits in that particular population.

Keywords: Murrah, seasonal variation, fat, SNF

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***Leucaena leucocephala*: A PROMISING FORAGE FOR IMPROVING SWAMP BUFFALOES PRODUCTION IN THAILAND**

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ABSTRACT

Leucaena (Leucaena leucocephala) a tree legume is interesting as a good feed ingredient, however, the toxic substances [mimosine and di-hydroxypyridine (DHP)] must be considered to limit its use, a series of studies aim to investigate whether which method is suitable to use as a feed for improving livestock production particular in swamp buffalo. In the first study, swamp buffaloes were fed the mixed dietary supplement leucaena with cassava meal (*Manihot culeit*) in the different ratios. Results showed that buffaloes consumed the increasing ratios of leucaena and cassava meal in mixed dietary supplement enhanced microbial supply to the small intestine. However, the tannins contents in leucaena is another anti-nutritional agent, therefore the low fibre digestibility in dietary supplement containing leucaena. Therefore, the second study showed that there are two ways of deactivates tannins containing in the leucaena, 1) sodium hydroxide (NaOH) treatment and 2) polyethylene glycol (PEG) treatment. The third study, toxicity of leucaena did not affect to swamp buffalo, when the proportions of leucaena in the mixed diet leucaena with ruzi grass (*Brachiaria ruziziensis*) containing not more than 25%, consequently, the mimosine + di-hydroxypyridine (DHP) in the urine was less 100 mg/kgBW^{0.75}. If the concentration of the mimosine + di-hydroxypyridine (DHP) in the urine were higher than the threshold concentration of 100 mg/kgBW^{0.75}, resulting in leucaena toxicity is indicative. Several studies demonstrated the advantages of using leucaena as the alternative feed ingredient, it is not improve the quality of feeds and minimize cost of feed in buffalo production, but also to increase N retention in soils results in a higher biodiversity and a lower methane releases in the atmosphere.

Keywords: Cassava meal, di-hydroxypyridone (DHP), *Leucaena leucocephala*, mimosine, rain tree pod (*Samanea saman*), swamp buffalo

INTRODUCTION

This paper aims to comply from 3 studies of using leucaena as a main feed ingredient to investigate that being suitable to use as feeds for feeding improvement in swamp buffaloes in order to reinforcement farmers to use, which were continuously conducted by the Research and Development Center for Livestock Production Technology, Faculty of Veterinary Science, Chulalongkorn University, Thailand, since 2007-present.

The studies of this paper are

1. The effects of different proportions leucaena and cassava meal in mixed supplemental diet in swamp buffaloes fed ammoniated rice straw as a basal diets on microbial production in the rumen
 2. The effects of PEG and NaOH treated leucaena on fibre digestibility
 3. The effects of *Leucaena leucocephala* added to Ruzi grass (*Brachiaria ruziziensis*) in different proportions to form mixed diets on purine derivatives and di-hydroxypyridine (DHP) in urine of Thai Swamp Buffaloes
- Study 1: The effects of different proportions leucaena and cassava meal in mixed supplemental diet in swamp buffaloes fed ammoniated rice straw as a basal diets on microbial production in the rumen (Source : the present study)

The use different proportions cassava meal added into chopped leucaena to form as a feed supplement in Thai swamp buffalo *ad libitum* receiving a basal diet of ammoniated rice straw. The study was conducted using a 4×4 complete Latin square design with 4 supplemental diets. Four male Thai, swamp buffaloes weighing 368±8.6 kg (30-36 month old) were allocated to one of four supplemental diets consisting of (kg on fresh weight/animal/day): i) 6.0 kg chopped fresh leucaena used as the control (100% LL), ii) 660 g cassava meal (CSM) + 5.0 kg chopped fresh leucaena (75% LL:25%CSM), iii) 1.32 kg sun-dried RPHP + 4.0 kg chopped fresh leucaena (50%LL:50%CSM), and iv) 2.0 kg sun-dried RPHP + 3 kg chopped fresh leucaena (25%LL:75%CSM).

Table 1 shows that proportions of cassava meal (readily fermentable carbohydrates) in dietary supplements increased, therefore the purine derivatives in urine (P<0.001), and efficiency microbial production in the rumen in buffaloes (PD/kg DOMI ratios) increased (P<0.05). It is possibly that proportions cassava meal increased, therefore cellulytic activity of microbail in the rumen enhanced and microbail production in rumen increased respectively, (Williams et al., 1984), but too high amounts of readily fermentable carbohydrates in diets may be reduce intakes

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of forages and digestibility of fibre (Khalli, 1993). This study showed that buffaloes consumed the increasing ratios of leucaena and cassava meal in mixed dietary supplement and fed with ammoniated rice straw enhanced microbial supply to the small intestine.

Table 1. Urinary purine derivatives excretion, the ratios of PD to DOMI urinary mimosine and DHP excretion in swamp buffalo fed *ad libitum* of ammoniated rice straw and supplemented with the different proportions of cassava meal added into chopped leucaena leaves

Items	Proportions of leucaena in dietary supplements				SED	Difference (P-value)		
	100% LL	75%LL	50% LL	25%LL		linear	quadratic	cubic
Number of animals	4	4	4	4				
Purine derivatives in urine (mmol kg BW ^{0.75} d ⁻¹)								
Allantoin	1.455 ^{bc}	1.414 ^c	1.646 ^b	1.869 ^a	0.106	0.001	<i>ns</i>	<i>ns</i>
Uric acids	0.071 ^a	0.063 ^b	0.068 ^{ab}	0.065 ^{ab}	0.004	<i>ns</i>	<i>ns</i>	<i>ns</i>
Total PDs	1.525 ^{bc}	1.476 ^c	1.715 ^b	1.935 ^a	0.107	0.001	<i>ns</i>	<i>ns</i>
PD/DOMI (mmol/kg DOMI)	39.13	38.40	43.81	44.12	3.396	0.05	<i>ns</i>	<i>ns</i>

¹Standard error of mean² averaged throughout experiments

^{2 abc}Values within the same column with different superscripts are significantly (P < 0.05) different.

³Values within the same column without different superscripts are not significantly (P < 0.05) different

(Source: Jetana et. al., 2011)

Study 2: The Effects of PEG and NaOH Treated Leucaena on Fibre Digestibility

There are usually two methods which have been used to inactive tannins and other secondary compounds in leucaena; the first method is the addition of chemicals with a high affinity for tannins, *such as* polyvinylpyrrolidone and polyethylene glycol (PEG) or gelatin, a synthetic polymer to which tannins have a greater binding affinity than protein (Kumar and D'Mello 1995; Salunkhe et al., 1990; Butler et al., 1986) and the second method is the use of alkaline treatments, *such as* NaOH solution or ash solution (Price et al., 1979). The second study was to find out which of the differently treated leucaena was suitable to be combined with ground RTP to form a protein supplement on whole tract digestibility of nutrients and urinary PD excretion in Thai swamp buffaloes fed rice straw as a basal diet.

Four male Thai, swamp buffaloes weighing 354±4.0 kg (24-36 months old) were used in this study. The experimental design was a 3×4 incomplete Latin square design and animals were allocated to 3 supplements. The three supplemental diets consisting of (kg on fresh weight): i) 500 g sun-dried leucaena leaves, 445 g oven dried RTP, 50 g cassava meal and 5 g premix (untreated leucaena) was used as the control, ii) 500 g leucaena treated with NaOH, 445 g oven dried RTP, 50 g cassava meal and 5 g premix (+NaOH), iii) 500 g sun-dried leucaena leaves, 445 g oven dried RTP, 50 g PEG and 5 g premix (+PEG).

Table 2. Dietary supplements (kg/day/animal) and chemical composition in supplements (g/kg on DM basis)

Ingredients	Supplements (kg on fed basis)		
	Control	+NaOH	+PEG
LL	2.00	-	2.00
LL treated with NaOH	-	2.00	-
RTP	1.78	1.78	1.78
Cassava meal	0.20	0.20	-
Poly ethylene glycol (PEG)	-	-	0.20
Premix	0.02	0.02	0.02

Control untreated sun-dried leucaena leaves, *CP* crude protein, *DM* dry matter, *N* nitrogen, *GE* gross energy, *LL* sun-dried leucaena, *MJ* Mega joules, *NDF* neutral detergent fiber, *PEG* poly ethylene glycol, *RTP* oven-dried rain tree pod, *+NaOH* LL treated with NaOH, *+PEG* LL added with PEG; Premix contained (g/kg DM basis): vitamin A 40,000,000 units, vitamin D3 4,000,000 units, and vitamin E 40,000 units, vitamin B12 0.02 g, Mn 160g, Fe 240 g, Zn 100 g, Cu 20g, Se 0.5 g, Co 2 g and I 5 g.



Whole Tract Digestibility

Table 2 shows dietary supplements (kg/day/animal). The digestibility coefficient of DM was lower ($P < 0.05$) in buffaloes consumed leucaena+PEG supplement than in those fed untreated leucaena diet, however there was not different between animals fed treated leucaena supplemental diets. None of supplements affected the digestibility coefficient of OM, but NDF digestibility was lower ($P < 0.05$) in animals supplemented with untreated leucaena than in those with the other supplements (Table 3). However, NDF digestibility increased in animals fed treated leucaena supplemental diets. The fibre digestion improved when animals fed leucaena treated with NaOH diet, possibly due to the NaOH solution breaking down the lignin bond and the disintegration of fibre reduced particle size and the passage rates increased (Klopfenstein 1978) and sodium salts increased osmotic pressure and rumen washout (Jackson 1977). Likewise, the fibre digestion increased in animals when fed leucaena+PEG diet, possibly sufficient rumen degradable protein (RDP) is available in the rumen due to tannins was inactive by PEG. It is possible that soluble carbohydrates in the rumen may decrease as it is binding with PEG (McSweeney et al., 2001; Brooker et al., 2000), this condition can elevate pH in the rumen, and this may be suitable to optimise cellulolytic microbial activity. Similar findings were reported by Silanikove et al. (1996); Waghorn et al. (1987); Barry et al. (1986), they found an increase in fibre digestibility of diet containing rich-tannins when supplemented with PEG.

Both treatments of leucaena by adding PEG and treating with NaOH solution to control tannins results in increasing fibre digestion. The use of NaOH solution treated leucaena are required chemicals, time of soaking and cost of NaOH, though the NaOH might be replaced by ash wood for saving cost but time and improvement of techniques still required. In the same way the addition of PEG in diets containing high phenolic compounds is a convenient method, but it increases cost. However, the forms of untreated leucaena combined with RTP in the present study have shown enhancing microbial production supply into small intestine but depressing fibre digestion.

Table 3. Intakes of DM, OM and NDF and the digestibility coefficients of DM, OM and NDF and N balance in Thai, swamp buffaloes fed a based diet of rice supplemented with different treated leucaena leaves

	Control	+NaOH	+PEG	S.E.D
Number of animals	4	4	4	
BW (kg)	353±10.4	356±13.2	355±9.00	
BW ^{0.75} (kg)	81.3±3.4	81.9±2.3	81.8±1.6	
Intakes (g/kg BW ^{0.75} /day)				
DM	66.6	66.2	66.5	0.56
OM	63.9	64.1	61.4	0.55
NDF	45.1	49.5	46.6	0.39
Apparent digestibility (decimals)				
DM	0.54 ^a	0.52 ^{ab}	0.50 ^b	0.02
OM	0.57	0.56	0.57	0.02
NDF	0.41 ^b	0.45 ^a	0.46 ^a	0.02
Urinary purine derivatives excretion (mmol/kg BW ^{0.75} /day)				
Allantoin	0.58 ^a	0.42 ^{ab}	0.34 ^b	0.09
Uric acid	0.09 ^a	0.04 ^b	0.07 ^{ab}	0.02
PD	0.67 ^a	0.46 ^b	0.40 ^b	0.10
Allantoin/DOMI (mmol/kg)	18.6 ^a	14.4 ^{ab}	11.4 ^b	3.12
PD/DOMI (mmol/kg)	21.3 ^a	15.8 ^{ab}	13.7 ^b	3.32

S.E.D : Standard error of difference (Least Significant Different/ $T_{\alpha 0.05}$)

^{ab}: Values within the same row with the different superscripts are significantly ($P < 0.05$) different

Values within the same row without superscripts are not significantly ($P > 0.05$) different

(Source: Jetana et. al., 2012)

Study 3: The effects of *Leucaena leucocephala* added to Ruzi grass in different proportions to form mixed diets on purine derivatives and mimosine+di-hydroxypyridine (DHP) in urine of Thai Swamp Buffaloes

Toxicity of leucaena (mimosine+DHP) in animals can determined by two methods; 1) direct methods, such as reduction of production, milk yield decreased, loss weight etc., and 2) indirect method such as, size of thyroid gland enlarge, lower hormone thyroxin in blood, the quantity of DHP in blood and urine. Earlier study, the information of excretion of mimosine and DHP in urine is a simple determination method to clarify that is the



toxicity of leucaena in animals (host) in justifying being safe when animals gave leucaena in diet. Though, leucaena can be *ad libitum* fed at level 100% of dry matter intake in goats and did not show any sign of effects on animal health in Thailand (Phaikeaw et al., 2012; Jetana et al., 2010). However, the studies suggested that only certain proportion of leucaena in mixed diets, when fed to goats, its enhances intakes, digestion and microbial production in the rumen (Jetana et al., 2014, Jetana et al., 2016). The study therefore was conducted to investigate the effects of feeding different proportions of leucaena in mixed diets [leucaena vs. Ruzi grass {RZG (*Bachiaria ruziziensis*)}] on purine derivatives and mimosine, 3, 4-DHP and 2, 3-DHP in urine.

Table 4. Urinary purine derivatives excretion, the ratios of PD to DOMI urinary mimosine and DHP excretion in swamp buffalo fed a different proportion of ruzi grass and leucaena leaves

	Proportions of leucaena level in the mixed diet					SEM	P-Value
	0.0	25%	50%	75%	84%		
Number of Animals	4	4	4	4	4		
PD in the urine ($\mu\text{mol/kgBW}^{0.75}$)							
Allantoin	373 ^a	231 ^b	248 ^b	291 ^b	216 ^b	49.7	<0.01
Uric acid	179 ^a	129 ^{ab}	145 ^{ab}	138 ^{ab}	99.2 ^b	44.4	<0.01
PD	551 ^a	360 ^{bc}	393 ^{bc}	428 ^b	315 ^c	68.8	<0.01
PD/DOMI	33.1 ^a	17.7 ^c	20.7 ^{bc}	27.3 ^{ab}	18.6 ^{bc}	6.76	<0.01
Mimosine intake (g/d)	0 ^e	34.6 ^d	68.5 ^c	103 ^b	145 ^a	4.35	<0.01
Mimosine intakes ($\text{g/kgBW}^{0.75}\text{d}^{-1}$)	0 ^e	0.54 ^d	1.06 ^c	1.61 ^b	2.23 ^a	0.07	<0.01
Mimosine and metabolites in the urine ($\text{mg/kg BW}^{0.75}/\text{day}$)							
Mimosine	0 ^e	0.70 ^b	0.81 ^b	1.35 ^a	1.56 ^a	0.38	<0.01
3,4-DHP	0 ^e	44.8 ^c	194 ^a	111 ^b	208 ^a	37.3	<0.01
2,3-DHP	0 ^d	15.3 ^c	29.6 ^a	19.0 ^{bc}	24.7 ^{ab}	4.70	<0.01
Mimosine+DHP	0 ^d	60.8 ^c	224 ^a	131 ^b	234 ^a	37.9	<0.01

BW^{0.75} metabolic body weight, DHP dihydroxy pyridine, SEM standard error of mean

^{abcd} within a row, means with different superscript differ ($P<0.05$)

PD Excretion in the Urine and the Ratios of PD: DOMI

Table 4 shows that increased proportions of leucaena leaves to ruzi grass in mixed diet depressed the efficiency microbial digestion in buffaloes (17.1, 20.7 and 18.6 mmol PD/kg DOMI, respectively) when compared to animals fed a based diet (33.1 mmol PD/kg DOMI). No difference in the efficiency microbial digestion in buffaloes, when animals fed the diet containing 75% of leucaena in mixed diet. The microbial yield in animals fed the mixed diets containing high proportions of leucaena were low, this is not surprising due to leucaena toxicity as a result of degradation of mimosine and DHP. Mimosine impedes cell division (Jones 1979), is acutely toxic and may cause rapid or violent deaths (Pratchett et al., 1991). According to Shelton (2009) had shown that mimosine toxicity is rare, due to mimosine usually being rapidly degraded by plant enzymes, certain unidentified members of the ruminal population and simple hydrogenation to the toxic goiterogen 3-hydroxy-4(1H)-pyridone (DHP). In addition to inactive proteins for microbial protein synthesis as a result of formation of tannin-protein complexes.

Mimosine and DHP Excretion in the Urine

The mimosine and DHP rapidly appeared in the urine of buffalo just the ingestion of fresh leucaena was about 1 h 20 min, after consumption (Alcântara et al., 1997). DHP excretions in the urine of buffaloes increased by the proportions of leucaena in the diets. This demonstrated that mimosine and DHP toxicity in leucaena was more responsive in buffaloes fed the diet containing leucaena more 25%. Animals consumed the mixed diet containing leucaena 25% leucaena (LL) vs. 75% ruzi grass, consequently the DHP concentration in urine of buffaloes containing 60.8 mgDHP/kg BW^{0.75}, the value was below 100 mgDHP/kg BW^{0.75} this value is used to classify that subclinical toxicity is considered to be anticipated. In short feeding of this study, none of the animals showed any visible toxicity symptom during the period the leucaena proportions in diets was fed. The toxicity of leucaena will not affect to buffaloes when different appropriate proportion of leucaena to ruzi grass were fed, in this study had shown that the diet containing leucaena not more 25% was highest benefit for buffaloes as the DHP in urine was less 100 mg/kgBW^{0.75}.



The benefits of incorporating leucaena in the diet achieved when toxicity in leucaena must be controlled (Shelton 2009) *i.e.* by limiting amount of intake, inoculating bacteria with high capable of degrading and detoxifying DHP into the rumen, treating with chemicals and supplementing with minerals: Fe, Mn, Zn *etc.* (Phaikaew et al., 2012).

CONCLUSION

Several studies demonstrated the advantages of using leucaena as the alternative feed ingredient, it is not improve the quality of feeds and minimize cost of feed in buffalo production, but also to increase N retention in soils results in a higher biodiversity and a lower methane releases in the atmosphere.

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UTILIZATION OF RAIN TREE PODS (*Samanea saman*) AS A SWAMP BUFFALO FEED IN THAILAND

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ABSTRACT

Characteristics of the mature rain tree pods (*Samanea saman*) are black-brown, oblong, lumpy, 10-20 cm long, 15-19 mm wide, ca. 6 mm thick, straight or slightly curved, not dehiscing but eventually cracking irregularly, and they are filled with a sticky, brownish pulp that is sweet and edible. Chemical analysis of the rain tree pod contained 760 g dry matter (DM)/kg as fresh-basis, 87.3 g ash, 22.0-32.0 g nitrogen, 330-460 g total nonstructural carbohydrates (TNC), 180-400 g total sugar (TS; sucrose 68.5%, glucose 5.5%, xylose 9.0%, galactose 4.2, fructose 8.9% and other sugar 4.0%), 15-70 g starch, 140-150 g reducing sugar (RS) and 170-250 g non reducing sugar (NRS) and 10-50 g total phenolic compounds. Metabolizable energy for ruminants is 10 mega joules/kg DM and organic matter digestibility is 840-870 g/kg DM. With high sugar content in rain tree pod, its can be used for substituting molasses. A series of studies was conducted on the processing and utilization rain tree pods (RTP) as an ingredient in swamp buffalo feed. Sun-dried rain tree pod pellets were fed as a dietary supplement in swamp buffaloes fed different treated rice straw. Results showed that digestibility of nutrients, N balance, and urinary PDs excretion depended on types of treated rice straw. Oven-dried ground rain tree pod also can be used as an appetizing material for combination with leaves of leucaena, mulberry (*Morus sp.*) and cassia siam (*Senna siamea*), therefore oven-dried ground rain tree pod as an ingredient in dietary supplement enhanced microbial supply to the small intestine. However, proportions of sun-dried rain tree pellets and leucaena in mixed diet increased, therefore the purine derivatives (PDs), mimosine+di-hydroxypyridine (DHP) in the urine and the ratios of purine derivatives to digestible organic matter intakes (PDs/DMI) increased. The processed rain tree pods, have been demonstrated to be a great ingredient feed for enhancing microbial protein production in the rumen.

Keywords: Concentrate pellet, di-hydroxypyridine (DHP), *Leucaena leucocephala*, oven-dried, rain tree pod (*Samanea saman*), sun-dried, swamp buffalo

INTRODUCTION

Rain tree actually is not a forage plant; however, product (pod) obtained after ripening and falling down from tree is valuable as an animal feed (Jetana et al., 2008; Staples and Elevitch. 2006.). Rain tree pod is a product of the rain tree (*Samanea saman*). This plant can be usually grown on tropical climate area, with relatively low inputs and no irrigation. Although rain tree has been cultivated for multiple-purpose trees, such as shadow, curved wood, furniture, fertilizer, cultivation of lac (*Laccifer lacca Kerr*) and livestock fodder. To use rain tree pod as a potential animal feed requires information on its chemical composition, nutritive value, anti-nutritional agents, and digestibility and product quality aspects. In the current paper, two of processing rain tree pods; sun-dried rain tree pod pellet and oven-dried ground rain tree pod were used to study.

Chemical Composition and Nutritional Value of Rain Tree Pod

Chemical analysis of the rain tree pod contained 760 g dry matter (DM)/kg as fresh-basis, 87.3 g ash, 22.0-32.0 g nitrogen, 330-460 g total nonstructural carbohydrates (TNC), 180-400 g total sugar (TS; sucrose 68.5%, glucose 5.5%, xylose 9.0%, galactose 4.2, fructose 8.9% and other sugar 4.0%), 15-70 g starch, 140-150 g reducing sugar (RS) and 170-250 g non reducing sugar (NRS) and 10-50 g total phenolic compounds. Metabolizable energy for ruminants is 10 mega joules/kg DM and organic matter digestibility is 840-870 g/kg DM.

Feeding Rain Tree Pod to Animals

With their special properties of rain tree pods consisted with 3 reasons; (1) the high nutritive values with both protein and fermentable carbohydrate content, (2) containing high OMD and ME content, (3) rain tree is one of the multiple-purpose trees, being planted in Thailand; the rain tree pod is available in dry season and it is suitable to make a pellet feed and can be kept for a long time. In briefly, after collecting the pods of rain tree were firstly ground through a 10-14 mm (Ø pore size) grinding plate prior to grinding twice passing through a 5-7 mm (Ø pore size) grinding plate using 10-Horsepower electrical meat grinder. The rain tree pod pellets were drying by sun for 12-18 h, and keeping in air-tight storages before using them. On the other hand, RTP were dried in a hot oven at

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75 °C for 72 h after collecting, then immediately ground (<4 mm Ø) prior to using or keeping in air-tight storages (oven-dried rain tree pods).

The present paper aims to evaluate the processed rain tree pods 2 types, sun-dried rain tree pod pellet and oven-dried ground rain tree pod are used as a supplement diet in buffalo feed in 3 experiments : (1) sun-dried rain tree pod pellet as a feed supplement to swamp buffaloes fed different treated rice straw as a basal diet, (2) oven-dried ground rain tree pod compared with three rich protein leaves of leucaena, mulberry and cassia as a feed supplement to swamp buffaloes fed rice straw as a basal diet, and (3) different proportions of sun-dried rain tree pod pellets added to fresh chopped leucaena leaves to form dietary supplements on purine derivatives and di-hydroxypyridine (DHP) in urine in Thai swamp buffaloes.

Effects of sun-dried rain tree pod pellet as a supplement diet fed to swamp buffaloes receiving different treated rice straw as a basal diet on digestibility of nutrients, N balance, urinary purine derivatives excretion and microbial production in the rumen (Jetana et al., 2015; Jetana et al., 2017)

Four Thai swamp buffaloes with an average weight of 300±12.6 kg were used in this study. Each of the animals were fed twice daily with 1.5 kg of sun-dried rain tree pod pellets per time (3.0 kg as fed basis/day) as a supplemental diet. After animals finishing rain tree pod pellets approximately 5-10 minutes, animals fed *ad libitum* fives a day with one of the four treated rice straw; untreated rice straw (RS), rice straw treated with 1% ammonium hydroxide solution (NH₄OH) [RSAM], rice straw treated with bio-extract solution of rain tree pods (RSEM), and RSAM treated with 1% bio-extract solution of rain tree pods (RSEM). The experimental design was 4 × 4 Latin square with arrangement of rice straw treatments. The experiment consisted of four 21-day periods; each comprising 14 day dietary adaptation and 7 days collection.

The chemical compositions of rain tree pod pellets which used as a supplement diet is given in Table 1 (g /kg DM).

Table 1. Chemical components of feed (g/kg DM basis)

Components	RS	Rice straw treated Bio-extracted solution	RS treated NH ₄ OH	RSAM treated EM	Rain tree pod pellets
	(RS)	(RS+EM)	(RSAM)	(RSAM+EM)	(RTP)
DM	874	393	868	415	708
OM	989	845	960	868	962
NDF	775	750	700	750	374
ADF	575	475	550	525	287
N	8.9	12.8	14.3	14.8	25.9
N×6.25	55.9	80.0	89.4	92.5	162

Table 2 shows animals fed RSEM, were lower in intake and digestibility than animals fed other treated rice straw, its expected that the low pH caused from high sugar in the RTP bio-extracted solution. Resulting in depression of fibre degradation due to the utilization of readily fermentable carbohydrates (sugar in RTP) caused the reduction of fibre digestibility (Mertens, 1977).

Table 2. Intakes and digestion, N balance and purine derivatives excretion in the urine of swamp buffaloes fed different treated rice straw as the basal diets with rain tree pod pellets supplement.

Item	Non-Ammonium Hydroxide		Ammonium Hydroxide		SED
	RS	RSEM	RSAM	RSAE	
Number of animals	4	4	4	4	
Live Body weight(kg)	297	302	298	303	13.2
Metabolic Body weight (kg)	71.5	72.2	71.4	72.4	2.02
Rain tree pod pellets intakes (g/kg BW ^{0.75} d ⁻¹)					
DM RTPP	29.9	30.0	30.4	29.9	0.99
OM RTPP	28.8	28.8	29.3	28.7	0.95
Rice straw Intakes (g/kg BW ^{0.75} d ⁻¹)					
DM Treated Rice straw	58.5 ^a	52.4 ^b	56.7 ^{ab}	53.2 ^{ab}	3.08
OM Treated Rice straw	50.6 ^a	44.3 ^b	47.2 ^{ab}	46.1 ^{ab}	2.65
Total intakes (g/kg BW ^{0.75} d ⁻¹)					
DM	88.4 ^a	81.5 ^b	87.1 ^{ab}	82.2 ^{ab}	2.99



Item	Non-Ammonium Hydroxide		Ammonium Hydroxide		SED
	RS	RSEM	RSAM	RSAE	
OM	79.3 ^a	72.4 ^b	76.5 ^{ab}	74.2 ^{ab}	2.58
Digestion (g/kg)					
DM	520 ^a	473 ^b	520 ^a	484 ^{ab}	19.7
OM	571 ^a	527 ^b	548 ^{ab}	544 ^{ab}	17.4
N balance					
N in treated Rice straw	497 ^c	644 ^b	777 ^a	756 ^a	37.6
N in RTPP	573	574	583	572	38.4
Total N intakes	1070 ^c	1218 ^b	1360 ^a	1316 ^a	17.5
N in urine	206 ^b	229 ^b	336 ^a	366 ^a	38.2
N in feces	828 ^b	897 ^{ab}	921 ^a	875 ^{ab}	42.8
N balance	35.9 ^b	92.0 ^a	103 ^a	75.7 ^a	18.6
	Purine derivatives in urine (mmol kg BW ^{0.75} d ⁻¹)				
Allantoin	0.267 ^c	0.372 ^b	0.376 ^b	0.531 ^a	0.043
Uric acids	0.134 ^c	0.300 ^{ab}	0.239 ^b	0.330 ^a	0.043
Total PDs	0.401 ^c	0.672 ^b	0.615 ^b	0.860 ^a	0.077
PD/DOMI (mmol/kg DOMI)	9.107 ^c	17.91 ^b	15.91 ^b	24.70 ^a	2.17

¹Standard error of mean² averaged throughout experiments

^{2,abc}Values within the same column with different superscripts are significantly (P < 0.05) different.

³Values within the same column without different superscripts are not significantly (P < 0.05) different

Animals fed untreated RS had lower (P < 0.05) N balance than in those fed RSAM, RS+EM and RSAM+EM. N balance was generally lower (P < 0.05) in animals fed untreated RS than in animals fed treated rice straw, this indicated that the N balance was better in animals fed treated RS, particular in treated RS containing higher N. The amounts of PD excretion in the urine of swamp buffaloes always are too low to use as microbial marker to estimate the microbial yields, with several reasons; renal clearance (Jetana et al., 2006), N recycling (Kennedy et al., 1990) and GFR (Norton et al., 1979). Nevertheless, this study demonstrated allantoin and total PD excretion in the urine highly relationship with digestible organic matter even though the total PD excretion in the urine were rather low. The present study implies total urinary PD excretion and the efficiency of microbial production in the rumen (PD/DOMI) improved in animals when fed treated RS than that in animals fed untreated RS, as resulting in increasing N content in treated RS. This was in agreement with Jetana et al. (2009), who found PD excretion and microbial protein synthesis increased in animals fed increasing level of N content diets.

Effects of oven-dried ground rain tree pod compared with three rich protein leaves of leucaena, mulberry and cassia as a supplement fed to swamp buffaloes receiving rice straw as a basal diet on urinary purine derivatives excretion and microbial production in the rumen (Jetana et al., 2011).

The use of oven-dried ground rain tree pod compared with three rich protein leaves of leucaena, mulberry and cassia as a feed supplement to Thai swamp buffalo receiving a basal diet of rice straw. The study was conducted using a 4x4 complete Latin square design with 4 supplemental diets. Four male Thai, swamp buffaloes weighing 266±1.6 kg (24-30 month old) were allocated to one of four supplemental diets consisting of (kg on fresh weight): i) 990 g oven-dried RTP and 10 g premix (RTPP) used as the control, ii) 490 g oven-dried RTP and 500 g sun-dried leucaena leaves and 10 g premix (LLRT), iii) 490 g oven-dried RTP and 500 g sun-dried cassia leaves and 10 g premix (CLRT) and iv) 490 g oven-dried RTP and 500 g sun-dried mulberry leaves and 10 g premix (MLRT) (Table 3).

Table 3. Dietary supplements (kg/animal/day) and chemical composition in supplement (g/ kg on DM basis)

Ingredients	Supplement Supplement (kg on fed basis)			
	RTPP	LLRT	CLRT	MLRT
RTP	3.96	1.96	1.96	1.96
LL	-	2.00	-	-
CL	-	-	2.00	-
ML	-	-	-	2.00
Premix	0.04	0.04	0.04	0.04



CL sun-dried cassia leaves, *CLRT* RTP+*CL* and premix, *LL* sun-dried leucaena leaves, *LLRT* RTP+*LL* and premix, *ML* sun-dried mulberry leaves, *MLRT* RTP+*ML* and premix, *RTP* oven-dried rain tree pod, *RTPP* RTP and premix (Premix contained (g/kg DM basis): vitamin A 40,000,000 units, vitamin D3 4,000,000 units, and vitamin E 40,000 units, vitamin B12 0.02 g, Mn 160g, Fe 240 g, Zn 100 g, Cu 20g, Se 0.5 g, Co 2 g and I 5 g)

None of supplements affected the digestibility coefficients of DM and OM, but NDF digestibility was lower ($P<0.05$) in animals supplemented with *RTPP* than in those supplemented with the other supplements (Table 4). The urinary PD excretion was higher ($P<0.05$) in animals supplemented with *RTPP* than in those supplemented with *LLRT*. However, there were no differences in urinary PD excretion among animals supplemented with *RTPP*, *CLRT* and *MLRT*. The ratio of PD/DOMI was lower ($P<0.05$) in animals supplemented with *LLRT* than in those supplemented with *RTPP* and *MLRT*, but the ratio of PD/DOMI in animals supplemented with *CLRT* and *LLRT* was similar.

Table 4. Intakes of DM, OM and NDF and the digestibility coefficients of DM, OM and NDF, N balance and purine derivatives excretion in the urine of Thai, swamp buffaloes fed a based diet of rice supplemented with different protein-rich leaves

	RTPP	MLRT	CLRT	LLRT	S.E.D
Number of animals	4	4	4	4	
BW (kg)	265±3.4	263±3.6	264±3.0	270±3.0	
BW ^{0.75} (kg)	65.7±0.6	65.3±3.6	65.4±0.3	66.6±0.6	
Intakes (g/kg BW ^{0.75} /day)					
DM	82.0	83.7	82.8	81.8	1.08
OM	70.0	72.1	72.6	72.0	0.92
NDF	59.5	57.9	57.9	56.3	2.44
Apparent digestibility (decimals)					
DM	0.54	0.53	0.55	0.54	0.02
OM	0.56	0.56	0.58	0.57	0.02
NDF	0.38 ^b	0.44 ^a	0.47 ^a	0.47 ^a	0.02
Nitrogen balance (g/kgBW ^{0.75} /day)					
N Intakes	1.26	1.35	1.33	1.31	0.02
N in urine	109 ^c	134 ^{ab}	149 ^a	126 ^b	8.51
N-balance	218 ^b	412 ^a	468 ^a	431 ^a	29.7
Urinary purine derivatives excretion (mmol/kg BW ^{0.75} /day)					
Allantoin	0.21 ^a	0.18 ^a	0.18 ^{ab}	0.16 ^b	0.16
Uric acid	0.04 ^b	0.04 ^{ab}	0.05 ^a	0.03 ^b	0.01
PD	0.24 ^a	0.22 ^{ab}	0.23 ^a	0.19 ^b	0.02
Allantoin/DOMI (mmol/kg)	5.42 ^a	4.85 ^{ab}	4.18 ^{bc}	3.77 ^c	0.50
PD/DOMI (mmol/kg)	6.34 ^a	5.82 ^a	5.40 ^{ab}	4.60 ^b	0.54

S.E.D: Standard error of difference (Least Significant Different/ $T_{\alpha,0.05}$)

^{ab}: Values within the same row with the different superscripts are significantly ($P<0.05$) different

Values within the same row without superscripts are not significantly ($P>0.05$) different

CL sun-dried cassia leaves, *CLRT* RTP+*CL* and premix, *DOMI* digestible organic matter, *LL* sun-dried leucaena leaves, *LLRT* RTP+*LL* and premix, *ML* sun-dried mulberry leaves, *MLRT* RTP+*ML* and premix, , *RTP* oven-dried rain tree pod, *RTPP* RTP and premix,

The efficiency of the microbial N produced in the rumen was usually estimated by urinary PD excretions and the ratios of allantoin/DOMI and PD/DOMI. The microbial supply into the small intestine was lower in animals fed *LLRT* supplemental diet than in those fed the other supplemental diets. One can expect that the formation of tannin-protein complexes when leucaena leaves are used as a feed in ruminants, the decrease of RDP (Waghorn et al., 1987) and consequently the ammonia-N concentration in the rumen might be limited and microbial production supply into the small intestine decreased (Waghorn 2008).

The effects of different proportions sun-dried rain tree pod pellets (*RTPP*) added to fresh chopped leucaena leaves to form dietary supplements on purine derivatives and di-hydroxy pyridine mimosine+(DHP) in the urine of Thai swamp buffaloes (Jetana et al., 2017)



The use different proportions of sun-dried RTPP added into chopped leucaena as a feed supplement to Thai swamp buffalo receiving *ad libitum* ammoniated rice straw as a basal diet. The study was conducted using a 4×4 complete Latin square design with 4 supplemental diets. Four male Thai, swamp buffaloes weighing 442±8.6 kg (36-40 month old) were allocated to one of four supplemental diets consisting of (kg on fresh weight/animal/day): i) 600 g sun-dried RTPP + 6 kg chopped fresh leucaena used as the control (RTPP = 1 Time), ii) 1,200 g sun-dried RTPP + 6 kg chopped fresh leucaena used as (RTPP = 2 Time), iii) 2,200 g sun-dried RTPP + 6 kg chopped fresh leucaena used as (RTPP = 4 Time) and iv) 3,200 g sun-dried RTPP + 6 kg chopped fresh leucaena used as (RTPP = 6 Time) (Table 5).

Table 5. Dietary supplements (kg/animal/day) (g/ kg on fed basis)

Ingredients	Supplement Supplement (kg on fed basis)			
	1 T (600RTPP)	2 T (1,200RTPP)	4 T (2,200RTPP)	6 T (3,200RTPP)
Sun-dried RTPP	0.60	1.20	2.20	3.20
Chopped fresh LL	6.00	6.00	6.00	6.00

Table 6 shows that the increased proportions of sun-dried RTPP in dietary supplements linearly increased ($P < 0.001$) the purine derivatives in urine, and the efficiency microbial production in the rumen in buffaloes linearly increased ($P < 0.05$) when proportions of sun-dried RTPP in mixed supplement diet increased. The urinary mimosine+DHP excretions linearly increased ($P < 0.01$) in animals when the proportions of sun-dried RTPP in leucaena of dietary supplements increased. The values of mimosine+DHP in the urine was nearly closed to 100 mg mimosine+DHP/kg BW^{0.75} in the urine, but the average excretion rate of mimosine+3,4-DHP did not reach to 100 mg mimosine+DHP/kg BW^{0.75} in the urine. Therefore, leucaena toxicity considered not to be anticipated to the animals. This study indicated sun-dried rain tree pod can be used as a good ingredient for combination with leaves of lucaena.

Table 6. Urinary purine derivatives in urine, the ratios of PD to DOMI, mimosine and DHP in urine of swamp buffalo fed *ad libitum* of ammoniated rice straw and supplemented with the different proportions of rain tree pod added into leucaena leaves

Items	Proportions of sun-dried rain tree pod pellets				S.E.D	Difference (P-value)		
	600 RTPP	1,200 RTPP	2,200 RTPP	3,200 RTPP		linear	quadratic	cubic
Number of animals	4	4	4	4				
Purine derivatives in urine (mmol kg BW ^{0.75} d ⁻¹)								
Allantoin	1.45 ^{bc}	1.41 ^c	1.65 ^b	1.87 ^a	0.106	0.001	ns	ns
Uric acids	0.07 ^a	0.06 ^b	0.07 ^{ab}	0.07 ^{ab}	0.004	ns	ns	ns
Total PDs	1.52 ^{bc}	1.48 ^c	1.72 ^b	1.94 ^a	0.107	0.001	ns	ns
PD/DOMI (mmol/kg DOMI)	39.1	38.4	43.8	44.1	3.396	0.05	ns	ns
Mimosine and DHP in urine (mg/kg BW ^{0.75} d ⁻¹)								
Mimosine	4.65 ^b	9.42 ^a	1.37 ^c	4.00 ^{bc}	1.430	0.01	ns	ns
3,4-DHP	18.2 ^b	20.9 ^b	28.7 ^a	28.2 ^a	1.886	0.001	ns	ns
2,4-DHP	52.6	57.6	61.0 ^a	61.7	6.143	ns	ns	ns
Mimosine+DHP	75.5 ^b	87.9 ^{ab}	91.1 ^a	93.8 ^a	6.812	0.01	ns	ns

¹ S.E.D: Standard error of mean averaged throughout experiments

^{2 abc} Values within the same column with different superscripts are significantly ($P < 0.05$) different.

³ Values within the same column without different superscripts are not significantly ($P < 0.05$) different

In conclusion, this paper demonstrated that the processed rain tree pods, sun-dried rain tree pod pellet and oven-dried ground rain tree pod. Both processing types have been confirmed to be a great ingredient feed for enhancing microbial protein production in the rumen.

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KNOWLEDGE ATTITUDE AND PRACTICE (KAP) ANALYSIS ON NUTRITION, BREEDING AND HEALTH MANAGEMENT OF SMALL HOLDERS BUFFALO FARMERS AROUNDGUNJANAGAR DAIRY PLANT, CHITWAN NEPAL

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ABSTRACT

Buffalo is an integral part of livestock commodity in producing milk, meat and hides. This study aimed to assess the knowledge and perception of artificial insemination, health and nutrition of buffaloes in small holder buffalo farmers and identify its practices at the household level. 100 households were purposively surveyed with well-structured pretested questionnaires. Result shows that 72% of respondents have knowledge on fecal test whereas 18 % perform test at every 3 months, 21 % at 6 month, 41 % once a year and 20 % never. Frequency of livertonics use shows that 45 % during deworming, 14 % at every 3 months and 41 % as prescribed by the technicians whereas 79% of respondents have knowledge on its use. Majority of farmers (69%) know the benefits of mineral supplements. Among them 29 % use just after calving, 28 % on last month of pregnancy, 18 % at the peak milk production and 25 % as prescribed. As feeding practice, 89 % farmers provide cultivated pasture and 11% farmers use concentrates as major ration. 84 % farmers have stall feeding and 14 % grazing and feed supplement and 2 % as grazing practice only. Regarding breeding practices, 70 % farmers have knowledge on AI, whereas (54%) prefer natural breeding, 30% AI and 16% both. Farmers are practicing AI for better calving rate (41%), accuracy on breeding (27%), better milk production (17%), reduce cost on breeding (7%) and others(8%). Majority of buffaloes (92%) show silent estrus. 30% of them bred buffaloes once animal shows estrus sign, (17 %) after 6 hours, (43%) 12 hours and (10 %) upto 24 hours. Pregnancy diagnosis practice is performed only by 65% farmers on different time interval. Intensive training packages are essential to transform knowledge on buffalo health, nutrition and breeding into practice.

Keywords: Buffalo, knowledge, attitude, practice, small holders buffalo farmers

INTRODUCTION

Buffaloes are the main source of milk and meat in Nepal. Buffalo are the major source of household income for the small holder farmers (Rasali, 1997). In 2015/16, the milk production from buffalo (1,210 thousand MT) constituted 65% of the total milk production, and the buffalo meat production (175 thousand MT) constituted 54% of the total meat produced in the country (MoAD, 2017). Though buffalo is the main source of milk and meat in Nepal, the country plans to meet the deficits in minimum annual per capita consumption, about 30% milk and 15% meat by 2018/19 (MoLD, 2017).

Chitwan is growing as milk producing district, while Gunjanagar VDC is one of the pocket area of milk production in Chitwan. Majority of dairy farmers are small holders and they depend on the diversification of agriculture to reduce risks and derive economic benefits from the limited resources they possess (Singh *et al.*, 2001).

MATERIALS AND METHODS

Study was performed from both primary and secondary data on animal husbandry practices in the Gunjanagar VDC of Chitwan district. A set of relevant questionnaire underlying knowledge, attitude and practice of farmers, was developed to record primary data on breeding, health and nutrition of buffalo. On the basis of secondary information, questionnaire pretesting was done and 100 household with at least one milch buffalo was surveyed for the collection of primary data. Collected information were subjected to frequency and percentage distribution via SPSS version 20.0 and excel 2010, for the assessment of the scientific management practices in the study area.

RESULTS AND DISCUSSION

Results on the knowledge, attitude and practice of small holder's buffalo farmers are shown in Table 1.

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Table 1. : Knowledge attitude and practice on breeding management o small holders buffalo farmers.

Different breeding parameters	No. of observation	Percentage
Knowledge on AI	Yes	70
	No	30
Breeding Method	Bull	54
	AI	30
	Both	16
	Better progeny	41
Purpose of AI	accuracy of breeding	27
	Better milk Production	17
	Decrease cost of rearing bull	7
	Other	8
Knowledge on heat detection	Yes	95
	No	5
Practice on heat detection	Bellowing	8
	Silent Heat	92
	At time of observation	30
Practice on Mating of animals after heat detection	Up to 6 hours	17
	Up to 12 hours	43
	Up to 24 hours	10
Trend on Pregnancy diagnosis	Yes	65
	NO	35
	Within 60 days	6
	Within 90 days	23
Trends on Pregnancy diagnosis	Within 120 days	30
	Above 120 days	14
	Never	27

Artificial insemination (AI) is the modern reproductive technique applied to control and improves reproduction as well as genetics (Heise, 2012). The result showed that the majority of farmers (70 %) had knowledge on the AI despite of it; their attitude on preference of breeding method showed breeding by bull (54%) to be higher followed by AI (30%) and both by (16%). This clearly shows that the farmers lacked the practice on AI though they have the knowledge about it. Reason behind this gap might be attributed by no utilization of veterinarian service, skills and knowledge in farm level, repeat breeding problems after failure of AI, poor semen quality, economical factors, social influences, limited source and supply of AI facilities along with limitation of well equipped knowledge of AI performer. Report from National livestock and breeding centre (NLBC, 2014) stated that farmer doesn't have a preference to use AI for breeding purpose. Similarly A study conducted in Chitwan and Nawalparasi reported that the constraints of adoption of AI are a scenario of higher AI failure (with more than 3 repeated failures) in buffalo; poor capacity and practice of AI service providers; and inefficient knowledge among farmers about handling of AI equipments (Sapkota, Gairhe, Kolakshyapati, & Upadhyay, 2016).

Result on perception of farmers over AI shows better calf production (41%) to be of highest followed by accuracy on breeding (27%), better calf production (17%), decrease cost of breeding (7%) and others (8%). The use of artificial insemination (AI) as a method of reproduction, particularly in the dairy sector, has been of enormous economic benefit through the genetic improvement of milk production, the control of venereal and other diseases and the reduction of lethal genes (Vishwanath, 2003; Watson, 1990; Shannon, 1978). Though AI have several advantages over the existing practice farmers fails to adopt the technology. The natural method on breeding using locally available bull does not generate improvement in coming generations. To increase the farmers practice good technological service must be strengthened (NLBC, 2015) to increase the acceptance of AI.

Heat detection was found to be satisfactory 95 % farmers are aware on it. Majority of farmers (92%) noted silent heat estrous expression; swelling of vulva, decrease in feed and water intake, mucous discharge; whereas only 8 % respondent mentioned bellowing as prominent sign of heat detection. Harshan et al, 2007 reported that bellowing is very common in mid heat (12-18 hours), whereas sometime in early heat and never in late heat (18-24 hours).



Results shows that the farmer practice on mating animal after observing heat sign is mostly inappropriate; (30%) at time when they observe, (17 %) upto 6 hours, (43%) upto 12 hours and (10 %) upto 24 hours. Missing the desired time(12-18) hours after detection of heat sign may lead to the AI failure which conversely relate the low level of farmers practice on adaptation of AI. Silent heat in the buffalo is one of the most important unsolved impediments to efficient breeding. It occurs in the hot seasons. The duration of heat in buffalo lasts from 12-24 hours (Bhikane and Kawitkar, 2000). A combination of estrus detection methods is necessary for identification of animals in heat. (Remesh et al., 2002).

Table 2. Summary on Health and management issues of small holder buffalo farmers.

Health and Management aspects	No. of observations	Percentage
Knowledge on De-worming	Yes	72
	No	28
Practice on De-worming	Every 3 months	18
	Every 6 months	21
	Every year	41
	Never	20
Knowledge on Livertonics use	Yes	79
	No	21
Practice on Livertonic use	During Deworming period	45
	Every 3 months	14
	As prescribed	41
Knowledge on Mineral supplement	Yes	69
	No	31
Practice on Mineral Supplement	Last month of pregnancy	28
	Recently after parturition	29
	At peak of lactation	18
	As prescribed	28
Type of feed used	Concentrates + Roughages	11
	Cultivated pasture + Concentrate	89
	Grazing + feed supplement	14
Feeding Practice	Stall Feeding	84
	Grazing only	2

Another important aspect on breeding is pregnancy diagnosis to decrease the calving interval. Ideal calving interval for buffalo is considered around 60 days. Pregnancy diagnosis practice was found not performed by 27% farmers. Whereas majority (67%) performs at different time interval, 6 % at around 60 days, 23 % around 90 days, 30 % around 120 days, 14 % above 120 days. Buffalos are difficult breeder because of its inherent susceptibility to environmental stress, which causes anoestrus and sub-estrus. These two conditions are responsible for a prolonged inter-calving period resulting in great economic losses for the dairy industry for which the pregnancy diagnosis must be practiced to minimize. Results Regarding health and nutrition approach are presented in Table 2.

Result reveals that farmers possess knowledge on fecal examination (72%) out of which only (18%) respondent were found to perform fecal examination at every 3 months, 21 % at an interval of 6 month while 41 % once a year and 20% never. Majority of farmers having knowledge on fecal examination found frustrating result on its practice. Parasite load and pregnancy are inversely proportional. The adaptation of proper de-worming pattern along with use of nutritional supplements as like livertonics and minerals supplement will enhance the reproductive performance. 79% respondent have attitude of using livertonics at time of deworming time. Frequency of livertonics use shows that 45% during deworming time, 14% at every 3 month and 41% as prescribed by the technicians/veterinarians. Knowledge on Mineral supplement for animals was found in 69% respondent only. Out of which 29% farmers use after parturition time, 28% on last month of pregnancy, 18% use at the peak of milk production stage and 25 % as prescribed by vets.

Farmers knowledge on livertonics and mineral supplement to animal is found to be interesting but the practice for their use is limited. In lack of proper nutrients supplement, production of buffalo declines. Poor nutrition, management, stress and season are mainly responsible for anestrus problem in buffalo (Zicarelli, 1997; Qureshi et al., 2002; Borghese, 2005; Devkota et al., 2009; Devkota et al., 2013).



Results on Feeding practices of buffalo shows that majority of farmers (89 %) use cultivated pasture and concentrate as feed source where as 11% farmers are found to have concentrates as major ration. The trend of supplying green grass (roughages) along with the concentrate ration helps in the better milk production. Stall feeding practices 84 % farmers have stall feeding practice as way of rearing animals, whereas 14 % respondent have grazing and feed supplement rearing practice and 2% as grazing practice only.

In conclusion, majority of farmers had knowledge on various aspects of buffalo husbandry practice including health, management, nutrition and breeding. However, the attitude and practices of good husbandry were found to be moderate. It was promising that most of the buffalo farmers were aware of detecting silent heat. However, the practice of regular deworming, supplementation of vitamin-mineral mixtures and timely pregnancy diagnosis needs to be promoted in buffalo farming communities of western Chitwan to enhance buffalo productivity.

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EFFECT OF INCREASED DOSES OF FINAL GONADOTROPIN-RELEASING HORMONE (GnRH) OF OVSYNCH ON CORPUS LUTEUM FUNCTION IN DAIRY BUFFALOES

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ABSTRACT

Reproductive efficiency in buffalo (*Bubalus bubalis*) has constraints such as silent estrus, and heat stress affecting estrus behavior. Lesser concentration of circulating steroid hormones (estradiol and progesterone) is due to smaller ovaries, fewer primordial follicles and smaller dominant follicle and these lead to reproductive constraints in buffalo. Artificial insemination (AI) has been largely incorporated in cattle reproduction in Nepal but not much in buffalo mainly due to poor estrus expression and difficulty in predicting timing of AI in buffalo. Synchronization protocol using gonadotropin releasing hormone (GnRH) and prostaglandin F2 alpha (PG) have been designed to synchronize ovulation to allow fixed timed artificial insemination (FTAI) in both cattle and buffalo. One such protocol is Ovsynch which helps to synchronize ovulation and perform FTAI. To tackle challenges of lesser concentration of steroid hormones in higher producing cows, studies focused on increasing steroid hormones to imitate that of heifers have been reported. No such studies have been reported in buffalo. The objective of this study was to assess the effect of increased doses of final GnRH of Ovsynch in dairy buffaloes on CL function [CL size and progesterone (P4) secretion]. Dairy buffaloes (n=14) were enrolled in an Ovsynch protocol. The buffaloes that responded to the first GnRH of Ovsynch (n=6) were divided to receive two doses (100 vs 400 µg) of final GnRH of Ovsynch followed by FTAI. There was no significant difference in P4 per CL volume ($p > 0.3$) post treatment between the two treatment doses. Due to small sample size in the study, the data is not adequate to prove the effect of greater GnRH dose on CL function in buffalo. So we suggest further studies on GnRH doses in buffalo synchronization protocols.

Keywords: Ovsynch, synchronization, buffalo, corpus luteum (CL) function, GnRH (gonadotropin releasing hormone), estradiol, progesterone, PGF2 alpha (prostaglandin F2 alpha)

INTRODUCTION

Buffaloes have an economic importance in a developing country like Nepal because they are better at converting poor-quality roughage into milk and meat. Milk with greater fat and protein percentage is produced with a lesser nutrition level. Buffalo are reported to have a 5% greater digestibility of crude fiber than greater-yielding cattle; and a 4 to 5% greater efficiency of utilization of metabolic energy for milk production (Mudgal, 1988).

Reproductive management is a tool to increase productivity in any dairy animal. It is more important in buffalo because buffalo reproduction has always faced problems of longer gestation period, silent estrous, and heat stress affecting estrus behavior (Perera, 2011). A wide variability in estrus length and ovulation time makes it difficult to predict timing of AI in buffalo (Campanile et al., 1988). Poor expression of estrus is another hurdle in buffalo reproduction that requires investment of time and labor to determine the correct timing of AI. Conventional heat synchronization did not result in good conception rate (CR) after timed AI and so AI to heat detection is still preferred by buffalo farmers (Barile et al., 2005). Precise breeding technique such as synchronization of ovulation (Ovsynch) and use of fixed timed AI has a good scope in buffalo.

Concentrations of estradiol and progesterone is less in buffalo than in cattle (Baruselli et al., 1997) due to smaller ovaries, fewer primordial follicles and smaller dominant follicle (Danell, 1987). Ali and Fahmy, (2007) reported CR as 60% in cyclic and 37.5% in non-cyclic buffalo following Ovsynch. Baruselli et al. (2003a) reported CR as 45.4% in buffalo following Ovsynch. Due to earlier onset of ovulation in non-cyclic buffalo following Ovsynch, two inseminations at 0 and 24 h was suggested to increase CR (Ali and Fahmy, 2007). Study that compared Ovsynch with pre synchronization-Ovsynch showed no significant difference in ovulation and conception rate (Oropeza et al., 2010). Ovsynch in buffalo is reported to give improved fertility in favorable reproductive season (Barile et al., 2005). As an effort to overcome reduced steroid concentration in buffalo, we used two different doses of GnRH (100 µg vs. 400 µg) in the final GnRH of Ovsynch in this study. 100 µg is the dose of GnRH which was started as treatment of cystic ovary in cows (Kittok et al., 1973; Cantley et al., 1975; Bierschwal et al., 1975) as well as the dose used in most synchronization protocols (Pursley et al., 1995; Bello et al., 2006). Some studies on greater dose of GnRH done on cows have not been successful in determining an optimum dose to improve CL function.

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Likewise, in buffalo, 100 µg of GnRH is used in synchronization programs (Ali and Fahmy, 2007) and no studies on greater doses of GnRH have been reported to our knowledge.

The objective of the study was to determine whether a greater dose of GnRH (100 vs. 400 µg) using Ovsynch results in improved CL function marked by increased CL size and greater concentration of progesterone. We hypothesized that increased dosage of final GnRH of Ovsynch would not affect CL function.

MATERIALS AND METHODS

Animals

This study was conducted from October 2015 to January 2016 at the Agriculture and Forestry University Livestock Farm, Rampur, Chitwan, Nepal. Ten parous Murrah cross buffalo and four heifers were used in the study. Parity of the buffaloes used ranged from 0 to 6. Buffaloes were housed in a tie stall barn except when allowed to freely graze in the farm's grasslands from 7 am to 3 pm. They were fed hay straw and grass in the evening. Only a few buffalo in the group were lactating. Lactating females were milked twice daily. Milk yield ranged from 2 to 4 liters per day in the lactating females. The buffalo used in the study were at random stages of estrous cycle. Of the ten parous buffalo, one was a first lactation buffalo and nine were 2+ lactation buffaloes.

All animals received 100 µg of GnRH intramuscularly at a random stage of the estrous cycle (OvaCyst, gonadorelindiacetatetetrahydrate, Bayer Health Care). A week after the first GnRH (G1), 25 mg PG (Lutalyse, *dinoprostromethamine*, Zoetis) was administered. In multiparous buffaloes, 25 mg PG was repeated 24 hours after the first injection of PG. All injections were given intramuscularly in neck region with an 18-gauge needle.

Ultrasonography (Honda Electronics HS- 1500 Vet with 7.5 MHz transducer, Japan) was performed to evaluate diameter of pre-ovulatory follicles at time of treatment. Only buffaloes with a growing pre-ovulatory follicle were enrolled for treatment. Buffaloes were blocked by parity and pre-ovulatory follicle diameter and assigned randomly to one of the two treatments. Control buffaloes received 100 µg GnRH and treated buffaloes received 400 µg of GnRH. A 56-hour interval was maintained between the PG injection and treatment. All buffaloes received AI in the morning of the day following the treatment (16 to 20 hour after treatment).

Blood Collection

Blood was collected by jugular venipuncture using an 18-gauge needle and Vacutainer tubes without anticoagulant (BD Vacutainer, Preanalytical Solutions, Franklin Lakes, NJ). Blood was collected on d 0, 7, 14, 21 and 28 following treatment d 0 being the day of treatment. The blood samples were intended for analysis of progesterone concentration. Blood samples were stored overnight and centrifuged at 3400 rpm for 15 minutes to harvest serum the next day. Serum was stored at -20 °C until the time of assay except for the time when it was shipped from the research site in Chitwan to Kathmandu in an ice box.

Ultrasound Examination

Ultrasonography was used to scan ovaries and measure the number and size of the follicles and CL on days -9, -2, 0, 7, 14, 21 and 28. Buffaloes were scanned on day -9 to check for the presence and measure sizes of ovulatory follicles before giving G1; day-2 to check the presence and measure size of CL before giving PG; day 0 to ascertain CL regression in response to PG and to measure the size of the pre ovulatory dominant follicle (DF). After the buffaloes were bred on day 1, they were scanned on days 7, 14, 21 and 28 to follow the development of the CL. Day 28 ultrasonography was the first day for pregnancy diagnosis of those buffalo.

Progesterone Assay

The Abraxis Progesterone (bovine) ELISA kit (Abraxis USA) was used to determine serum concentrations of progesterone. The intra-assay coefficient of variation (CV) % was 7.6, 5.4 and 4.9 for low, medium and greater P4 respectively. The inter-assay CV % was 6.8, 8.3 and 2.7 for low, medium and high P4. Forty-two samples were run in duplicate in one plate along with six standards in duplicate. The optical density (OD) of each sample was measured by a spectrophotometer at a wavelength of 450 nm (with a reference filter of 650 nm). The measuring range of the assay was 0 to 20 ng/mL with an analytical sensitivity of 0.06 ng/mL.

Statistical Analysis

SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used for analysis of data. Proc Means was used to calculate means and SE. Proc Mixed procedure was used to test the effect of treatment on variables. The effect of treatment was considered significantly different when $P < 0.05$ at the 95% confidence level.



RESULTS

Among the n=14 buffaloes, n=6 buffaloes that responded to G2 (having an ovulatory size follicle which ovulated in response to the GnRH) were 3 from each dose group. The CL sizes determined by ultrasonography every 7 days starting from the day of treatment (day of G2 of Ovsynch) up to 28 days was used to calculate the CL volume by the formula:

CL volume = $\frac{4}{3} \cdot \pi \cdot (r^3)$ where r (radius) = d/2, d (diameter) = average of height and width of the CL.

4/14 buffaloes were diagnosed pregnant at the day 45 ultrasonography. Of the 4 animals that were pregnant, there were two from each treatment.

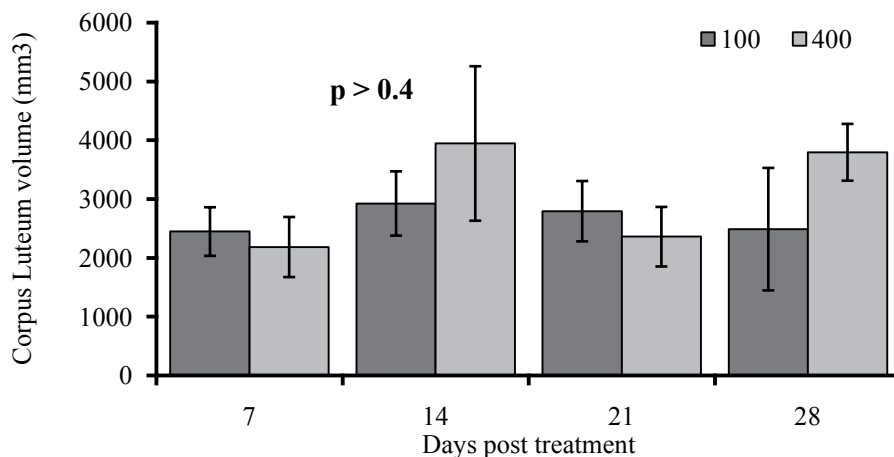


Figure 1. Corpus luteum (CL) volume post treatment of n=6 (n=3 vs. 3) in 100 µg vs 400 µg treatment groups denoted as mm³, CL volume was calculated using the formula: CL volume = $\frac{4}{3} \cdot \pi \cdot (r^3)$ where r (radius) = d/2, d (diameter) = average of height and width of the CL.

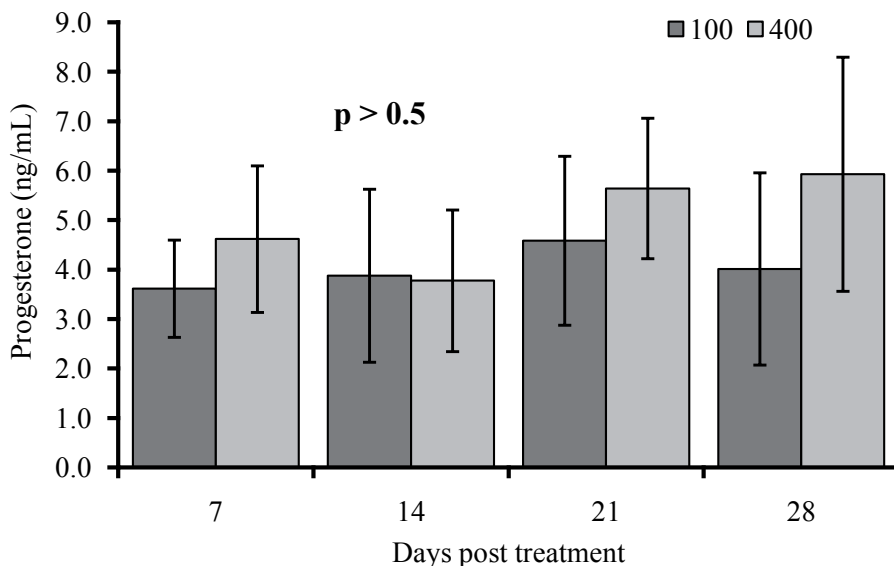


Figure 2. Progesterone concentration post treatment of n=6 (n=3 vs. 3) in 100 µg vs 400 µg treatment groups. Progesterone concentration is expressed as ng/mL

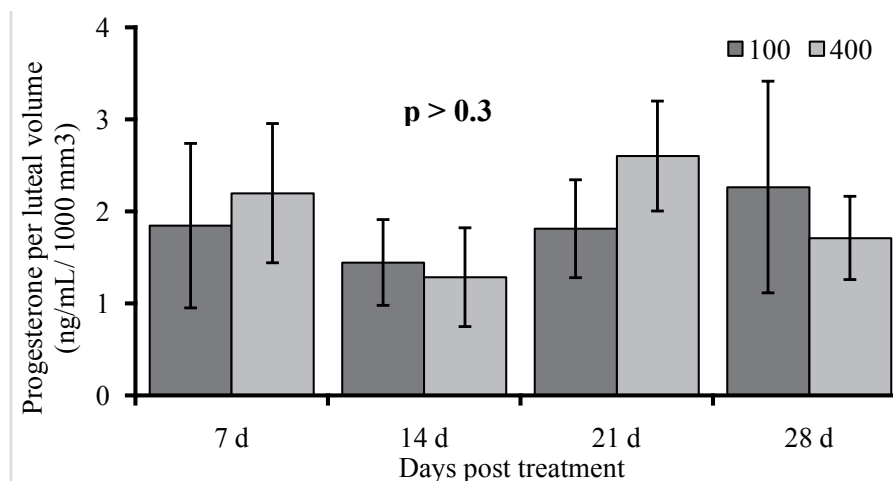


Figure 3. Per unit Progesterone concentration post treatment of $n=6$ ($n=3$ vs. 3) in 100 µg vs 400 µg treatment groups. Progesterone concentration is expressed as ng/mL/1000 mm³ CL volume ($p>0.4$), P4 concentration ($p>0.5$) and P4 per CL volume ($p>0.3$) of buffaloes in treatment groups were not significantly different from that of control group on different days post AI.

DISCUSSION

While it is well known that ovulation in response to G1 is a prerequisite for success of Ovsynch, our data had a few exceptions. Only 6/14 animals responded to G1 given at random stages of estrous cycle. In cattle, when Ovsynch was used for the first time, 18 of 20 lactating cows and 13 of 24 heifers had responded to G1 (Pursley et al., 1995). G1 resulted in ovulation in 90% of cyclic and 62.5% of non-cyclic buffaloes. PG induced luteolysis in 80 and 87.5% for cyclic and non-cyclic buffalo. And 80% of cyclic and 100% of non-cyclic buffalo responded to G2 (Ali and Fahmy, 2007). The animals that did not respond to G1 in this study were possibly acyclic. In our study, two animals that responded to G1 did not respond to G2. Both of those animals failed to regress CL in response to PG as seen in the ultrasonography at G2. However, Baruselli et al. (1997) did not find any relationship between dominant follicle development and presence of a CL or follicle from a previous wave. Out of the six animals that responded to G2, four animals were ones that had also responded to G1, which is in agreement with data that show response to G1 increases the chances of response to G2. A synchronization rate of 78.8 % was reported in a synchronization protocol in buffalo (Baruselli et al., 2003a). This study had 6 of 14 animals synchronized by Ovsynch.

Size of the Ovulatory Follicle

The ovulatory follicles of buffalo are smaller than those of cattle. Our data shows that the minimum size of pre ovulatory follicle is 8 mm. The maximum size observed was 14 mm in a multiparous animal, but she did not ovulate (even in response to 400 µg of GnRH). Another buffalo with a follicle of 13 mm ovulated in response to G2. It can be speculated that an ovulatory follicle size of 9 to 13 mm is more responsive to GnRH in buffalo. This finding is supported by a study which reported size of the dominant follicle as 9.82 ± 1.23 and 11.96 ± 2.15 mm for heifer and parous buffalo respectively at the time of final GnRH injection. The ovulation rate was 100% for heifer and 88.8% for parous buffaloes (Derar et al., 2012). Ovulation time averaged 22.6 h (range 16-36 h) and 10.4 h (range 6-24 h) in heifer and parous buffalo, respectively ($P = 0.01$). The mean diameter of the CL developed at Day 7 of the protocol was 15.45 ± 0.8 and 19.7 ± 1.3 mm in heifer and parous buffalo, respectively ($P = 0.03$).

In cattle DF size had a positive effect on the CL function (Pandey et al., 2011; Binelli et al., 2009; Busch et al., 2008). Also greater tendency of pregnancy loss between day 28 and 56 was detected with ovulatory follicles <15 mm diameter in comparison to the ones that were >15 mm (Stevenson et al., 2006). Follicles <11 mm when induced to ovulate led to reduced conception rates and increased embryonic mortality (Perry et al., 2005) as GnRH can induce LH surge and ovulation in follicles >9 mm diameter in cattle (Bodensteiner et al., 1996).

Although no significant effect of increased dose of G2 was seen on CL volume or progesterone level, we still suggest that more studies should be carried out to determine an effective synchronization protocol and doses of GnRH that can help to optimize fertility in buffalo. Buffalo reproductive physiology is different from that of cattle. Ovsynch has been widely studied in cattle but more studies are required in buffalo. Studies involving doses of the hormones used in synchronization protocols are important to optimize reproductive potential in buffalo.

This study has a small data set and is not suitable for a binomial result expression. We had 4/14 buffaloes pregnant at the day 45 ultrasonography.



Greater P4 concentration at the time of G2 of Ovsynch is indicative of incomplete luteolysis. Lack of complete luteolysis are proven to reduce fertility in synchronization protocols (Brusveen et al., 2009; Giordano et al., 2012; Martins et al., 2011b). In the present study, the mean P4 concentration of non-pregnant buffalo was found to be greater than that of pregnant buffalo at the time of treatment. In a study with synchronization protocol in buffalo, Ovsynch resulted in pregnancy of 12 of 30 nulliparous and 6 of 14 pluriparous buffalo (Presicce et al., 2005). PRID-PMSG protocol resulted in pregnancy of 12 of 17 buffalo. It was concluded that the efficacy of Ovsynch was the same in nulliparous and pluriparous buffalo. It was also suggested that progestogen treatment along with Ovsynch increases PR in acyclic buffalo. Rensis et al. (2005) also found that supplementation with progesterone along with Ovsynch helped to increase conception rates in non-cyclic buffalo.

Out of the 4 pregnant buffaloes, 3 were parous and 1 was nulliparous. Acyclic condition was found to be more prevalent in primiparous and old buffaloes when compared to heifers. Also, two types of anestrus have been reported - superficial and deep. Superficial acyclic buffalo have been found to respond to adequate synchronization protocol (Zicarelli L., 1998). In our study, buffalo that were acyclic showed some follicular or luteal activity. Those animals might have been the superficial anestrus buffalo. However, inadequacy of synchronization might have hindered the response. We used simple Ovsynch in this study. Pre synchronization followed by Ovsynch can increase the synchronization rate in acyclic animals.

Buffaloes have a wider variable time interval between LH surge and ovulation in the natural estrous cycle as well as in hormone-induced ovulations in comparison to cattle (Seren et al., 1995; Zicarelli L., 2003). Because of this fact, it was suggested that performing 2 to 3 insemination can help to increase pregnancy rates (Barile et al., 1997; Zicarelli L., 1997). Single AI was done after synchronization in the present study and we speculate that more than one AI might have helped to increase pregnancy in those animals.

There is not enough evidence to prove positive effect of greater dose of GnRH on luteal function in buffaloes. More studies to explore various aspects of reproductive management is necessary to better understand buffalo reproduction. GnRH is an efficient hormone that can be used to control follicular development in cattle as well as buffalo. Mostly result from cattle synchronization studies have been used in buffaloes. However, due to the difference in cattle and buffalo reproductive physiology, studies with buffalo need to be carried out. This will help to determine an optimum dose of hormones in synchronization protocol specific for buffaloes so that farmers can tackle with problems in buffalo reproduction.

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RESPONSE OF NOVEL HORMONAL PROTOCOL IN ANESTRUS BUFFALOES DURING DIFFERENT BREEDING SEASONS

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ABSTRACT

Dairy buffaloes contribute 68.68% of total milk production in Nepal. Buffalo reproduction is characterized by delayed puberty, silent estrus, and long post-partum ovarian inactivity. In Nepal, post monsoon to early winter is a good breeding season where as late winter to dry early summer is poor breeding season for buffaloes. The aim of this research was to understand the response of treatment of novel hormonal protocol during different breeding season in anestrus buffaloes along with its some limiting factors. The study was conducted from 2013 to 2017 in 91 anestrus buffaloes. In good breeding season, ovsynch protocol was applied whereas CIDR cosynch was applied in transition to poor breeding season. Out of total animals, blood sample of 14 animals from each breeding season were taken for determination of serum nutritional parameters (total protein, cholesterol, glucose) and cortisol level as stress factor. Body condition score was recorded. Pregnancy diagnosis was confirmed by transrectal ultrasonography. The non-parametric data were analysed by chi-square test and mean value was analysed by t-test and one way anova using SPSS version 20. Pregnancy was found 45.71% in good season, 50.0% in transition season and 42.85% in poor season. 73.21% of animals show estrus sign in CIDR cosynch protocol whereas 54.28% of animals show estrus signs in ovsynch protocol. There was no significant difference in pregnancy among CIDR cosynch and ovsynch protocol. Body condition score has highly significant ($P<0.01$) effect on the pregnancy after treatment. Serum cholesterol was found to be significantly ($P<0.01$) higher and cortisol level was found to be significantly lower ($P<0.01$) in good and transitional season. And serum glucose and total protein was tended to be significantly ($P<0.05$) higher in pregnant animal. In conclusion, pregnancy can be successfully achieved even in poor season using progestin based hormone protocol addressing nutritional and stress factor.

Keywords: Anestrus buffalo, novel hormone, breeding season

INTRODUCTION

Buffalo is the most important dairy animal in Nepal making an important contribution to milk, meat, power, fuel and leather production in Nepal. Buffalo contributes 68.68% of the total milk and 58.30% of the total meat production in the country (MOAD, 2017). Buffaloes however suffer from reproductive problems such as poor estrous expression, seasonal breeding pattern, and prolonged inter-calving intervals, which limit its production potential. These problems are severe during the summer season. This limitation is exacerbated during the hot season, when fertility decreases dramatically (De Rensis and Lopez-Gatiuss, 2007). It has been long recognized that the environment has an important role to play in the regulation of the mammalian reproductive function. Environmental cues, such as changing daylight or increase in temperature are known to trigger off growth or regression of the reproductive organs in every animal species. And also, nutrition and stress can influence the onset of breeding and affect the fertility. Similarly, seasonal anestrus in buffalo has been associated with high bio-meteorological factors such as day length, ambient temperature, relative humidity and rainfall.

In Nepal the environmental fluctuation is from cold and semi-dry to dry winter (December-February), rapidly increasing hot and dry spring (March-May), very hot and rainy monsoon summer (June-August) and moderate autumn (September-November) (Devkota and Bohara, 2009). January to June are poor breeding months whereas July to December are the active breeding months for Nepalese buffaloes (Devkota and Bohara, 2009). Buffaloes suffer from cessation of ovarian activity and silent heat during the months of dry spring and early summer. Most of the buffaloes exposed to extreme hot conditions cease ovarian activity (Sastri, 1983; Nanda and Kumar, 2000; Nanda and Nakao, 2003; De Rensis and Lopez-Gatiuss, 2007). True anestrus and silent ovulation were reported as the predominant causes of poor reproductive performance in Nepalese buffaloes (Sah and Nakao, 2009; Bhuminand et al., 2009). Estrus detection efficiency and accuracy are the vital hints for increasing conception rate in milch animals. Estrus synchronization may be used to overcome these problems by controlling the time of ovulation and avoiding the need for estrus detection. A successful synchronization protocol requires a clear knowledge of ovarian secretory function in relation to control of follicle development, luteal phase of the cycle and ovulation (De Rensis and López-Gatiuss, 2007).

The overall effect of poor reproductive performance along with seasonal fluctuation in calving of buffaloes

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is affecting its contribution to national economy although having high potentiality. However, complete studies on factor affecting the poor reproductive efficiency of Nepalese buffaloes are lacking. The main purpose of this study was to evaluate the response of anestrus buffaloes to novel hormonal protocols during different breeding seasons.

MATERIALS AND METHODS

Study Site and Time

This study was conducted at buffalo farms of Chitwan and Kaski districts. The experimental trial was conducted from 2013 to 2017.

Selection of True Anestrus Buffaloes

91 anestrus buffaloes were selected for the experimental trial, 35 for good breeding season and 14 for transition season and 42 for poor breeding season. Selection of anestrus buffaloes was based on history of animals, ovary examination by rectal palpation (RP) and ultrasonography (Honda, HV-1500, Honda Electronics, Tokyo, Japan).

Blood Sample

Blood sample was collected from jugular vein. In each of above three seasons, blood samples from 14 randomly selected buffaloes were collected before the start of synchronization protocols. In this study, we have only evaluated the total cholesterol, glucose, total protein and cortisol from plasma samples at laboratory of Chitwan Medical College, Bharatpur. Glucose, cholesterol and total protein were measured by Spectrophotometry. Similarly, stress factor was evaluated by measuring cortisol level in blood. Cortisol level was measured by Chemiluminescence.

Body Condition Score (BCS)

Body condition score (BCS) was measured as a parameter indicating nutritional status of animal. BCS was recorded in 1 to 5 range scale. The buffaloes were classified into three BCS groups, <2.5, 2.5-3.75 and >3.75.

Synchronization Protocol and Artificial Insemination

Buffaloes were divided into two treatment groups: i) Ovsynch, Standard ovsynch protocol characterized by the administration of GnRH on day 0 and 9, PGF2 α at day 7 and inseminated 16 hrs after second GnRH administration and ii) CIDR cosynch, Standard CIDR cosynch protocol characterized by the administration of GnRH on day 0 and 10, CIDR (controlled internal drug release) was placed intravaginally on day 0 and was kept inside for 7 days, PGF2 α on day 7 and inseminated at day 10 simultaneously with GnRH administration. Buffaloes of good breeding season (n = 35) were treated with ovsynch protocol and buffaloes of transition and poor breeding season (n=56) were treated with CIDR cosynch protocols.

Pregnancy Diagnosis

Pregnancy was diagnosed by transrectal palpation and ultrasonography between day 45 and 60 post insemination.

Data Analysis

The data obtained were subjected to MS-Excel. The output variable was pregnancy and input variables were nutritional status (total cholesterol, glucose, total protein), stress factor (cortisol) and two different protocols. Differences in nutritional and stress parameter between different season and between pregnant and non-pregnant animal was analysed by t-test and one way ANOVA. Conception rate among three different seasons and between different Body condition score and two different protocols were analyzed by chi-square test. The probability value of $P < 0.05$ was considered as significant.

RESULTS

The conception rate was not found significant among the three different seasons and two different protocols as shown in Table 1 and 2. Similarly estrus expression % at the day of fixed time artificial insemination was also found to be non-significant.



Table 1. Conception rate and estrus expression percentage in different breeding seasons after synchronization

Season	Conception rate	Estrus expression	p-value
Good	45.71 % (16/35)	54.28% (19/35)	p > 0.05
Transition	50.00 % (7/14)	73.80% (10/14)	
Poor	42.85% (18/42)	71.42 % (31/42)	

Table 2. Conception rate and estrus expression percentage in different protocol of synchronization

Protocol	Conception rate	Estrus expression	p-value
Ovsynch	45.71 % (16/35)	54.28% (19/35)	p > 0.05
CIDR cosynch	44.64% (25/56)	73.21 % (41/56)	

The comparison of conception rate was found to be significant (P<0.05) among three different body condition score groups. The conception rate of animal having body condition score of 2.5-3.75 (59.32%) was significantly higher (P<0.01) than that of the animals having BCS below 2.5 (9.1%) and above 3.75 (40%).

Table 3. Comparison of conception rate on the basis of body condition score

Body condition score	Conception rate	P-value
Below 2.5	9.09% (2/22)	p <0.01
2.5-3.5	59.32 % (35/59)	
Above 3.75	40.00 % (4/10)	

The mean serum glucose level was found to be higher during good season (88.71 mg/dl) where as low during May-Jun month (76.57 mg/dl). Similarly, cholesterol level was found to be significantly higher during good season (158.71 mg/dl) and transition season (157.43 mg/dl) as compared to poor season (105.43 mg/dl). However protein level was found to be non-significant among different breeding seasons. The cortisol level was found to be significantly lower in good season (1.93 µg/dl) and transition breeding season (2.02 µg/dl) than in poor breeding season (2.85 µg/dl).

Table 4. Comparison of Mean ± SD of nutritional parameter and cortisol level of different breeding seasons

Parameter	Good season	Transition season	Poor season
Glucose (mg/dl)	88.71 ± 15.85	88.42 ± 21.51	76.57 ± 20.80
Cholesterol(mg/dl)	158.71 ± 21.57**	157.43 ± 29.84**	105.43 ± 18.37
Protein (mg/dl)	6.56 ± 0.40	6.70 ± 0.37	6.28 ± 0.58
Cortisol (µg/dl)	1.93 ± 0.54**	2.02 ± 0.74**	2.85 ± 0.94

Note: **Significant at 1% probability

The serum glucose level of pregnant animals was found to be significantly higher (P<0.05) than that of non-pregnant animals. Similarly, protein level of pregnant animals was significantly higher (P<0.01) than that of non-pregnant animals whereas cholesterol level and cortisol level were found to be non-significantly different (P>0.05).

Table 5. Comparison of blood parameter between pregnant and non-pregnant animal

Parameter	Pregnant	Non-pregnant	P-value
Glucose (mg/dl)	91.52 ± 20.52*	79.84 ± 17.24	0.03
Cholesterol (mg/dl)	149.64 ± 35.32	134.32 ± 31.20	0.07
Protein (mg/dl)	6.77 ± 0.43**	6.34 ± 0.44	0.002
Cortisol(µg/dl)	2.17 ± 0.90	2.32 ± 0.82	0.28

Note: **Significant at 1% probability, *Significant at 5% probability

DISCUSSION

It is well documented that the breeding frequency in buffaloes is highest during good breeding season (July to December) and lowest during summer season (January to June) as per the findings of Devkota and Bohara, 2009. Since this research was conducted in three different seasons i.e November-December, January-February and May-June where November-December comes under good breeding season and May-June comes under poor breeding Season and January-February can be considered as transition period between good and poor breeding



season according to Devkota and Bohara, 2009.

There was no significant difference in conception rate among different breeding season. Progesterone based protocol was equally effective in transition to poor breeding season as ovsynch protocol was effective during good breeding season. This demonstrated that with exogenous progesterone use and stimulation using GnRH and prostaglandins, buffaloes can be taken out of reproductive seasonality if the management can improve during poor breeding season.

Body condition score (BCS) was a strong positive indicator of the likelihood of conception rate. Female with BCS 2.5-3.75 had 59.32 % conception rate, whereas females with BCS >3.5 and <2.5 had only 40.00% and 9.09% which attain highly significant difference ($P < 0.01$) among the different BCS. Lower BCS has been correlated with postpartum anestrus. Rasby et al. (1992) reported that nutrition restriction has a negative influence on LH release. Other studies also demonstrated the negative effect of low BCS on ovarian cyclicity and pregnancy rates in beef cows (D'occhio et al., 1990; Viscarra et al., 1998). Furthermore, investigations on postpartum reproduction indicate that BCS is a useful indicator of energy status and rebreeding potential (DeRouen et al., 1994). The results suggest that buffaloes may have to present good BCS for a satisfactory response to the treatment with GnRH and prostaglandins for FTAI.

In dairy animals, nutrition is one of the major causes of anestrus (Kumar et al., 2014) and other reproductive disorders (Pradhan and Nakagoshi, 2008). During the study period (November-December and January-February) there was availability of fodder in comparison to pre monsoon season (May-June) which was dry and the fodder was scarce due to which blood nutrient level was found lower than that during the winter season (November-December and January-February). There was no significant difference in serum glucose level among different seasons. However the blood glucose level was found to be higher than in the cyclic buffaloes as reported by Dhoble and Gupta, 1979; Veerapandian et al., 1987; Jani et al., 1995; Sing et al., 2006. And there was significant difference in blood glucose level between pregnant and non-pregnant animals. The mean blood glucose level of non-pregnant buffaloes was lower than that of pregnant animals. Hypoglycemic condition has been found to depress hypothalamic and hypophyseal functions in animal causing loss of ovarian activity as a result of the failure in gonadotrophin secretion (Howland et al., 1966; Arthur, 1975)

There was highly significant difference in the cholesterol level among different seasons. The cholesterol level of winter season (Nov-Dec & Jan-Feb) has similar cholesterol level as in cyclic buffaloes and the cholesterol level of summer season (May-June) has similar cholesterol level as of anestrus buffaloes as per the findings of Devanathan et al., 1984. Cholesterol is a precursor molecule is needed in the biosynthetic pathway of steroid hormones and its utilization is related to ovarian functional activity (Doisy, 1972). Like in cattle, a direct relationship between cholesterol levels and reproductive performance exists in buffaloes (Murthy and Rao, 1981). As there is availability of green grasses in winter season (Jan-Feb and Nov-Dec) as compared to summer season (May-June), the protein level was high in January-February and November-December than the May-June however there was no significant difference between them.

The cortisol level was significantly different among the different seasons. The cortisol is the stress factor and due to temperature and nutritional stress there might have been high cortisol level in summer season (May-June) as compared to winter season (November-February).

This study gives an indication that conception rate as in good breeding season can be achieved during poor breeding season as well using progestin based protocol if sound management of nutrition and stress factor is approached. BCS and serum nutritional factors at the beginning of synchronization protocol are the determining factor to achieve satisfactory conception rate.

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EVALUATION OF REPRODUCTIVE TRAITS OF LIME AND PARKOTE (*Bubalus bubalis*) BUFFALOES IN THE WESTERN HILLS OF NEPAL

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ABSTRACT

The study was carried out to estimate the phenotypic values of reproductive traits of indigenous Lime and Parkote breeds of buffaloes (*Bubalus bubalis*) considering various non-genetic factors. The study was conducted in Faliyagaun of Myagdi and Ramjhathati of Parbat districts during October to November, 2016. Altogether 100 buffaloes, 50 from each village were chosen by Purposive sampling and semi-structured questionnaire was prepared for data collection. Data were analyzed by Least Square Mixed Model and Maximum Likelihood (LSMMML PC-2) computer program using Harvey-1990 software and SPSS V.20. Results revealed that overall least square mean (LSM) of, Age at first service (AFS), Age at first calving (AFC), Gestation length (GL), Post-partum estrus (PPE), and Calving interval (CI) were 1346.56±67.35, 1677.88±67.12, 332.47±1.24, 212.32±27.53, and 557.06±27.26 days, respectively. Season of calving was found significant with Post-partum estrus and Calving interval at (P<0.05) level, longest Post-partum estrus and calving interval was found in winter and shortest in autumn. Age at first calving, Age at first service, Post-partum estrus, and Calving interval were highly significant (P<0.01) with location. Post-partum estrus was significantly affected (P<0.05) by parity and was observed to be high in the early, decreased in mid and increased in the late parity. Gestation length was non-significant with all the factors indicating it to be the constant factor for buffaloes irrespective of the genetic and non-genetic factors. Coat color and breed were non-significant with all the reproductive traits. It can be concluded that buffaloes of Parbat district were superior in terms of the reproductive traits, and the reproductive traits were influenced by the environmental factors so, to improve the reproductive performance, adjustment to management schemes could be made.

Keywords: Non-genetic factors, indigenous breeds, Lime, Parkote, reproductive traits

INTRODUCTION

Agriculture sector in Nepal contributes about 31.2% to National GDP (MOAD 2015) and share of Livestock subsector to AGDP is 26.8% , whereas livestock sector alone contributes 11% to National GDP (MOLD 2072/73) .Thus, livestock sub-sector is an important output of agriculture that contributes to national economy. Livestock is an integral part of the Nepalese farming system. Livestock with combination with agricultural lands and forests gives the model of a complete integrated farming system.

There are 5.16 million buffaloes in Nepal (MoAD, 2015). All the identified indigenous buffalo breeds of Nepal are riverine type and have 25 pairs of chromosomes (2n = 50). Are mainly black and grey in color and consists of peculiar markings or spots on different parts of body. Body color, horn shape, spotted marks in the body and chevron can be used as the medium to distinguish the breed. The mature body weight of buffaloes ranges from 310 to 450 kg. The indigenous buffalo breeds of Nepal are suspected to be the descendants of the wild buffalo Arna (*Bubalus bubalis arnee*) which are found in the eastern hills of the country. So far three indigenous breeds of buffaloes are identified and characterized in Nepal. Which are Lime, Parkote and Gaddi.

The expressions of the phenotypic character is the function of genotype under the influence of environment. As the breeder is primarily interested in the genetic improvement, the separation of the phenotypic changes of economic traits (productive and reproductive) into the genetic and environmental characters is important (Singh et al., 1989).

Large no of exotic breeds have been introduced from outside for rearing and also for crossbreeding programs and this is gradually affecting the population of the indigenous buffaloes and has led to the decrease in their blood level (Neopane et al., 2007). Due to the indiscriminate breeding there is clear reduction of the indigenous breeds in the western hills. The absence of definite breeding plan and blood level for these local buffaloes mainly concentrating on agro-geological belts the population of the Lime, Parkote and Gaddi buffaloes is diminishing gradually (Neopane et al., 2007).

The objective of the present study was to make the comparative study of the reproductive performance of the indigenous hill buffaloes and estimate the effect of the non-genetic factors as, parity, age, season of calving and breed on the reproductive traits, age at 1st calving, age at 1st service, calving interval, gestation length and post-partum estrus.

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MATERIALS AND METHODS

Research Site and Husbandry System

This study was carried out in two of the western hill districts of Nepal, Myagdi and Parbat. In Myagdi the site was Faliyagaun village of Muna VDC and in Parbat, Ramjhathati was selected for research. The altitude of the site in Myagdi district was 1620 Masl and the site in Parbat district was at 1249 Masl.

In both the villages, animals were raised in the separate housing near to the house. During summer and spring they were left in the pastures to graze for the duration of 4-7 hours. Whereas during winter when there is lean season for fodder crops, they were fed on the dried straw and some of the available grasses. Also during the food deficiency in the lower hills, animals were moved upward to the forest in search of food. Besides feeding grasses animals were fed kudo (feed made by cooking the flours of millet or maize in water) as the additional food. During morning and evening, cut-and carry feeding was done before milking the buffaloes.

Sampling Procedure and Sample Selection

Study used purposive sampling technique in order to conduct household survey. The list of indigenous buffalo rearing farmers was prepared with the help of DLSO office staffs and key informants. Altogether 100 animals from Myagdi and Parbat, 50 from each district were selected for study of different reproductive parameters accordingly with respect to various genetic and non-genetic factors.

Data Collection Technique and Types of Data

The data regarding general information and reproductive traits of buffalo were collected from the household survey and Semi-structured questionnaires was prepared to collect the data.

Study used primary and secondary data and information. Primary data were collected through the household survey of indigenous buffalo raising farmers in Myagdi and Parbat districts. Whereas the secondary data such as geographical condition, Status of buffaloes were collected through the desk study of the publications of NARC, MOLD, CBS and other unpublished thesis.

Table 1. Genetic and Non-genetic factors considered for the study.

Factors	Categories
Coat colour	1= Grey, 2= Black, 3= Black with white fur on legs and neck
Breed	1= Lime, 2= Parkote
Parity	1= first to third, 2= fourth to sixth, 3= seventh and above
Location	1= Parbat, 2= Myagdi
Season of calving	1= Spring (Mar-May), 2= summer (June-Aug), 3= autumn (Sep-Nov), 4= winter (Dec-Feb)

Table 2. Reproductive traits considered for the study.

Traits	Description
Age at first Service	Age of heifer when conceives for first time.
Age at first calving	Age of heifer when delivers for the first time.
Calving Interval	Period between two successive calving
Post-partum estrus	Time taken from calving to next successful insemination
Gestation length	Period between conception and calving

Data Analysis

Data generated for this study were first entered into MS-excel and converted into the text document (MS-DOS). The data were analyzed by least square procedure using Harvey (1990) software package and SPSS 20, to study the main effect of the non-genetic factors on productive and reproductive traits and also to study the main cause of variation.

RESULTS AND DISCUSSION

The overall least square mean (LSM) of, age at first service (AFS), age at first calving (AFC), gestation length (GL), post-partum estrus (PPE), and calving interval (CI) were 1346.56±67.35, 1677.88±67.12, 332.47±1.24, 212.32±27.53, and 557.06±27.26 days respectively (Table 3).

Data analysis revealed that the Breed and the Coat Colour of the animal did not significantly affect any of the reproductive traits. However, the age at first service and age at first calving of the Parkote (1401.99±49.85 days



AFS & 1734.04±49.62 days AFC) buffaloes were slightly higher as compared to the Lime buffaloes (1379.89±11.23 days AFS & 1712.04±42.18 days AFC). Similarly a higher AFS and AFC of Parkote buffaloes than the lime buffaloes was also reported by Amatya et al. (2000).

The highly significant effect ($P < 0.01$) of the location on the reproductive traits such as, AFS, AFC, post-partum Estrus and Calving Interval was found. But the effect of the location on the Gestation length was indifferent to the location. Season of calving had a significant effect on the Post-partum estrus and and Calving interval at ($P < 0.05$) level. A higher post-partum estrus and calving Interval was observed in the winter followed by summer, spring and autumn respectively. Another non-genetic factor, parity of the dam also affected the post- partum estrus at ($P < 0.05$) level. Of all the reproductive traits considered, Gestation length was the one which was not affected by any of the genetic and non- genetic factors.

The variation in the AFC for the same breed among the different studies might be due to the differential inputs, management practices and the health conditions of the animal. The better management practices and the well fed conditions led to the faster growth and early service and which in turn resulted in the earlier age at first calving (Negussie et al., 1989).

The higher AFS and AFC in the Myagdi district may be due to the differences in the altitude, insufficiency of the feeds and poor management practices than in Parbat. Rasali (1996), reported that the age of first calving was significantly affected by the altitude.

Thiruvankadan et al. (2014) reported a significant ($P < 0.05$) effect of the season of calving on the post-partum estrus. According to the study, the murrh heifers calving in the monsoon had significantly lower service period, than those freshening in the other seasons. Whereas the highest service period was observed in the winter season calvers. This finding was in accordance with the present study.

PPE was significantly higher (240.82±34.21 days) during the 1st – 3rd parity followed by the 7th and above parity (210.06±31.61days) and the lowest PPE was found during the 4th -6th parity (186.07±55.47 days). The significant effect of the parity in the PPE found in the present study was in accordance with the findings of (Kundu et al., 2003; Sures et al., 2004).

Table 3. Least square mean and standard error of reproductive traits of Lime and Parkote buffaloes with respect to the different non- genetic factors in Western Hills of Nepal, 2016

Non-genetic factors	No. of Obs.	Age at 1 st Service	Age at 1 st Calving	Gestation Length	Post-partum Estrus	Calving Interval
Overall Mean	100	1346±67.35	1677±67.12	332.47±1.24	212.32±27.53	557.06±27.26
Breed						
Lime	58	1379.8±11.23	1712.04±42.1	330.51±0.83	191.60±15.82	535.57±15.84
Parkote	42	1401.9±49.85	1734.04±49.6	330.71±0.97	187.68±18.61	523.33±18.64
Significance		NS	NS	NS	NS	NS
Location						
Parbat	50	1281.75±46.41	1612.32±46.20	330.26±0.9	144.11±17.33	504.60±17.35
Myagdi	50	1500.14±45.51	1833.76±45.30	330.96±0.89	235.18±16.99	610.29±17.02
Significance		**	**	NS	**	**
Parity						
1 st – 3 rd Parity	55	-	-	331.12±1.43	240.82±34.21	592.56±33.87
4 th – 6 th Parity	38	-	-	331.63±1.55	186.07±55.47	523.55±54.92
Above 7 th	07	-	-	334.65±2.51	210.06±31.61	555.07±31.30
Significance		-	-	NS	*	NS
Season						
Spring	10	-	-	331.60±1.39	194.91±30.75	541.28±30.42
Summer	40	-	-	332.04±3.81	227.82±47.14	569.3±47.02
Autumn	47	-	-	331.22±1.26	135.11±27.94	472.77±27.67
Winter	03	-	-	335.09±2.15	291.42±84.13	644.60±83.29
Significance		-	-	NS	*	*

Note: *= Significant at 0.05 level, ** = Significant at 0.01 level and N.S = non- significant



CONCLUSION

From the results obtained through the observations in the experiments, The reproductive traits of the indigenous buffaloes were influenced by the non-genetic factors; Location, Season of Calving and Parity of dam so, the phenotypic expression of the reproductive trait was not only genetic but was also affected by the environment. The differences in the reproductive traits over the location may be due to the differences in the altitude, feeding and management practices whereas the effect of the season may be due to the environmental stresses and the availability of the fodder.

The expression of the gestation length was solely genetic as it was not affected by any of the factors considered. It was the constant factor for the indigenous buffaloes irrespective of the factors considered. As, there was non-significant effect of the coat colour to the reproductive traits it can be said that coat colour was not an important factor to be considered while evaluating the reproductive traits. Also, from the above findings it can be seen that the Lime buffaloes were early maturing than the Parkote buffaloes, whereas the Parkote buffaloes were superior in terms of PPE and CI. Whereas, location wise the buffaloes of the Parbat district were superior to the buffaloes of the Myagdi district in terms of the reproductive traits.

Hence, it can be concluded that as the reproductive traits were influenced by the environmental factors. In order to improve the reproductive performance, the adjustment to the management schemes can be made and for the selection of the animal, the phenotypic characters in relation with the environmental conditions should be considered.

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STUDY ON FACTORS AFFECTING NOVEL HORMONAL PROTOCOLS IN ANESTRUS BUFFALOES DURING ACTIVE BREEDING SEASON

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ABSTRACT

July to December is regarded as active breeding season whereas January to June is regarded as low breeding season for buffaloes in Nepal. The management of buffalo reproduction is difficult due to various problems such as poor expression of estrus, irregular estrus cycles and low conception rates. Nowadays use of hormonal protocols in animal reproduction has become popular. This study was conducted with objective to study the possible factors affecting the outcome of hormonal protocols in anestrus buffaloes during active breeding season. Murrah cross bred buffaloes (n=14) of Livestock Farm of Agriculture and Forestry University, Chitwan, Nepal were selected for the research. Buffaloes were divided into two groups, 7 buffaloes received G6G protocol and other 7 received Ovsynch protocol treatment. Body condition scoring (BCS) and managemental practices were recorded. Fecal examination and blood sample collection was carried out on day 0 of treatment. Ovulation was confirmed on day 7 after artificial insemination. Transrectal ultrasonograph was applied for early pregnancy diagnosis and to understand ovarian dynamics. Pregnancy confirmation was done after 90 days of insemination. Overall pregnancy was 14.29%. Similarly, protocol wise pregnancy was 14.29% in G6G and 14.29% in ovsynch protocol. Endoparasite prevalence was found to be 57.14% however anestrus buffaloes showing positive pregnancy result were free from endoparasitic load. Mean BCS of buffaloes was 2.8 ranging from 2.5 to 3.5. Serum biochemical parameters like glucose, total protein, calcium and cholesterol were in normal range but phosphorus was in sub normal level. Anestrus buffaloes showing positive pregnancy result were free from endoparasitic load. Endoparasitic problem and nutritional status likely play a vital role on pregnancy outcome. However, further study is required to clarify this preliminary finding.

Keywords: G6G, Ovsynch, anestrus buffaloes, active breeding season

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SHORTENING FIRST CALVING AGE IN BUFFALO THROUGH FEEDING MANAGEMENT

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ABSTRACT

Among several constraints in buffalo raising delayed puberty and age of first conception causes an economic burden to farmers. To find out the way to minimize the age of first conception, 12 Murrah cross-breed calves having similar birth weight, maternal parity and age were selected. Calves were fed *ad-libitum* colostrum for four days after birth. Thereafter milk was fed as 1/10th of their body weight for 2 months followed by 1/15th of the body weight with few concentrate and green grass until weaning at 6 months of age. Randomly calves were distributed in 3 groups with 4 calves in each group. Each group were offered with DM 2.5% of the body weight for three years. The three treatments (T1, T2, T3) groups were given 35%, 20% and 5% DM requirement from concentrate respectively for two years. Result showed that the calves which were chosen for trial were not differ significantly on body weight and also during weaning period didn't differ significantly. At 1 year of age, 35% concentrate fed group had a highest average weight gain (593 gm/day) whereas 5% concentrate fed group had a lowest average body weight gain (361 gm/day). Among 4 calves of 35% concentrate fed group, 2 calves conceived at around 24 months of age and parturated at 36 months of age whereas 2 other conceived at 36 months of age. Moreover, 35% concentrate fed group calves had 350 kg body weight at 24 months whereas 20% and 5% concentrate fed group had 263 kg and 248 kg respectively which is considered inappropriate weight to conceive. Hence it was concluded that among the total requirement of DM, 35% DM from concentrate feeding increases the body weight, shortens the first conception age leading to parturition by 36 months of age.

Keywords: Buffalo, Dry matter, Concentrate, Parturition

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STUDY ON ESTRUS BEHAVIOUR WITH REFERENCE TO VAGINAL ELECTRICAL RESISTANCE AND ANALYZING SERUM BIOCHEMICAL PROPERTIES DURING ESTRUS SYNCHRONISATION IN CROSSBREED BUFFALOES

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ABSTRACT

Vaginal Electrical Resistance (VER) is an important aid to estrus detection and early pregnancy diagnosis in cattle and buffaloes. The present research was carried out to evaluate estrus behavior with reference to vaginal electrical resistance and to analyze serum biochemical properties during estrus synchronization in crossbreed buffaloes. Vaginal electrical resistance reading was noted on every twelve hour interval for continuous 21 days after GnRH injection of both Ovsynch and G6G protocol. Swollen vulva, mounting and mucosal discharge was observed on every VER reading. Blood sample on day 0, PGF_{2α} injecting day and Artificial Inseminating (AI) day were collected from jugular vein. Data were tabulated in MS-Excel and analysed using SPSS version 20. Nearly 70% of buffalo shows lower VER reading at the time of AI. Mounting and swollen vulva was not significant but mucosal discharge was significant ($P < 0.05$) with respect to VER. Serum values of protein, glucose and calcium were found to be increased whereas phosphorus found to be decreased during estrus. In conclusion, VER values can be used for estrus detection on commercial buffalo farm. Further research needs to be done on natural estrus cycle regarding these parameters.

Keywords: Vaginal electrical resistance, estrus, buffaloes

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EFFECT OF NON-GENETIC FACTORS ON LACTATIONAL EFFICIENCY OF INDIGENOUS BUFFALOES AT DIFFERENT STAGE OF PARITY IN WESTERN HILLS OF NEPAL

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ABSTRACT

This study was undertaken during October-November 2016 to evaluate the effect of breed, parity and breed × parity interaction on lactational efficiency of indigenous buffaloes of Muna VDC-3, Faliyagaun of Myagdi district and Ramjhathati VDC-9 of Parbat district of Western Nepal. Altogether 100 buffaloes, 50 from each study site were considered by purposive sampling and data were collected using the semi structured questionnaire. Data analysis was done by Henderson's Least Square Mixed Model and Maximum Likelihood (LSMML PC-2) computer program using Harvey-1990 software. Colostrum period (days), days to reach peak milk yield (DPMY), lactation length (days) and dry period (days) were the parameters under consideration to study the lactational efficiency. The overall means for colostrums period, DPMY, lactation length and dry period were 3.11, 13.91, 362.10 and 165.40 days respectively. All non genetic factors; breed, parity and breed × parity interaction had non-significant effect on the traits of lactational efficiency. But higher value of lactation days was found at later parity (above 7th parity) than early parities (1st -3rd parity) and mid parities (4th -6th parities) where values of later parity were (394.10±48.02), mid parities were (353.90±24.51) and early parities were (361.20±12.53). Similarly, lower value of dry period was found in later parities (105.70±36.05) as compared to mid (135.20±18.40) and early (175.90±9.41) parities. The lactation length was found higher in later parities for both Parkote (394.20±47.53) and Lime breed (394.00±75.15). Dry period was found lowest in Lime (60.00±56.42) in later parities. Better management skill with appropriate breeding program is the need to increase the lactational efficiency of indigenous buffaloes.

Keywords: Lime, Parkote, lactational efficiency, parity, breed

INTRODUCTION

Buffaloes are the main source of milk and meat in Nepal with the total annual contribution of about 65.22% (milk) and 54.33% (meat) which is equivalent to 1.21 million metric ton of milk and 175 thousand metric ton of meat from 5.16 million heads of buffaloes (DOA, 2017). In Nepal, buffaloes are mainly raised for milk, meat, draught, milk product, manure and hides (FAO, 2005). Indigenous buffalo (*Bubalus bubalis*) in Nepal are riverine type having 25 pairs of chromosome (2n=50). Lime, Parkote and Gaddi are the identified indigenous breeds of buffalo domesticated in Nepal where Lime and Parkote are abundantly found in western mid-hills of Nepal and Gaddi are found in far-western hills (Neopane et al., 2007). It has been estimated that Lime is about 35% of the total indigenous buffalo population found in the hills and mountains of the country and only 25% of the total population are Parkote (Rasali, 1998). Indigenous buffaloes of Nepal are hardy and have the ability to adapt themselves in different agro-ecological regions. Because of their special qualities like production potentiality in low input system, efficient forage digestion ability, tolerance to cold and harsh climate, and relatively smaller body size than other exotic breeds, they are highly suitable to flourish on narrow and steep slope of the hills and mountains of the country. Within the indigenous breeds of buffaloes, some very high yielding individuals have been reported. However, due to the absence of the proper mating application and knowledge of selection, those high potential genetic stocks have not been utilized (Poudel et al., 2011). Only limited studies have been done regarding the improvement of the production potential of such indigenous breeds. Even though, identification and characterization of different breeds of buffaloes have been done (Shrestha and Sherchan, 1997). In this context, present study was carried out with the aim of understanding the effect of non-genetic factors on the traits related to lactational efficiency including colostrum period, days to reach peak yield lactation length and dry periods of indigenous Lime and Parkote.

MATERIALS AND METHODS

Study was executed during October-November of 2016 in Muna VDC-3, Faliyagaun of Myagdi district and Ramjhathati VDC-9 of Parbat district at Western Nepal. Information from DLSO staffs and key informants was utilized for site identification and to obtain the list of indigenous buffalo rearing farmer. Total of 100 buffaloes, 50 from each study site were considered by purposive sampling and household survey was conducted to collect the data using the semi structured questionnaire. Different lactational traits such as colostrum period (days), days to reach

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peak milk yield, lactation length (days) and dry period (days) were recorded. Thus collected data were used to study the effect of non genetic factors (breed and parity) and their interaction (breed × parity) on lactational traits of Lime and Parkote buffalo breeds. Collecting all the information required for the study, the data were coded and entered into MS-Excel & converted into text documents (Text MS-DOS). The data were analyzed by least square procedure using Harvey (1990) software package and mean comparison was performed by Duncan's Multiple Range Test (DMRT) software. Among different models of Harvey (1990), following fixed effect model was used to estimate the effect of different non genetic factors on colostrum period, days to reach peak milk yield, lactation length and dry period.

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

where, μ is the overall mean

a_i is the effect of i^{th} breed ($i=1$ and 2)

b_j is the effect of j^{th} parity ($j=1, 2$ and 3)

$(ab)_{ij}$ is the effect of interaction between i^{th} breed and j^{th} parity

e_{ijk} is the random (residual) element assumed as randomly and independently distributed

RESULTS AND DISCUSSION

The traits of lactational efficiency such as colostrum length (days), days to reach peak milk yield, lactation length (days) and dry period (days) are ultimately the important economic traits of the dairy animals. So, the above traits were considered for the study. The non genetic factors such as breed, parity and breed×parity interaction were considered to study their effects to the above mentioned traits of lactational efficiency. The results of the study are presented in Table 1 and 2.

Colostrum Length

The overall mean colostrum length (days) in this study was found to be 3.11 ± 0.21 days with the range of 1-7 days (Table 1). The result is within the range of finding by FAO (1993) in which it is reported that after 3-5 days of calving the milk will reach its normal composition.

There was no significant effect of breed on colostrum length of buffalo (Table 1). Both Lime and Parkote breeds have almost similar value of colostrum period. Moreover, the non significant difference on colostrum length was observed with respect to parity (Table 1). However, slightly higher value of colostrum length was observed in early parity (1st to 3rd parity) than the later parities (7th and above parities).

Similarly, interaction between breed and parity (B×P) also has no significant effect on colostrum length (Table 1). However, slightly higher value of colostrum length was observed in early parity (1st to 3rd) in case of both the breeds.

Table 1. Least Square Mean and Standard errors of colostrums period (days) and days to reach peak milk yield with respect to breed, parity and breed-parity interaction of indigenous buffalos

Factors	No. of observations	Colostrum period (days)	Days to reach peak milk yield
Pooled mean	100	3.11 ± 0.21	13.91 ± 1.25
Range	100	1-7	4-30
Breed			
Lime	58	3.08 ± 0.20	13.95 ± 1.20
Parkote	42	3.12 ± 0.20	13.45 ± 1.20
Significance		NS	NS
Parity			
1 st to 3 rd	55	3.18 ± 0.12	13.73 ± 0.71
4 th to 6 th	38	2.95 ± 0.24	13.00 ± 1.39
Above 7 th	07	2.70 ± 0.47	15.84 ± 2.73
Significance		NS	NS
Breed X Parity Interactions			
Lime X 1 st to 3 rd	37	3.18 ± 0.16	14.19 ± 0.92
Lime X 4 th to 6 th	19	2.91 ± 0.30	13.42 ± 1.74
Lime X Above 7 th	02	2.50 ± 0.74	13.00 ± 4.27
Parkote X 1 st to 3 rd	18	3.17 ± 0.19	13.10 ± 1.12



Factors	No. of observations	Colostrum period (days)	Days to reach peak milk yield
Parkote X 4 th to 6 th	19	3.00 ± 0.39	12.43 ± 2.28
Parkote X Above 7 th	05	3.00 ± 0.47	19.80 ± 2.70
Significance		NS	NS
CV%		33.84	43.49

Note: NS – Non significant; CV - coefficient of variation

Days to Reach Peak Milk Yield

The overall mean 'days to reach peak milk yield' was 13.91 ± 1.25 (around two weeks) with the range of 4-30 days (Table 1). However, Poudel *et al.* (2011) found higher value of 'days to reach peak milk yield' in his study at Gulmi and Argakhanchi districts. While working on production performance of indigenous as well as Murrah cross breed, he observed that the value of 'days to reach peak milk yield' was between 4-6 weeks. Similarly, Shah *et al.* (2011) also found 'days to reach peak milk yield' value of Lime breed to be around 36 days.

There was no significant effect of breed on 'days to reach peak milk yield' of indigenous buffaloes (Table 1). Both Lime and Parkote breeds have almost similar value of 'days to reach peak milk yield'. No difference in the value of 'days to reach peak milk yield' in both Lime and Parkote breed was also observed by Poudel *et al.* (2011). However, he found higher value of 'days to reach peak milk yield' (5-6 weeks) in case of Murrah crossbred.

The non significant difference on days to reach peak milk yield was observed with respect to parity too (Table 1). However, days to reach peak milk yield value was higher in late parity (7th and above parity) than in early (1st-3rd) and mid (4th -6th) parities.

Breed and parity interaction (B×P) also has no significant effect on 'days to reach peak milk yield' (Table 1). However higher value of 'days to reach peak milk yield' was observed in case of Parkote × late parity (7th and above parity) but lower and similar values in other B×P observations.

Lactation Length (days)

The overall mean lactation length was 362.10 ± 22.10 days (Table 2) with the range of 210-635 days. This value is higher in comparison to the findings of Shah *et al.* (2011) who found the lactation length of Lime of western hills to be 303 days.

Breed had no significant effect on lactation length (Table 2). Both Lime and Parkote breeds have almost similar value of lactation length but Shrestha *et al.* (2005) found significant ($P < 0.01$) effect of breed on lactation length where he observed the lactation length of Lime and Parkote to be 276 days and 285 days respectively. The difference in the value of lactation length within Nepalese indigenous breed is much lesser as compared to exotic breeds. Metry *et al.* (1994) found the lactation length value of Egyptian buffalo to be between 288-301 days. Similarly, Silva *et al.* (1995) found the lactation length value of Murrah between 248-441 days and Tailore *et al.* (1992) found the lactation length value of Surti buffalo between 261-379 days.

The non significant difference on lactation length was observed with respect to parity (Table 2). However, value of lactation length is found higher in case of late parity (7th and above parity) than early (1st-3rd) and mid (4th-6th) parities. Jamuna *et al.* (2015) while carrying out the performance study of Murrah buffalo and Afzal *et al.* (2007) studying the milk yield and lactation traits of Nili Ravi buffalo also revealed the non significant difference of lactation length with respect to parity. However, Bashir *et al.* (2015) found the significant difference ($P < 0.05$) of lactation length with respect to parity. In his findings, lactation length was maximum after first calving (283 days) and has decreased with increase in parity reaching minimum (258 days) in ≥ 10 lactations.

Breed and parity interaction (B×P) also has no significant effect on lactation length (Table 2). However, bit higher value of lactation length was observed in late parity (7th and above) in case of both the breeds (Lime and Parkote). The value of lactation length in this case has gone in the line of parity as the lactation length value in case of breeds is almost constant. In this case, it seems that proper management and breeding practices would be determining factor to increase the lactational efficiency of indigenous buffalo.

Dry Period (days)

The overall mean dry period was 165.40 ± 16.59 days with the range of 31-365 days (Table 2). The value of dry period in this study is much lower in comparison to Bashir *et al.* (2015) who revealed the dry period value of Nili Ravi buffalo to be 258 days. So, there is much difference in dry period within the breeds though the value of dry period within Nepalese indigenous breeds (Lime and Parkote) in this study is almost similar with no significant differences (Table 2).



Statistically no significant difference of dry period was observed with respect to parity (Table 2). However, much lower value of dry period was observed at late parity (7th and above). The value of dry period decreases with increase in parity. Bashir *et al.* (2015) also recorded that the dry period is longer (290 days) for 1st parity Nili Ravi buffalo followed by gradual decline of dry period value in later parities. Similar result (gradual decrease in dry period with the increase in parity) was also reported by Kandasamy *et al.* (1993). Differential climatic conditions and managerial operations may also leads to variation in dry period value (Hussian *et al.*, 2006). Reproductive management can be a dependent factor to optimize the dry period to 45-60 days (Kanaujia and Balaine, 1975). It is also possible that some animals can conceive very early after calving, have longer lactation length and very few dry days (Bashir *et al.*, 2015).

Breed and parity interaction (B×P) also has no significant effect on lactation length (Table 2). As the value of dry period within the breeds (Lime and Parkote) is almost constant, the dry period value at different breed-parity interaction is in the same line as in the dry period value at different parities.

Table 2. Least Square Mean and Standard errors of Lactation length (days) and Dry period (days) with respect to breed and parity and breed–parity interaction of indigenous buffalos

Factors	No. of observation	Lactation length (days)	Dry period (days)
Pooled mean	100	362.10 ± 22.10	165.40 ± 16.59
Range	100	210-635	31-365
Breed			
Lime	58	363.30 ±22.01	161.90 ±16.50
Parkote	42	360.50 ±22.01	164.50 ±16.50
Significance		NS	NS
Parity			
1 st to 3 rd	55	361.20 ± 12.53	175.90 ± 9.41
4 th to 6 th	38	353.90 ± 24.51	135.20 ± 18.40
Above 7 th	07	394.10 ± 48.02	105.70 ± 36.05
Significance		NS	NS
Breed X Parity Interactions			
Lime X 1 st to 3 rd	37	364.80 ± 16.21	179.00 ± 12.17
Lime X 4 th to 6 th	19	346.40 ± 30.68	134.50 ± 23.03
Lime X Above 7 th	02	394.00 ± 75.15	60.00 ± 56.42
Parkote X 1 st to 3 rd	18	356.20 ± 19.74	171.50 ± 14.82
Parkote X 4 th to 6 th	19	364.30 ± 40.17	136.30 ± 30.16
Parkote X Above 7 th	05	394.20 ± 47.53	169.20 ± 35.68
Significance		NS	NS
CV%		29.35	48.26

Note: NS – Non significant; CV - coefficient of variation

Non – genetic factors considered in this study (i.e breed and parity) does not make significant influence in increasing the lactational efficiency. Nevertheless, few traits of these indigenous breeds like “days to reach peak milk yield, lactation length and dry period are more acceptable compared to similar traits of other exotic breeds. So, appropriate breeding/mating plans with good management practice and proper nutrition would be a key in the improvement of lactational efficiency.

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BUFFALO POPULATION AND BREEDING STRATEGIES FOR ITS GENETIC IMPROVEMENT IN NEPAL

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ABSTRACT

The domestic buffalo (*Bubalus bubalis*) contributes significantly to the Nepalese agrarian economy in various ways such as milk, meat, draught power production and as a source of income that requires minimum inputs. Nepal is having 5.2 million of buffalo population, contributes the highest national meat share (54%) and supply the highest 65% of the total milk produced of the country. In Nepal, the domesticated buffaloes are mainly of riverine type with fifty chromosomes (n=50). These dual purpose river buffaloes are maintained with low inputs for milk production, and also possess good quality meat characteristics however their potential is still remain unexplored and unexploited. Even though area of the country is small, Nepal has three distinct buffalo breeds viz. Lime, Parkote, Gaddi in low to mid hills and large number of non-descript populations. There is Terai buffalo in low land which has been identified and characterized in phenotypic and production level and yet to be released in the FAO breed list. These genetic resources are progressively being eroded mainly due to unplanned breeding and lack of policy. There is almost unrestricted interbreeding among different breeds and there is a marked decline in the availability of unique animals conforming to the attributes of defined breeds, particularly in their native breeding tracts. The situation is further complicated by the fact that there exists no breed societies or breed registration/improvement societies to register animals of specific breeds, maintain herd books and ensure the purity of the breeds. The germplasm of such well-defined breeds constitute a valuable genetic resource which needs to be conserved on priority basis. Hence, proper conservation measures have to make to preserve the valuable buffalo genetic resources of Nepal for the sustainable utilization. This paper combines the phenotypic data of all four breeds taken for this study and all the information available regarding the indigenous buffaloes of Nepal and recommends breeding strategies for the conservation and improvement of indigenous buffaloes.

Keywords: buffalo genetic resources, conservation, strategies

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A REVIEW ON BUFFALO BREEDING SYSTEM IN NEPAL AND FUTURE STRATEGIES

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ABSTRACT

Buffalo is an important livestock commodity of Nepal. Buffalo industry is leading food sectors through milk and meat supply in Nepal. At past, most of the peoples used to raise buffalo and there is at least one buffalo in each household. Majority of the buffalo reared by farmers belongs to native breeds, among them Lime, Parkote and Gaddi are reared since historical times. Terai buffalo and other cross breeds are also reared by farmers mainly in terai region. Murrah is an exotic buffalo breed reared by commercial farmers. The productivity of all buffalo breeds have been rated low due to poor management, health care and haphazard breeding system that prevails all over the country. However, farmers are raising the native breeds for their hardiness, quality milk and taste, disease resistance, low cost for raising, social reason and favorable climatic condition. These characteristics are the advantage to maintain native buffalo as a reservoir of valuable genes that might be available in the future. It is believed that constant genetic deterioration due to indiscriminate breeding of native buffalo, competition with efficient high yielding exotic breeds and inadequate nutrition are increasing threat to native buffaloes. Selection within native breeds at some extent helps in increasing productivity of buffalo population. To improve the present level of productivity Pedigree and Performance Recording Schemes (PPRS) need to be carried out. Larger herd of indigenous buffalo need to be established by government, universities and Nepal Agricultural Research Council farms to better utilize Pedigree and Performance Recording Schemes (PPRS), while pure breed commercial farms of buffalo serves for the same purpose for exotic breed. Progeny of the best pure breed animals need to be used for semen production and also use for increasing productivity of native breed as well as upgrading of existing crossbreds buffalo population.

Keywords: Buffalo, pedigree and performance recording schemes (PPRS), upgrading

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FACTORS AFFECTING SUCCESS OF NATURAL BREEDING ON CONCEPTION IN BUFFALOES OF WESTERN CHITWAN

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ABSTRACT

A study was conducted to determine the factors affecting success of natural breeding on conception in buffaloes. A questionnaire survey was conducted in 89 households of four different villages of western Chitwan consisting of 100 buffaloes. Information regarding parity, time interval from calving to first breeding, number of breeding per conception, timing of breeding, season of breeding, breeding bull, peak milk yield and frequency of deworming was collected. Overall first breeding conception rate (FBCR) was 75.0% and the number of breeding per conception (mean \pm S.D) among pregnant buffaloes was 1.37 ± 0.75 . Chi-square test and ANOVA were used to analyze the data. FBCR in 1st, 2nd, 3rd and $\geq 4^{\text{th}}$ parity buffaloes was 61.1%, 80.7%, 76.5% and 76.7%, respectively; FBCR was significantly ($P < 0.05$) higher in 2nd parity than in 1st parity buffaloes. The FBCR in buffaloes bred within 3 months postpartum, those bred between 4 and 7 months postpartum and those bred ≥ 7 months postpartum was 81%, 77.2% and 76.52%, respectively. The FBCR in buffaloes those were mated within 6 hours, between 7 and 12 hours and beyond 12 hours after the onset of estrus was 68.8%, 79.7% and 66.7%, respectively. Maximum number of buffaloes were bred during winter season (42%) followed by autumn (26%), summer (19%) and spring (13%). Among the four breeding bulls used for mating, the bull C had the best fertility; the FBCR among buffaloes mated by bull C was the highest (90%). Among all buffaloes, the peak milk yield (mean \pm S.D.) was 8 ± 2.24 litres/day. The FBCR in buffaloes producing peak milk yield ≥ 8 litres/day and those producing < 8 litres/day was 84.2% and 72.2%, respectively. Conception rate was not affected by deworming schedule. In conclusion, parity, postpartum interval, timing of breeding, peak milk production and frequency of deworming had no effect on conception but bull performance made difference in conception rate of buffaloes in western Chitwan. Winter and autumn were active breeding season while spring and summer were the poor breeding season for buffaloes in Chitwan.

Keywords: buffalo, conception, natural mating, breeding bull, peak milk yield

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EVALUATION OF OVSYNCH PROTOCOL ON REPRODUCTIVE PERFORMANCE OF ANESTROUS BUFFALOES DURING GOOD BREEDING SEASON IN CHITWAN, NEPAL

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ABSTRACT

Ovsynch protocol is established tool for fixed time artificial insemination use in lactating dairy herds. Anestrus and silent estrus is the major cause of infertility in Buffaloes of Nepal. There are very scarce reports of using Ovsynch in anestrus and silent estrous buffaloes during breeding season. So this study was designed based on the hypothesis that Ovsynch protocol might be alternative breeding technique in silent estrous buffaloes and a tool for corrections of true anestrus in buffaloes. This study evaluated the effectiveness of Ovsynch/FTAI protocol (day 0 fertirelin inj. 100 μ g I/M, day 7 cloprostenol inj. 500 μ g I/M, day 9 fertirelin inj. 100 μ g I/M, and day 10 fixed time artificial insemination 18 hours after second injection of fertirelin) in anestrus buffaloes (n=14) of Chitwan, Nepal. Protocol was evaluated in terms of estrus characteristics, follicular wave synchronization, ovulation and pregnancy rate. Responses were analyzed by grouping the buffaloes based on type of anestrus, length of anestrus, body condition score (BCS), milking status, parity, age, management condition, follicle count on day of second GnRH treatment. Average total follicle count and count of follicles larger than 5 mm in diameter on day 9 was 11.7 \pm 2.9 and 1.9 \pm 0.8 respectively. Out of the total buffaloes, 92.9% showed follicles larger than 5 mm in diameter. Intensity of estrus characteristics on day of AI was mild. Major estrus signs observed were uterine tonicidity, alertness, mucus discharge on rectal manipulation of internal genitalia, swelling of vulva and teat engorgement. Mucus discharge on rectal manipulation of internal genitalia and teat engorgement were clearly noticed in 50 % buffaloes. There was higher tendency (P=0.08) of estrus expression by buffalos with anestrus period of 10 months or lower (80%) compared to those with anestrus period longer than 10 months (16.7%). Overall ovulation rate was 85.7%. Pregnancy rate to FTAI was 28.6% (22.2% and 40% in 9 true anestrus and 5 silent estrus buffaloes respectively). Overall, 64.3% (66.7% and 60% of true anestrus and silent estrus respectively) buffaloes were pregnant when checked at 102 days of FTAI including subsequent breeding. Although Ovsynch/FTAI seems to be alternative breeding technique in silent estrus buffaloes, further study with increased sample size is recommended. This protocol can potentially be used for resumption of ovarian cyclicity in true anestrus buffaloes.

Keywords: FTAI, ovulation rate, pregnancy rate, silent estrus, true anestrus, ultrasonography

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ESTIMATION OF GENETIC AND NON-GENETIC PARAMETERS OF MURRAH BUFFALO IN LIVESTOCK DEVELOPMENT FARM, POKHARA

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ABSTRACT

This study was carried out at Livestock Development Farm, Pokhara, Kaski, Nepal in 2011/012 with the objective to estimate the average performance of farm buffaloes in term of production and reproduction traits considering various non-genetic factors. The factors were parity of dam, season of calving, year of calving, and season of service. Data recorded at the farm for the period of 2001 to 2010 and were analyzed using the statistical package Harvey (1990). Results revealed that overall least square mean of lactation length (LL), lactation yield (LYD), Daily milk production (DMP), Annual milk production (AMP) and Birth weight (BWT) were 356±06.50 days; 1763.99±40.55 liters; 4.88±0.69 liters, 1600.82±24.30 liters, and 32.32±0.34 kg, respectively. The lactation length was highly and significantly affected ($P<0.01$) by the year of calving. Parity had significant effect ($P<0.05$) on lactation length; whereas, season of service and season of calving had no effect on lactation length, and lactation yield. The lactation yeild, daily milk yield and annual milk yield were highly and significantly affected ($P<0.01$) by the year of calving and parity. Birth weight was not affected by year of calving, season of service and season of calving whereas parity and sex had highly significant ($P<0.001$) effect on it. Results revealed that overall least square mean of age at first service (AFS), age at first calving (AFC), gestation length (GL), calving interval (CI) were 1275.56±19.28 days, 1582.45±19.27 days, 306.89±0.75 days and 611.97±11.59 days, respectively. Lactation length ($P<0.05$), lactation yield ($P<0.01$), and daily milk production ($P<0.01$), annual milk production ($P<0.01$) and birth weight ($P<0.01$) these entire production traits were significantly ($P<0.01$) affected by parity, indicating need of careful adoption of management for earlier parity to have better production traits. Moderate heritability estimation of AFS, AFC, LL, LYD, DMP, AMP and CI indicated that these traits, if used for selection criteria may bring genetic improvement in these traits and consequently improve productivity. High and positive genetic correlation between LL and LYD; LL and AMP; LYD and DMP; LYD and AMP; DMP and AMP; and AFS and AFC indicated that the improvement of the one trait can be achieved by selecting the other trait. Thus, selection based on phenotypic merit of buffaloes over various traits such as lactational yield, daily milk production, and annual milk production would be effective in making improvement in these traits.

Keywords: Genetic, non-genetic factors, Murrah

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EPIDEMIOLOGY OF ZONOTIC BRUCELLOSIS IN LIVESTOCK FARMERS IN PAKISTAN; “AN UNDERESTIMATED PUBLIC HEALTH PROBLEM”

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ABSTRACT

Brucellosis is an emerging zoonosis of public health importance in Pakistan. In Pakistan, brucellosis is under-reported due to insufficient awareness, scarce diagnostic protocols as well as lack of its epidemiological understanding. The role of these livestock as carriers (small and large ruminants) in the epidemiology of brucellosis in the ecosystems in Pakistan is not completely understood. An active disease surveillance study was conducted for a period of one year in rural and urban areas of Khyber Pakhtunkhwa, Pakistan in 2016-17. Samples were collected from 504 smallholder livestock farmers and simultaneously their animals were also sampled respectively. Diagnostic tests i.e. SPAT, RBPT, ELISA, and PCR were used for human samples diagnosis; while SPAT and PCR were adopted to diagnose the animal samples. Data was analyzed through R software conducting regression models. A seroprevalence of anti-brucella antibodies in humans detected were 4.3%, buffalo 5.2%, cattle 7.9%, goats 4.6% and sheep at 2.7%. The results suggested that farmers having infected cattle and buffaloes were 3.6 (C.I 95%; 1.8-6.8) times were at higher risk of getting the infection. While amongst the risk factors studied here; having knowledge about the disease and education status of the farmers were found significantly ($P < 0.05$) associated with occurrence of zoonotic brucellosis in humans. Here we have identified the anti-Brucella antibodies in humans and livestock in Pakistan. Transmission of brucellosis from livestock into humans is likely to continue due to the increasing human interaction with their animals and dairy products. Lack of awareness and control strategies as well as the unhygienic farming system adds into the widespread and chances of zoonosis. The results of our study could be an important contribution to the designs control and prevention strategies in Pakistan.

Keywords: Brucella, epidemiology, SPAT, RBPT, ELISA, PCR, Pakistan.

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SEASONAL PREVALENCE OF FASCIOLIASIS IN BUFFALOES IN DIFFERENT ALTITUDE OF DHANKUTA AND SUNSARI DISTRICTS IN NEPAL

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ABSTRACT

Buffaloes are considered as “black gold” in livestock herders in Nepal, contributing 67.7% of the milk production and 57.4% of the total meat production in the country. Fascioliasis in buffalo is caused by both *Fasciola gigantica* and *Fasciola hepatica*. Infestations of this disease in buffalo in the study areas based on altitudes have not been properly documented. Hence to estimate the prevalence of fascioliasis in buffaloes in Dhankuta and Sunsari districts of Nepal, a cross sectional study was carried out for a year in different altitudes and climatic conditions during 2013-14. Ranges of altitudes of selected sites were 1800-2200 masl in Murtidhunga; 800-1200 masl in Dhankuta of Dhankuta district and 175-200 masl in Madhesha of Sunsari district. Altogether 798 faecal samples were collected from buffaloes of livestock farmers in Murtidhunga (282), Dhankuta (239) and Madhesha (277). Seasons were divided into three viz. summer, rainy and winter. Samples were collected in every two months interval from same study sites and tested microscopically for the presence of Fasciola eggs using sedimentation technique at laboratory of Agricultural Research Station, Pakhribas, Dhankuta. The overall prevalences of fascioliasis in buffaloes were high 42.6% in Madhesha followed by 39.7% in Murtidhunga, and 37.2% in Dhankuta. In Madhesha, winter season showed significant prevalence result 57.4% (P=0.0003). Relatively high prevalence (40.0%) was found in Rainy season in Murtidhunga and low prevalence (28.3%) in same season in Madhesha. Majority of farmers offered straw and grasses for food. Only limited farmers provided commercial feed along with local food materials to buffaloes. Additionally 4.6% and 4.3% faecal samples were found positive for Paramphistomiasis in buffaloes in Murtidhunga and Madhesha, respectively. The prevalence of fascioliasis in buffaloes was relatively higher in low altitude than high altitude but it was not statistically significant. Fascioliasis in buffaloes in Nepal is highly endemic but seasonal variation occurs. Strategic deworming in high risk period like winter season in Madhesha is recommended along with measure to prevent pasture contamination with buffalo feces.

Keywords: Fascioliasis, season, altitude, buffalo, Dhankuta and Sunsari districts

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ASSESSMENT OF CLINICAL PROBLEMS IN MURRAH BUFFALOES (*Bubalus bubalis*) IN PADAMPUR, CHITWAN, NEPAL

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ABSTRACT

Clinical features and health condition of buffalo have direct impact on quality and quantity of milk production and household economy of small holder farmers in rural areas. Murrah buffaloes (*Bubalus bubalis*) that have important phenological and adaptation trait to thrive in adverse climatic condition were the subject of present research. This study was carried out at Kalika Municipality, Padampur, Chitwan where 37 buffalo rearing households were purposively selected. Close ended semi-structured questionnaire was used to collect information through direct interview with the household leader. Study findings revealed that majority of farmers reported that the buffaloes were facing repeated breeding (35.19%) followed by mastitis (24.3%), intestinal parasite (18.9%), ectoparasite (13.5%), milk fever (5.4%), metritis (5.4%), foot and mouth Disease (2.7%). Thus, based on the findings of present study, it is concluded that focus should be given to the abovementioned problems while developing buffalo health management strategies for improved production and productivity of this species. Meanwhile, further study covering wider geography and considering the major risk factors is recommended.

Keywords: Murrah buffalo, ecto-parasite, intestinal parasite, mastitis, repeat breeding

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CLINICAL PREVALENCE OF DISEASES AND DISORDERS IN BUFFALOES AT THE VETERINARY TEACHING HOSPITAL, AGRICULTURE AND FORESTRY UNIVERSITY (AFU), NEPAL

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ABSTRACT

A retrospective study was undertaken to determine the occurrence and distribution of diseases in buffaloes based on breed, sex, system affected, diseases type and seasonal variation from the record book of the Veterinary Teaching Hospital, Agriculture and Forestry University from July 2012 to July 2017. A total of 712 buffaloes cases were presented in five years duration of which 99% were Murrah crosses followed by local breeds. The cases were categorized on the basis of age group namely yearlings, heifer, adult and senile with the diseases distribution of 5%, 23%, 62% and 10% respectively. Out of the total cases, 86% were medical followed by 12% gynecological and 2% surgical. Parasitic infection (61.92%) and mastitis (10.32%) were the major medical problems, while the anestrus was the predominant gynecological problems. Surgical cases were remarkably negligible and most were wound related problems. In the fecal examination, 83% were positive for parasitic infection such as Nematodiasis (44%), Paramphistomiasis (35%), Protozoan (10%), Moneiziasis (6%) and fascioliasis (5%). In the hospital, the milk samples positive on California Mastitis Test (CMT) were recommended for bacterial culture and antibiotic sensitivity test. The cultural report revealed 85% mastitis was caused by coliform bacteria and 12% mastitis was due to staphylococcal bacteria. The mastitis cases were predominantly higher in the first and third parity. The highly sensitive antibiotics on antibiotic sensitivity test were gentamicin (33%), ciprofloxacin (31%) and tetracycline (16%) respectively. Outcome of this study could be insightful for outlining the appropriate disease control and management strategies around the university vicinity.

Keywords: Clinical prevalence, buffalo, veterinary teaching hospital

INTRODUCTION

Livestock is an integral and indispensable component of farming systems in Nepal which contributes about 12.8% to the total national gross domestic product (GDP) and 31.5% to the agricultural GDP (Sharma & Banskota, 2012). The domestic water buffalo (*Bubalus bubalis*) contributes a significant share of national milk production and is the major milk producing animal. Buffaloes are kept mostly by small-scale producers, who raise one or two animals in mixed crop–livestock systems (FAO, 2015). Buffaloes are raised for supply of animal protein, draft power, and manure. At present, the national buffalo population is 5.2 million which are contributing for 65.28% of milk and 54.34% meat production in the year 2015/16 (MoAD, 2016). The total annual milk production of Nepal is 1.8 million tons (65% from buffalo and 35% from cattle). Buffalo enterprise contributes 52.9% of the livestock share in the national GDP (Paudel et al., 2008). The major components of livestock GDP are milk and milk products from buffalo and cattle (32.7% and 24.7% respectively).

Chitwan has tropical climatic conditions with four main seasons; winter (cold and semi dry to dry), spring (hot and dry), summer or monsoon (very hot and humid) and autumn (moderate climate) in the month December to February, March to May, June to August and September to November respectively (Devkota and Bohora, 2009). The Veterinary Teaching Hospital (VTH) of Agriculture and Forestry University (AFU) is located at the western part of Chitwan where a large number of livestock, pets and poultry species are brought for the diagnosis and treatment of diseases. The cases are brought mainly from the university periphery and sometimes from the neighboring districts. All the cases brought to the hospital are recorded daily in the register book. Buffalo is one of the major livestock species of Chitwan district with the population of 68,809 (18,166 milking buffaloes), which are contributing to 66.91% of total milk and 31.26% of total meat production of the district (MoAD, 2016). Veterinary Teaching Hospital (VTH) is an ideal and reliable source of information about animal diseases with their treatment. Analysis of the case record gives a comprehensive idea about the disease problems at local areas. Recently, Gautam *et al.*, (2017) reported the case flow pattern of different cases registered at the VTH during two years period. But the species wise case flow patterns of various disease and disorders have not been studied yet. Therefore, the present study was aimed to determine the occurrence of diseases and disorders in buffaloes attended in the VTH, AFU including the distribution of diseases based on breed, age, sex, group and seasonal variation from the fiscal year 2012/13 to 2016/17.

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MATERIALS AND METHODS

The retrospective epidemiological study of diseases was conducted using 5 years data from July 2012 to July 2017 recorded at VTH, AFU, Rampur, Chitwan, Nepal. The patient’s data we collected from the register book after official permission from the administration of the VTH. All the cases brought to the hospital were diagnosed by the clinicians or faculty veterinarians mainly on the basis of general, physical and clinical and laboratory examinations. Gastrointestinal parasitic infections were diagnosed based on fecal examination by light microscopy. Mastitis was diagnosed by California Mastitis Test (CMT) and the positive milk samples were cultured for *invitro* antibiotic sensitivity test. Occasionally, the reproductive problems were diagnosed by transrectal ultrasonography. In the *invitro* antibiotic sensitivity test, the different strains of isolated microorganisms of mastitis were streaked onto Mueller Hinton agar plates and different antibiotic discs (ciprofloxacin, gentamicin, tetracycline, chloramphenicol, enrofloxacin, amikacin etc.) were diffused on them. After applying the discs, the plates were kept at 10°C for 3-4 hour to allow pre-diffusion of the antibiotics. The plates were then incubated at 37°C for 24 hour and observed for sensitivity by measuring the zone of inhibition. The results found were recorded as sensitive, intermediate and resistant following earlier study (Dhakal *et al.*, 2007).

All recorded clinical cases were primarily categorized into three major groups on the basis of treatment required. These groups were: (1) Medical cases, (2) Gynaeco-obstetrical cases and (3) Surgical cases. Mastitis, Fecal examination (FE), anorexia/inappetance, grass poisoning, lameness, enteritis, stress, constipation, skin infections were included in medical cases. FE cases were further classified into paramphistomiasis, nematodiasis, protozoan, moneiziasis, fascioliasis and negative cases. Similarly, mastitis cases included a number of milk samples subjected to the VTH; most of the cases in this category included only the milk samples brought by the owner, not the sick animal. Gyneco-obstetrical cases included cases of pregnancy diagnoses (PD), vaginal prolapse, silent heat, anestrus, and repeat breeding. Surgical cases included tail gangrene, eye injury, tumor, udder wound, castration, dehorning, horn injury, teat obstruction and urolithiasis. Likewise, the cases were broadly classified into; fecal examination, mastitis, PD, anestrus and repeat breeding, anorexia, tail gangrene and others under the disease and disorder wise classification. In the age wise occurrence of cases, animals having the age <1 year, 1-3 years, 4-8 years and >8 years were classified as yearling calf, heifer, adult and senile respectively. Finally, the data were entered into Microsoft excel 2007 and diseases and other health related problems in those five years was analyzed using proportion and simple percentage methods and presented graphically to determine age, sex, breed, group and season wise distribution of diseases.

RESULTS

Out of 712 cases, 699 (98%) were she-buffaloes and rest were he-buffaloes of which 99% were Murrah-cross followed by local breed. Age group variations classified into yearling calf, heifer, adult and senile were found to be 34 (5%), 166 (23%), 441 (62%) and 71 (10%) respectively (Figure 1). Fig-2 shows the year wise case flow pattern; there was an increasing trend of cases flow pattern from the fiscal year 2012/13 to 2015/16 however, the lowest cases flow was recorded during the year 2016/17. Season wise cases flow presented in the Figure 3 shows that the disease and disorders recorded were highest during summer season followed by spring, autumn and winter. In the group wise distribution, highest cases were found in the medical group (86%) and then gynecological group (12%) followed by least cases were in the surgical group Figure 6.

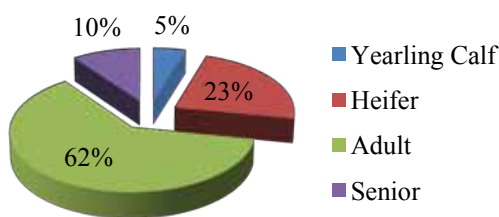


Figure 1. Age wise distribution of buffaloes diseases presented in VTH from 2012-2017

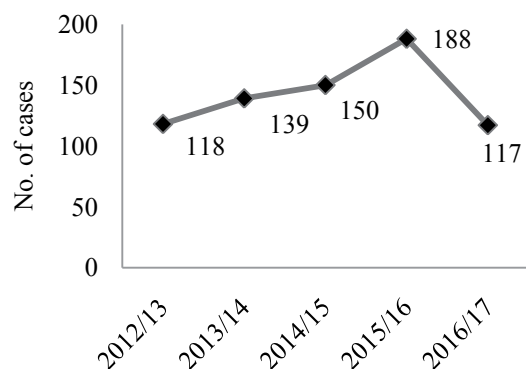


Figure 2. Year wise distribution of buffaloes diseases presented in VTH from 2012-2017

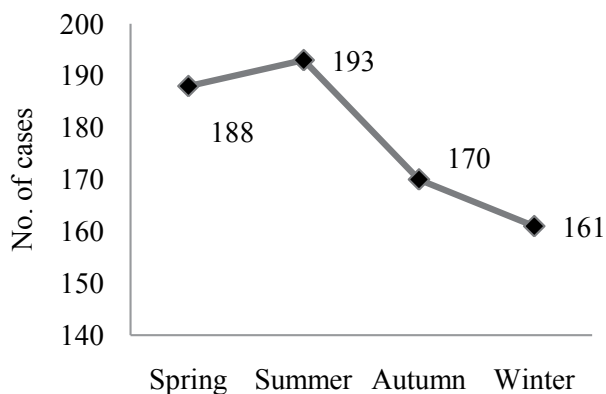


Figure 3. Season wise disease distribution of buffaloes diseases presented in VTH from 2012-2017

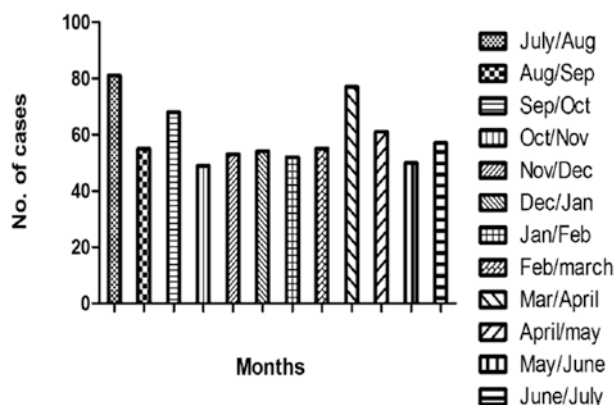


Figure 4. Month wise disease distribution of buffaloes diseases presented in VTH from 2012-2017

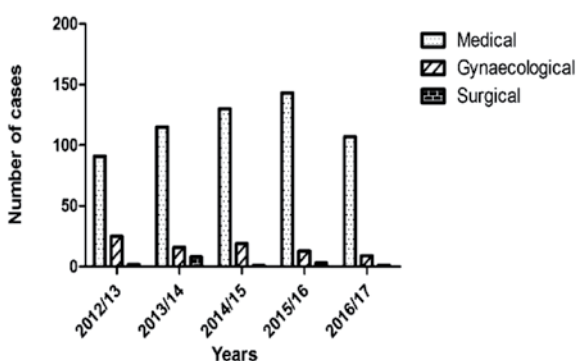


Figure 5. Year wise distribution of different group of buffaloes diseases presented in VTH from 2012-2017

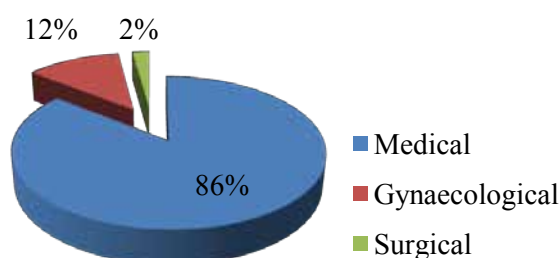


Figure 6. Frequency of different group of buffaloes diseases presented in VTH from 2012-2017

Among the various cases FE was found the most frequently registered problem 490 (72%). Similarly, mastitis, anestrus, PD and others were 82 (12%), 37 (5%), 45(7%) and 20(3%) respectively (Figure 7).

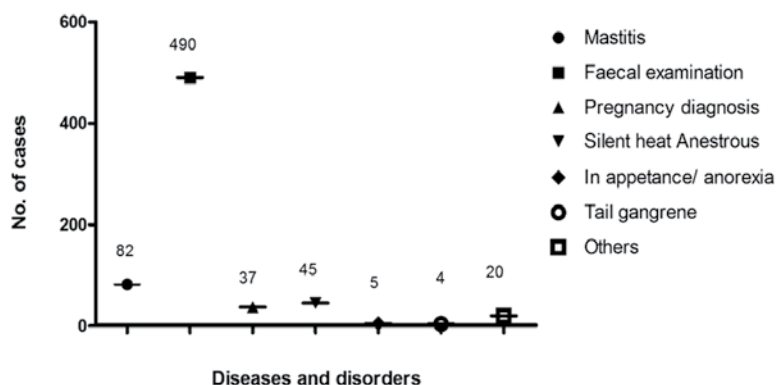


Figure 7. Disease wise case flow pattern of buffaloes cases presented in VTH from 2012-2017

Mastitis was the second most frequently registered case in the hospital. The distribution was found in all the seasons of the year but the highest no. of cases was presented in the month July/Aug and Sep/Oct (Figure 9). Seasonal occurrence of mastitis was found highest during autumn followed by summer, winter and spring (Figure 8). The highest cases of mastitis were recorded in the first parity followed by third and second parity (Figure 10). In the antibiogram, ciprofloxacin (31%), gentamicin (33%), tetracycline (16%), chloramphenicol (7%), enrofloxacin (4%) and amikacin (%) were found sensitive (Figure 11).

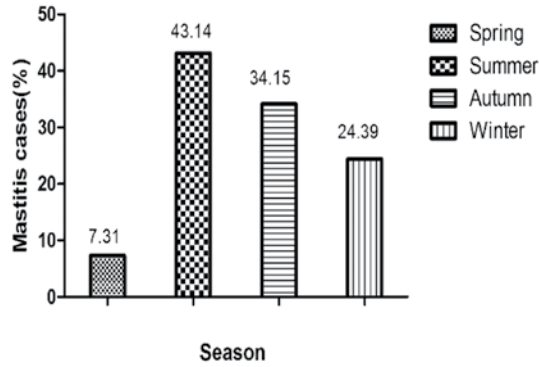


Figure 8. Seasonal variance of clinical mastitis brought to VTH from 2012-2017

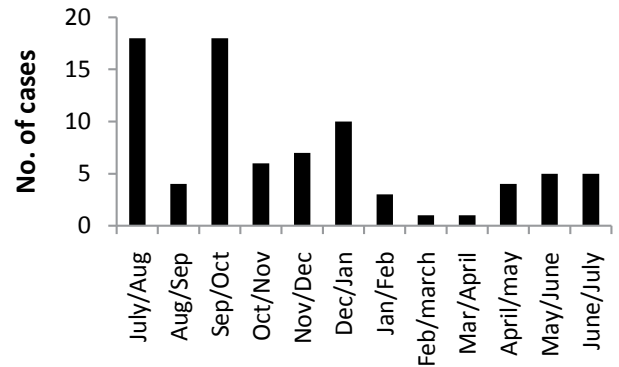


Figure 9. Month wise distribution of mastitis in buffaloes diagnose in VTH from 2012-2017

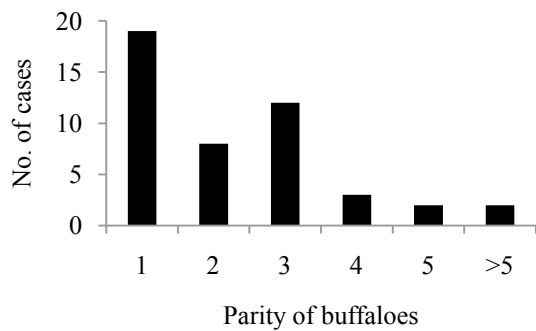


Figure 10. parity wise distribution of presented mastitis case in VTH from 2012-2017

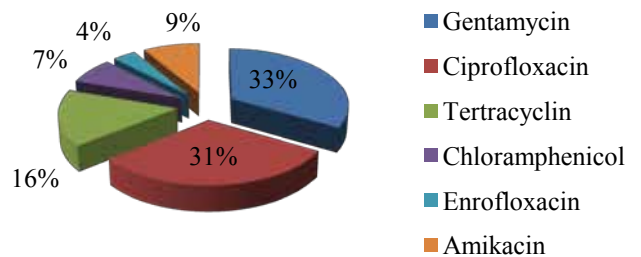


Figure 11. Pie-chart showing antibiotic sensitivity of mastitis cases diagnose in VTH from 2012-2017

The occurrence of clinical mastitis in different buffaloes during different months has shown in Table 1. The maximum number of clinical mastitis cases were observed in the month of July/Aug (21.95%) and Sep/Oct (21.95%) followed by Dec/Jan (12.19%). In particular, 43.14% of buffaloes had clinical mastitis during summer season followed by the autumn season (34.15%).

Table 1. Prevalence of clinical mastitis at different months and season at VTH from the year 2012-2017

Seasons	Months	Cases	Percent
Spring	Feb/march	1	1.21
	Mar/April	1	1.22
	April/may	4	4.88
Summer	May/June	5	6.09
	June/July	5	6.09
	July/Aug	18	21.95
Autumn	Aug/Sep	4	4.88
	Sep/Oct	18	21.95
	Oct/Nov	6	7.32
Winter	Nov/Dec	7	8.54
	Dec/Jan	10	12.19
	Jan/Feb	3	3.66

Mastitis is a disease caused by unhygienic condition of the shed condition and milking practices mainly rescue due to gram negative bacteria (*Escherichia*, *Klebsiella*, *Enterobacter*) and gram positive bacteria (*Coliform*, *Staphylococcus*, *Streptococcus* etc. and fungus. Among the cultured cases 85% were coliform, 12% were staphylococcal and 3% were *Klebsiella* and 3% with no growth that may be due to fungal cause (Figure 13).

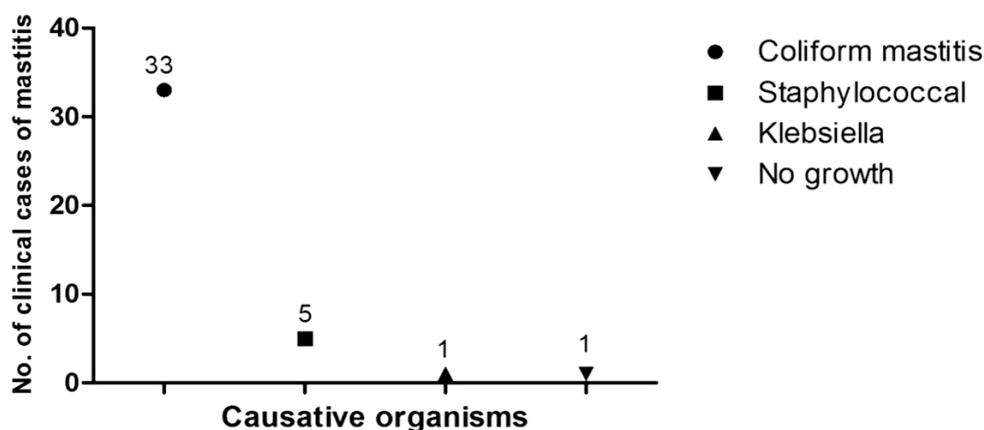


Figure 13. Cause of mastitis diagnosed in VTH from the year 2012-2017

In the present study, reproduction related cases were PD, anestrus and repeat breeding. The case flow for PD was highest during winter season followed by spring, summer and autumn (Figure 14). Month wise occurrence of PD cases has shown in the Figure 15. It suggests that the maximum numbers of cases were registered in March/April followed by equal number of cases in Jan/Feb and Aug/Sep. Similarly, anestrus, silent heat and repeat breeding cases were found maximum during autumn season followed by spring, winter and summer (Figure 17).

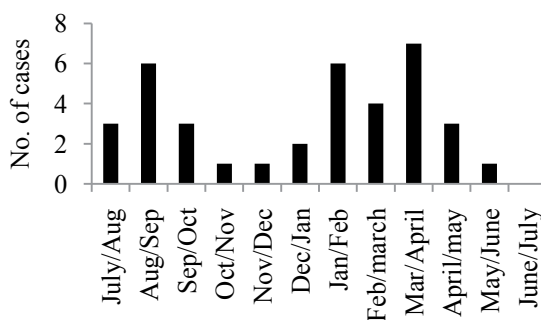
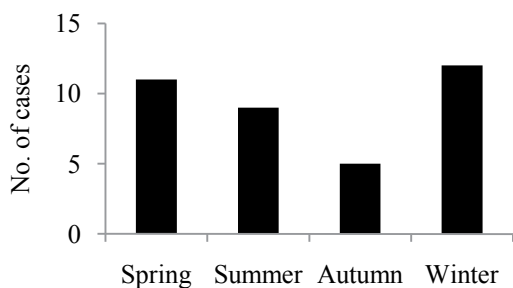


Figure 14. Season wise distribution of cases presented for pregnancy diagnosis (positive) in VTH from 2012-2017

Figure 15. Month wise distribution of cases presented for pregnancy diagnosis in VTH from 2012-2017

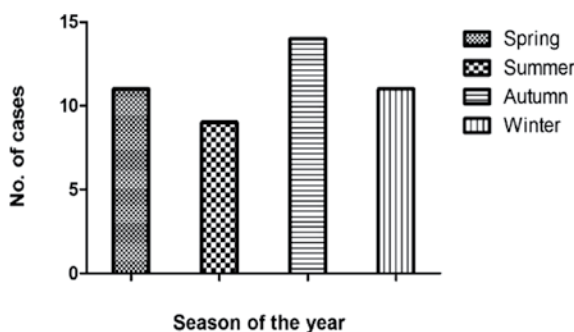
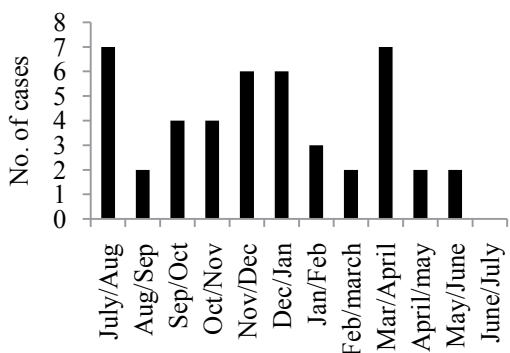


Figure 16. Month-wise anestrus, silent heat, repeat breeding case flow pattern at VTH during 2012-2017.

Figure 17. Season wise anestrus, silent heat, repeat breeding case flow pattern at VTH during 2012-2017

Out of the total samples (490), 72% carried for the FE, 83% were found positive and remaining was found negative for the endoparasites (Figure 18). The nematodes contributed 44%, paramphistomes (35%), fasciola (5%), protozoa (10%) and moneizia (6%) (Figure 19). Cases of parasitic infection were found maximum during spring season followed by summer and autumn (Figure 20).

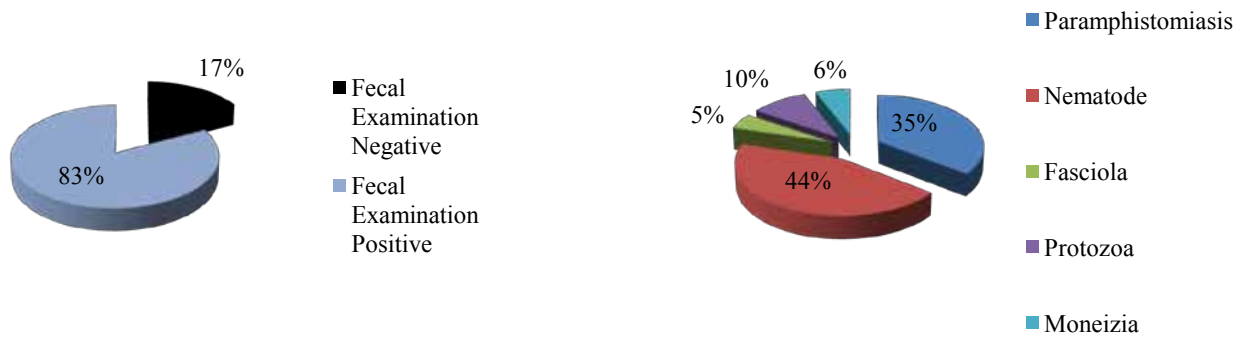


Figure 18. Distribution showing frequency of positive and negative in fecal examination in VTH from 2012-2017

Figure 19. Distribution showing frequency of different endo-parasites in positive fecal examination in VTH from 2012-2017

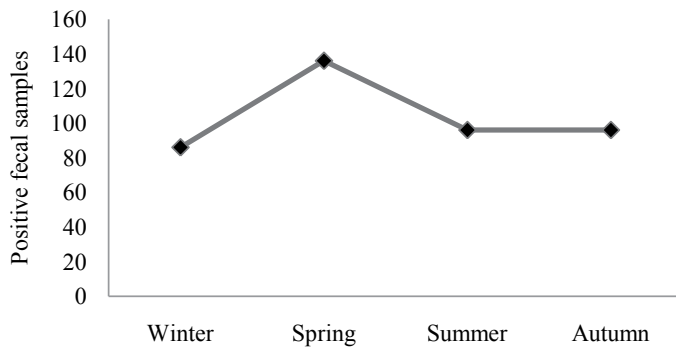


Figure 20. Seasonal variation of endoparasites in the sample positive for fecal examination brought to the VTH from FY 2012/13-2016/17

DISCUSSION

The age wise distribution of disease and disorders shows that the adults were maximum registered cases followed by heifers, seniors and yearlings. Heifer is the reproductive stage and adult is the productive stage at which the animals seem to be more susceptible to disease and disorders. The owner is also more concerned about the health of their livestock at these stages. The owner frequently visits the clinics or hospital for artificial insemination (AI), PD, FE, mastitis and milk culture, which are the problems and conditions occur during reproductive and productive life of buffaloes. Sex wise case flow pattern shows the male and female ratio is 1:49 which suggests that the owner do not rear he-buffaloes for the commercial meat production and natural insemination. Nowadays, owners are more concerned about the advantage of AI and they often call the technicians for AI in the area having facilities of AI service. The major buffalo breed reared was found to be the Murrah-cross (99%). Devkota *et al.*, (2015) also found all improved buffalo breed raised by farmers in a household survey conducted in the Chitwan district.

The total number of buffalo cases brought to VTH from the FY 2012/13 to 2015/16 was in increasing trend. Gautam *et al.* (2017) also reported the increasing trend of cases from the FY 2014/15 to 2015/16. But, the number of cases were decreased during the FY 2016/17. The Increased number of private veterinary clinics and agro-vet centers at various places around the vicinity of VTH might a possible reason behind decrease in the number of cases. It is because the animals owner prefer to take their sick animals to nearby clinics rather than to bring at VTH. The home service provided by the vets, vet students and technicians have also making the owners easier to treat their sick animals. There were a quite small number of surgical cases which might be due to the lack of 24 hours emergency service facility, inpatient and ambulance service at the VTH. As the surgical cases being the emergency condition, they need immediate correction and may require admission of the patient at the hospital. It is essential to increase the number of overall cases and especially surgical cases flow at VTH in order to make veterinary students exposure to such cases. Gautam *et al.* (2017) has also suggested the concerned authorities either to start ambulatory clinical service at nominal cost or run clinical practice classes at farmer’s community.

The highest numbers of cases were observed in the month of July-Aug followed by March-April and Sep-Oct. In general, total number of cases reported were maximum in summer followed by spring, autumn and winter season respectively. This seasonal variation might be due to intolerable hot and humid climatic conditions which being favorable to various diseases. Among the various cases presented to VTH, FE cases shared the highest proportion which is in agreement with the previous study (Gautam *et al.*, 1999; Gautam *et al.*, 2017). In the present study, more than three- quarter of the fecal samples brought to the VTH were found to be positive for endoparasitic eggs with the highest prevalence of paramphistomum followed by nematodes, protozoa, moneizia and fasciola. In the seasonal variation of parasitic infection, the highest prevalence of endoparasitic infection was found in the spring followed by equal number in summer and autumn and least in the winter season. The present finding suggest that the prevalence



of endoparasitic infection is higher during the rainy season than the winter. Therefore, it is important to administer the antihelmintics to the buffaloes and other livestock before the commencement of rainy season as a prophylactic measure. PD, anestrus, silent heat and repeat breeding cases were the major reproductive cases found in the study. Among them, the PD cases represented highest during the month March/April followed by Aug/Sep and Jan/Feb. Similarly, the anestrus, silent heat and repeat breeding cases were found maximum in the month of March/April and July/Aug followed by Nov/Dec and Dec/Jan. The reproductive failure cases were accounted maximum during autumn followed by spring, winter and summer.

In the present study the highest prevalence of organisms causing mastitis were found Coliform followed by Staphylococcus and Klebsiella. Sharma *et al.*, (2012) also found that the major mastitis causing organisms are Staphylococcus, Streptococcus, E.coli, Corynebacterium and Klebsiella. Dhakal I.P. (1997) was carried out a study on 165 buffalos at different villages of Chitwan district and reported that Coliform was the most frequently isolated organisms (27%) followed by Staphylococcus (19%) and Streptococcus (8%). Bei- Zhong Han *et al.*, (2007) have investigated in crossbred buffalos (native swamp buffalos with Murrah and Nili-Ravi, more productive) and they found high average loads of bacteria count as well as Coliform. Probably this is due to inadequate sanitary and hygienic conditions during milking, collection and transport. The highest incidence of clinical mastitis was found in the summer season followed by autumn, winter and spring season. Similarly, the greatest number of clinical cases of mastitis were observed in the month of July/Aug and Sep/Oct. The maximum numbers of cases were found affected with clinical mastitis at 1st lactation followed by 3rd and 2nd lactation. Dhakal *et al.*, (2007) also reported the similar findings in buffaloes brought to VTH, Rampur, Chitwan. They also reported the greatest number of buffaloes being affected with clinical mastitis at 1st calving followed by 2nd and 3rd calving. The incidence of mastitis during 2nd lactation varied with eth resul to Dhakal *et al.*, (2007) because they reported high in first calving followed by 2nd and 3rd calving. The parasitic infection and mastitis showed a seasonal variation.

Thus, the prophylactic measures should be taken for their prevention and control around the university vicinity. The total cases flow tendency is not satisfactory especially the surgical cases. Inpatient facility, ambulatory and 24 hours emergency services might increase such cases. The concerned authority should pay special attention to update their services and technologies for the disease diagnosis and treatment that not only benefits to the farmers but also to the students to develop their clinical skills.

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BREEDING MANAGEMENT PRACTICES AMONG SMALL HOLDER BUFFALO FARMERS IN VICINITY OF AFU, RAMPUR

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ABSTRACT

A study was carried out to find the buffalo breeding management practices adopted by small holder farmers in the vicinity of university farm of AFU, Chitwan. Majority of the farmers (74.1%) preferred natural service for breeding even though 62.1% of farmers were found to have knowledge on Artificial Insemination. About 75.6% buffaloes were found to breed for the first with in the age of 3 years and only 25.6% of buffaloes were found to be conceived in first attempts. Most of the farmers (91.4%) consider bellowing as the sign of heat but a few farmers (8.6%) judge the heat considering the silent features. About 62.1% of the farmers were found to adopt the practice of PD. Of which, 33.3% were found to adopt it within 3 months of breeding where as 41.7% and 25.0% were resorted between 3-4 months and after 4 months respectively. After calving 19.0%, 36.2%, 37.9% and 6.9% farmers resorted to breed within 2 months, between 2-3 months, between 3-4 months and after 4 months respectively. Only 6.9% of the farmers were found to maintain the breeding bull. Repeat breeding (50%) is identified as the major reproductive problem followed by anestrus (36.2%), retention of placenta (6.9%) and others (6.9%). Finding necessities the need to create awareness among the small holder buffalo farmers on recommended breeding management practices through organizing training programs.

Keywords: Buffalo, breeding, practice

INTRODUCTION

Nepal is a small holder farmer dominated agrarian country where animal husbandry forms the backbone of agricultural economy. Traditionally, animal husbandry is carried out by all the farmers, regardless of their economic status. Out of 5.4 million household in Nepal (NPHC, 2012), about 1.7 million (30.7%) households rear buffalo (CBS, 2013). Being one of the major commodities, buffaloes contribute to the livestock economy by providing milk, meat and draft power. Nepal has about 5.2 million buffaloes that provide about 1.2 million mt. milk and 175 thousand mt. meat annually (MoAD, 2017). Despite the vital importance of buffalo farming, the productivity remains low largely due to poor management of health, nutrition and breeding. Reproduction is one of the most important considerations determining profitability (Sreedhar et al., 2017) and better breeding practices helps to minimize the economic losses significantly (Karir et al., 2006). Reproductive efficiency greatly relies on the farmers' skills and capabilities (Olsson, 2007) but in Nepalese context, very little work has been done on this regard. Therefore, the present study was conducted to investigate the management practices of buffaloes adopted by the small holder farmers in relation to the breeding prospective.

MATERIALS AND METHODS

A survey study was conducted in August 2017 in the vicinity of livestock farm of Agriculture and Forestry University located at Rampur, Chitwan where predominantly the small holder farmers dwell. The most common route in the vicinity was selected, every household in the route were visited and only the farmers having at least one milch buffalo were purposively interviewed using a structured questionnaire. The information regarding the breeding management and practices were collected. The data, thus obtained, was analyzed using SPSS software version 20.0.

RESULTS AND DISCUSSION

Results of the buffalo breeding management practices adopted by the farmers are presented in the Table 1. The result indicates that majority of the farmers (43.1%) are rearing the non descriptive breed of buffaloes where as 29.3% rear cross breed and 27.6% rear pure breed Murrah. The reason may be lack of awareness regarding the advantage of cross breeds in Chitwan has very suitable agro climate for cross-breed buffaloes.

Most of the farmers (91.4%) answered silent features like swelling of vulval lips and redness of the vaginal mucosa as the sign of heat but a few farmers (8.6%) used to judge the heat considering bellowing of animals. Thus, the practice of heat detection followed by the buffalo farmers was not satisfactory because silent estrus is a common problem in buffaloes even under good management (Abdalla, 2003). The finding shows that 62.1% farmers have knowledge on artificial insemination (AI). In spite of this encouraging fact, the response to preference

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of AI technique for breeding was very poor. Only 8.6% of the farmers preferred AI service where as 74.1% farmers favored bull service and 17.2% farmers resorted to both services. The lower preference of AI may be due to scarce availability of AI service or other constraints. A study conducted in Chitwan and Nawalparasi reported that the constraints of adoption of AI are a scenario of higher AI failure (with more than 3 repeated failures) in buffalo; poor capacity and practice of AI service providers; and inefficient knowledge among farmers about handling of AI equipments (Sapkota et al., 2016). The existing practice of breeding by using locally available bull does not postulate improvement in coming generations. So, there is a wider space of intervention in genetic improvement of buffaloes via sound AI techniques.

About 43.1% of farmers were breeding their buffaloes as they observed the sign of heat where as 8.6%, 39.7% and 8.6% of farmers breed within 6 hrs, between 6-12 hrs and between 12-24 hrs respectively after signs of heat are observed. Difficulties in detecting heat which helps to determine appropriate time of insemination or service is the biggest restriction to attain high conception rate in a herd (Rao et al., 2013). Because of such difficulties, the farmers may have followed the practice of mating at the time judged by self perceived false experiences may had created such variations in the time of breeding. This indicated that majority of farmers are not aware of the fact that breeding of dairy animals between 12-18 hrs after onset of heat results in better conception. After breeding, pregnancy diagnosis (PD) is regarded as a necessary step to reduce calving interval. About 62.1% of the farmers were found to adopt the practice of PD. Among them, 33.3% were found to adopt the practice of PD within 3 months of breeding where as 41.7% and 25.0% were resorted between 3-4 months and after 4 months respectively. After calving, 19.0%, 36.2%, 37.9% and 6.9% farmers resorted to breed the buffaloes within 2 months, between 2-3 months, between 3-4 months and after 4 months respectively which was found to be encouraging than the report of 205 days postpartum by Shrestha et al., (2005). Only 6.9% of the farmers were found to maintain the breeding bull which may be due to the fact that majority of the farmers in the area have 1-2 buffaloes and hence not felt the need of breeding bull.

Table 1. Breeding management practices adopted by buffalo farmers

Practice (Number of respondents)	Category	Frequency (%)
Breed (58)	Pure breed (Murrah)	16 (27.6)
	Cross breed	17 (29.3)
	Non descriptive	25 (43.1)
Practice of heat detection (58)	Yes	56 (96.6)
	No	2 (3.4)
Sign noted at heat (58)	Bellowing	53 (91.4)
	Silent features	5 (8.6)
Knowledge about AI (58)	Yes	36 (62.1)
	No	22 (37.9)
Preferred of breeding method (58)	Natural service	43 (74.1)
	AI	5 (8.6)
	Both	10 (17.2)
AI/Bull service after heat (58)	At time when observed	25 (43.1)
	Up to 6 hrs after observation	5 (8.6)
	Between 6-12 hrs after observation	23 (39.7)
Practice of PD (58)	Between 12-24 hrs after observation	5 (8.6)
	Yes	36 (62.1)
	No	22 (37.9)
Closest date of PD (36)	With in 60 days	1 (2.7)
	Between 60- 90 days	11 (30.6)
	Between 90-120 days	15 (41.7)
Breeding after calving (58)	More than 120 days	9 (25.0)
	With in 60 days	11 (19.0)
	Between 60-90 days	21 (36.2)
Maintaining buffalo bull (58)	Between 90-120 days	22 (37.9)
	More than 120 days	4 (6.9)
	Yes	4 (6.9)
	No	54 (93.1)



Knowledge and practices of smallholder buffalo farmers that influences the breeding were presented in Table 2. Majority of the farmers (82.8 %) have knowledge on regular deworming but only 63.8% of the farmers were found to deworm their animals before breeding and 70.7% farmers have practice of supplementing liver- tonic preparations at the time of deworming. About 60.3% of farmers were found to adopt the practice of supplementing multi-mineral preparations of which 28.6%, 31.4%, 20% and 20% farmers preferred to offer it in prepartum, peripartum, postpartum and as per prescription, respectively.

Table 2. Breeding influencing knowledge and practices of buffalo farmers

Practice (Number of respondents)	Category	Frequency (%)
Knowledge on regular deworming (58)	Yes	48 (82.8)
	No	10 (17.2)
Deworming before breeding (58)	Yes	37 (63.8)
	No	21 (36.2)
Liver tonic supplementation at time of deworming (58)	Yes	41 (70.7)
	No	17 (29.3)
Practice of mineral supplementation (58)	Yes	35 (60.3)
	No	23 (39.7)
Most preferred time of mineral supplementation (35)	Pre-partum	10 (28.6)
	Peripartum	11 (31.4)
	Postpartum	7 (20.0)
	As prescribed	7 (20.0)

The reproductive performance of buffaloes, as experienced by the respondents, is given in Table 3. The result of the study revealed that most of the buffaloes (75.6%) were served for breeding for the first within the age of 3 years. The rest were breed at the age of 4 year. This indicates that first caving is expected within the age of 4 years which is shorter than the average reported by Shrestha et al. (2005) but is longer than the average of a good herd as mentioned by Viswanath (2002).

About 25.6% buffaloes were found to be conceived in first attempt where as 52.3%, 20.9% and 1.2% were found to be conceived in second, third and more than third attempts. From the result, it can be referred that the overall conception rate is about 51% ($(22 \times 1 + 45 \times 2 + 18 \times 3 + 1 \times 4) \times 100 = 50.59$) that is nearly equal to the report of NLBC (2017) which has mentioned that the national conception rate of AI in buffaloes is 47.64%. But, higher conception rate than the finding might be expected because majority of the farmers had served the buffaloes naturally; and acceptance of mounting by the buffalo and service by a bull indicate appropriate timing to far extent.

Table 3. Reproductive performance of buffalo

Parameters (Number of respondents)	Category	Number of animals (n=86)	Percentage
Age of first service (58)	With in 3 years	65	75.6
	With in 3-4 years	21	24.4
	With in 4-5 years	0	0
	More than 5 years	0	0
Service per conception (58)	1 st attempt	22	25.6
	2 nd attempt	45	52.3
	3 rd attempt	18	20.9
	More than 3 attempts	1	1.2

Reproductive problems perceived by the buffalo farmers were tabulated in Table 4. Majority of the farmers (50%) identified repeat breeding as the major reproductive problem followed by anestrus (36.2%), retention of placenta (6.9%) and others (6.9%). The finding is in line with reports of other authors as they have reported repeat breeding and anestrus due to ovarian dysfunction and silent ovulation, as a two common reproductive disorders in buffaloes (Sah and Nakao, 2006; Panchal et al., 1991).

Table 4. Reproductive problems perceived by buffalo farmers

S.N.	Problem	Frequency (N=58)	Percentage
1	Anestrus	21	36.2
2	Repeat breeding	29	50.0
3	Retention of placenta	4	6.9
4	Others	4	6.9

Preference of milch animal and reason behind the preference is given in Table 5. Out of 58 respondents, 20.7% preferred cattle with the reason of superior milk quality (10.4%), easy trade (6.9%) and easy management



(3.4%). 79.3% farmers favored buffalo in the ground of superior milk quality (29.3%), easy trade (50%) and easy management (10.3%). The result indicates that the most important factor determining the preference of animal is easiness in trade (50%) followed by milk quality (41.51%) and easy management (10.3%). This may be due to the legal prohibition of slaughter of cattle and wider acceptance of buff meat mostly by the ethnic communities in Nepal. In addition, most of the Nepalese farmers enjoy the domestic production of ghee for household consumption and buffalo milk contains higher level of milk fat than cow milk (Menard et al., 2010).

Table 5. Preferred Milch animal and reason for preference on respondents' prospective

		Reason of preference			Total
		Superior milk quality	Easy trade	Easy management	
Preferred animal	Cattle	6 (10.4)	4 (6.9)	2 (3.4)	12 (20.7)
	Buffalo	17 (29.3)	25 (43.1)	4 (6.9)	46 (79.3)
Total		22 (41.51)	29 (50.0)	6 (10.3)	58 (100)

CONCLUSION

From the study, it can be concluded that a major proportion of the farmers are rearing non descriptive breed of buffaloes and are breeding through natural service rather than AI even though they have knowledge on benefits of AI. Difficulty in heat detection due to silent estrus is a known problem in buffaloes and because of this it is very hard to determine the exact time of inseminating or servicing. But, most of the farmers consider bellowing as a sign of heat and were not aware of considering silent features of heat. Knowledge about best time of serving after onset of heat is a limiting factor among farmers, requiring two or more service to be conceived in buffaloes and repeat breeding as a major reproductive problem. Practice of pregnancy diagnosis needs to be enhanced. Hence, there is a need to create awareness among the small holder buffalo farmers on recommended breeding management practices through organizing training programs.

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RE-ASUMPTION OF OVARIAN CYCLICITY TO INDUCE PREGNANCY IN ANESTROUS BUFFALOES USING CIDR SYNCHRONIZATION PROTOCOL DURING POOR BREEDING SEASON

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ABSTRACT

Buffaloes are regarded as the seasonal breeder; with diminished ovarian activities in slump breeding season. Ovarian dynamics could be resumed with application of synthetic reproductive hormones. CIDR synchronization, one of the novel protocol, can be used to regulate the ovarian cyclicity. This study was designed to investigate the efficacy of the protocol for inducing normal reproductive cycle with factors concerned in Nepalese buffaloes during the slump breeding seasons. Total of 14 anestrous buffaloes; 7 from Rampur and 7 from Pokhera farms were taken. Parasitic load was examined and all were positive were taken. Only half of them were de-wormed as treatment group and half as control group. CIDR synch protocol was used over all selected animals. Two shorts of Intra muscular GnRH injection was made on day 0 and day 9. CIDR was inserted intra-vaginally on day 0 and removed on day 7 along with PGF2 alpha injection. Fixed time artificial insemination was carried out on day 10. After 10 and 40 days of FTAI, corpus leutem and early pregnancy diagnosis was carried out respectively. All the buffaloes in the experiment expressed estrous during the time of FTAI and overall 78.6 % and 42.9 % of buffaloes ovulated and pregnant respectively. Of the total, 85.71 % and 71.43 % were found to be ovulated; 57.14% and 28.57 % were found to be pregnant in treatment group and control group respectively. Mean (\pm SE) size of corpus leutem on day 10 post FTAI was 13.09 \pm 0.86 mm. There was significant ($P=0.035$) difference in the size of CL on day 10 post FTAI between pregnant and non-pregnant buffaloes. Pregnancy rate in buffaloes with BCS \geq 2.75 (55.6%) was significantly ($P= 0.02$) higher than in buffaloes with BCS < 2.75 (20%). Not significant relation was found with pregnancy rate in relation to parity, age, lactation stage, location. CIDR synchronization protocol can effectively induce estrus and ovulation in anestrous buffaloes; however endoparasitic load strongly affects the protocol.

Keywords: CIDR synch, anestrous buffalo, ovarian cyclicity.

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UDDER HEALTH IN RELATION WITH UDDER MORPHOMETRIC TRAITS IN RIVERINE BUFFALOES

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ABSTRACT

The aim of the present study was to investigate the possible association between udder morphometric traits and udder health in riverine buffaloes ($n=81$). The udder health was defined on the basis of quarter foremilk bacteriology and somatic cell counts results following International Dairy Federation criteria. The udder morphometric parameters were evaluated on the basis of udder shape (bowl, round, goaty and stepped); udder conformation (pendulous and non-pendulous); udder length (UL); udder width (UW); udder depth (UD); and distance between front, rear, left-sided and right-sided teat pairs. An animal-level incidence of subclinical mastitis (SCM) was 21%. The prevalence of bowl-shaped udders were high (55.5%) followed by round (38.3%), goaty (3.7%) and stepped (2.5%) shaped udders. The occurrence of SCM was higher in buffaloes with goaty udder shapes (33.3%) followed by round (24.1%) and bowl (18.6%) udder shapes. Non-pendulous udders were more frequently reported (92.6%) but the incidence of SCM did not vary significantly due to udder conformation. The mean \pm SE values of UL, UW and UD were 70.8 \pm 0.92 vs. 71.8 \pm 1.83, 50.5 \pm 0.97 vs. 50.3 \pm 2.22 and 12.6 \pm 0.4 vs. 12.8 \pm 0.94cm, respectively, for healthy and SCM udders which did not differ significantly among the health groups. Although the distances between different pairs of teats were lower in SCM udders as compared with healthy udders, but it was found to be statistically non-significant; the average values (cm) between front, rear, left-sided and right-sided teats for healthy and SCM buffaloes being: 12.9 \pm 0.36 vs. 12.6 \pm 0.77, 9.0 \pm 0.27 vs. 7.9 \pm 0.6, 7.2 \pm 0.23 vs. 7.1 \pm 0.36 and 7.3 \pm 0.22 vs. 7 \pm 0.37, respectively. In conclusion, it could be said that, unlike cows as reported in literature, udder health seems to be less associated with udder morphometric traits in riverine buffaloes.

Keywords: Buffalo, subclinical mastitis, udder morphometry, udder conformation

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PROFILE OF PREGNANCY SPECIFIC PROTEIN B (PSPB) CONCENTRATION IN NEPALESE BUFFALOES DURING FIRST THREE MONTH OF GESTATION

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ABSTRACT

Maintenance of pregnancy is essential to improve overall productivity of buffaloes (*Bubalus bubalis*). The objective of this study was to determine serum pregnancy specific protein B (PSPB) profile of Nepalese buffaloes during first trimester using novel BioPRYN® test. This study was conducted in Livestock farm of Agriculture and Forestry University (AFU). Ovulation Synchronization using OVSYNCH technology with fixed timed artificial inseminations (AI) in 14 buffaloes (10 parous and 4 heifers) was performed in this study. Blood samples were collected weekly from day 0 (AI) to 90 (13 week) from all buffaloes. Sample collections were suspended at day 34 from non pregnant animal after pregnancy was confirmed by transrectal ultrasonography at days 34 and by rectal examination at day 62. Each sample was analyzed in duplicate using an ELISA test (BioPRYN® assay). Four buffaloes had become pregnant out of fourteen synchronized (28.6%) as shown by ultrasonography and PSPB analysis. The basal serum PSPB concentrations of buffaloes were 0.24 ± 0.025 ng/mL. Significant differences ($p < 0.005$) in PSPB concentrations were observed between the pregnant and non pregnant buffaloes from day 27 (3.28 ± 0.67 ng/mL) as PSPB level of non pregnant was always close to basal level. PSPB concentrations were reached to peak at week seven (30.46 ± 12.67 ng/mL). Thereafter decreased gradually at week ten (18.88 ± 4.57 ng/mL) and remaining high till week thirteen (27.23 ± 8.23 ng/mL). In conclusion, this result suggests that the determination of serum PSPB profile by BioPRYN(®) test is useful to know the stage of pregnancy as well as further physiological investigation. These findings become milestone in buffalo to improve the reproductive efficiency in future.

Keywords: Nepalese buffaloes, pregnancy diagnosis, pregnancy specific protein b (PSPB), and first trimester.

INTRODUCTION

Buffalo constitutes the backbone for the meat and dairy sector of Nepal. The buffalo population of the world is about 171.86 million heads in 2007 (Pasha & Hayat, 2012; FAO, 2009) and about 5.2 million heads of buffaloes are reared in Nepal and contribute 70.71% in milk production and 59.33% in meat production (Krishi Diary, 2014).

Pregnancy is established and maintained by interactions of developing conceptus and mother. Mononucleate and binucleate cells in the outer epithelial trophoectoderm layer synthesized or sequestered a number of proteins and they are transported into the peripheral circulation (Kumar *et al.*, 2014) of pregnant ruminants from 3 to 4 weeks after mating until parturition (Zoli *et al.* 1992b; Perenyi *et al.* 2002). For the First time Laster 1977; Robert & Parker, 1976 discovered proteins in uterus and Sasser *et al.*, 1986 discovered protein in circulation. Pregnancy Specific Protein B (PSPB) is a type of Pregnancy associated Glycoproteins (PAGs) of molecular weight 67 kDa (Zoli *et al.*, 1991). PSPB was first isolated and partially purified in 1982 by Butler *et al.* This protein is more precise and reliable pregnancy marker in ruminants like hCG discovered in pregnant women's urine or blood and PMSG (Pregnancy Mare Serum Gonadotropin) in pregnant mare's serum (Sasser *et al.*, 1986) useful for pregnancy diagnosis. The profile of the concentrations of boPAGs in maternal plasma during pregnancy and postpartum periods was established by a highly sensitive and specific radioimmunoassay (RIA) (Zoli *et al.* 1992) in cow, by ELISA in Boer goats and in dairy, dual purpose and beef cattle (Shahin, 2012). Presence of PSPB in circulation means viable of feto-placental unit in ruminant uterus (Ectors *et al.* 1996) and may furthermore helpful for prediction of number of foetus, fetal weight (Amiri, *et al.*, 2013; Abdulkareem *et al.*, 2012), finding of early placental abnormalities, and embryonic and fetal mortality (Heyman *et al.* 2002; Szenci *et al.* 2003).

Various pregnancy diagnosis approaches are used in buffaloes and cows including Transrectal palpation (Abdulkareem *et al.*, 2011), transrectal ultrasound (Thomson, *et al.*, 2010; Piechotta, *et al.*, 2011), milk or plasma progesterone assay (Abdulkareem *et al.*, 2011; Sharma and Kaker 1990) etc. The BioPRYN ELISA is recommended for detection of pregnancy in cattle at or greater than 28 days post breeding and 73 days post calving (Sasser, 1986). Although serum PSPB profile has been described for many species, no any profile data about the Nepalese buffalo. The knowledge of this profile is of fundamental importance for further physiological investigations and practical applications. The objective of the present study was to establish serum PSPB concentration profile in pregnancy of Nepalese buffalo of first three month of pregnancy.



MATERIALS AND METHODS

Study Site and Experimental Animals

The study was conducted from November, 2015 to March, 2015 in at Agriculture and Forestry University (AFU) Livestock Farm, Rampur. Fourteen buffaloes, ten of parous and 4 heifer were used during this study. Buffaloes were housed and managed under AFU, Nepal.

Experimental Design

All buffaloes were synchronized by OVSYNCH technology fig. 1. Two mili Liter (100mg) GnRH hormone (OvaCyst®, **gonadorelin diacetate tetrahydrate, Bayer health care LLC**) injection intramuscularly was administered at a random stage in the estrus cycle at zero days. To avoid the selection of pregnant animal transrectal ultrasonography (TRUS) was used to confirm open stage in every steps of hormonal treatment. Seven days later 5 mL PGF2 α hormone (Lutalyse®, **dinoprost tromethamine inj., Pharmacia and Upjohn Company**) is administered intramuscularly to regress responsive corpus lutea. Parous (two or more than two times calved buffaloes) were administered with 5mL of PGF2 α after 24 hrs (at 8th day) of first PGF2 α , second GnRH injection is injected after 48 hours of the first PGF2 α injection to induce ovulation of the responsive dominant follicle recruited after the first GnRH injection. Buffaloes were received FTAI after 16-20 hours of the second GnRH injection as shown in fig. 1.

Blood Sampling

Blood samples for measurement of PSPB were collected from each buffaloes from a day before AI (day of second GnRH) upto confirmation of pregnancy by TRUS at 34 day of post AI weekly. After 34th day (5th weeks) bloods were collected only from pregnant buffaloes confirm by TRUS till 90 days weekly. Approximately 10 mL blood sample were collected by jugular venupuncture into vacuum tube (BD vacutainer serum, Blood Collection Tubes, BD, Franklin Lakes, NJ, USA) by multi sample needle (EXELINT) and were put into cool box overnight. Serum was separated at day after blood collection by centrifugation at 3300 rpm for 10 minutes, serum were transferred to 0.5 mL serum tubes and stored at deep freeze until PSPB analysis. Samples were transported to Laboratory in an ice box.

Pregnancy Diagnosis

Pregnancy diagnosis using TRUS (Honda electronics HS- 1500 vet, Japan, 10-5 MHz linear probe) was conducted using a linear 7.5 MHz transducer on day 27, 34 and 62 post AI to determine Persistent Corpus leuteum, amniotic fluid or embryo presence. All transrectal ultrasonography exams were performed by the same skilled operator.

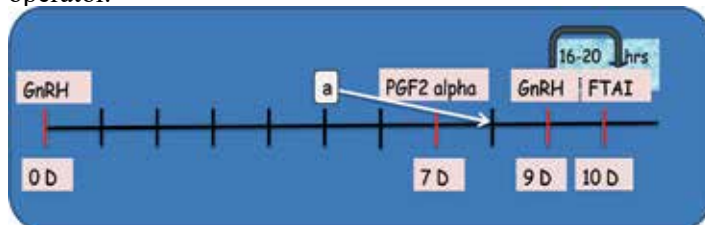


Figure 1: OVSYNCH Technology; a fixed-time A.I. synchronization protocol using GnRH and PGF2 α hormone. a. Administration of same dose of PGF2 α in 8th days for pluriparous (two or more than two times calved) buffaloes.

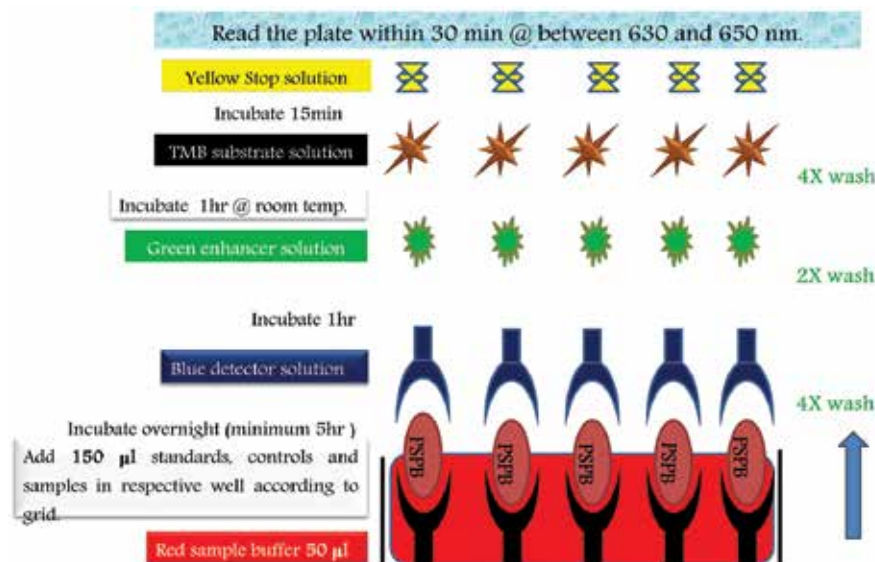


Figure 2: The procedure for the ELISA (BioPRYN® assay) starts with the addition of the serum to the plate coated with anti-PSPB antibody, after incubation of the test sample in the coated well and is detected by secondary binding of a labeled antibody (Detector). Binding of the labeled antibody conjugate is detected by the addition of the Enhancer) and 3, 3', 5, 5'-tetramethylbenzidine (TMB) unbound conjugate is washed away, and is quantified by the subsequent color development. Color development is proportional to the amount of PSPB in the sample. (Source: BioPRYN® training manual, BioTracking LLC, Moscow, Idaho 83843).



BioPRYN® Assay

Each serum sample was analyzed using a BioPRYN® test in duplication to assess optical density (OD) to find PSPB concentrations according to the. BioPRYN® assay were done at Animal Health Research Division (AHRD), NARC, Khumaltar. Procedure of BioPRYN® is shown in fig. 2.

Reading of plate that is optical density (OD) was export as a text file. Then it was transferred to excel for further data analysis. The concentration of PSPB in ng/mL was obtained only after the standard line is a good fit ($R^2 > 0.985$). Samples with higher PSPB concentrations than the estimated standard dose (4 ng/mL or > 4 ng/mL) were re-assayed in new kit with further dilution of serum with PBS for determination of accurate PSPB concentration.

Data Analysis

Numerical data are expressed in mean \pm standard error of the mean (SE). Data analysis was carried out by using Microsoft excel 2007. Statistical significance was assessed using analysis of variance (ANOVA). Probability values < 0.05 were considered significant. The statistical analysis was computed using OpenEpi (Open Source Epidemiologic Statistics for Public Health) version 3.03 software.

RESULTS

Transrectal ultrasonography at 34 days and 62 days after AI showed that four buffaloes were pregnant out of fourteen synchronized (28.6%). Among four pregnant three were parous and one was heifer buffalo.

Serum PSPB Profile During Pregnancy

Serum concentrations of PSPB ng/mL (mean \pm SE) of four buffaloes are presented in scatter chart fig. 3. The average basal serum PSPB concentrations of buffaloes were 0.24 ± 0.025 ng/mL prevailing at a day before AI. PSPB level seems to be constant up to day 20 (0.32 ± 0.05 ng/mL).

PSPB concentration increased significantly ($p < 0.005$) on day 27 (3.28 ± 0.67 ng/mL) compare to basal level. Thereafter PSPB concentration increased significantly ($p < 0.01$) on week 6 (24.26 ± 7.08 ng/mL), reaching its highest level on week 7 (30.46 ± 12.67 ng/mL). Then concentrations starts to decrease gradually to 18.88 ± 4.57 ng/mL at week 10 thereafter starts increasing and reaches to 27.23 ± 8.23 ng/mL at week 13.

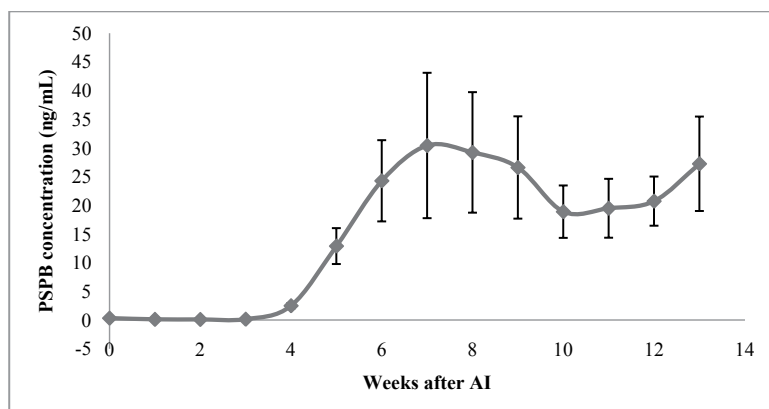


Figure 3: Serum profile of a novel Pregnancy Specific Protein B (PSPB) (Mean \pm SE) in pregnant Nepalese buffaloes (*Bubalus bubalis*) during the first trimester. Buffaloes were bled from day 0 to day 90 of gestation. Significant differences were detected on week 5 ($P < 0.01$) and week 6 ($P < 0.005$) of pregnancy.

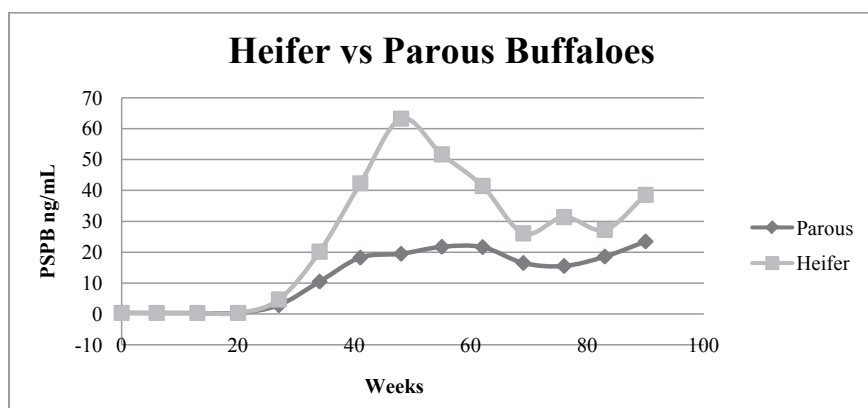


Figure 4: Line chart showing comparison of PSPB concentration of one heifer and mean of three parous buffaloes.

Heifer vs Parous

PSPB level up to days 27 is similar while concentration after 34 days of heifer is slightly higher than parous buffaloes upto 90 days. Heifer has peak value at 48 days i.e. 63.24 ng/mL while parous have peak value at 55th day and 62nd days post AI i.e. 21.72 ± 10.42 ng/mL and 21.64 ± 10.49 ng/mL respectively line chart is shown in fig. 4.



DISCUSSION

The present study describes serum PSPB concentrations profile first in Nepalese buffalo of first three month of gestation. The basal serum PSPB level of pregnant buffaloes (< 0.3 ng/ml) was agreed with EI-Battawy, *et al.* (2009) (< 0.20 ng/ml) in Egyptian buffalo. The mean PSPB concentration observed in pregnant buffaloes at day 27 post AI (3.28 ± 0.67) was similar to previous finding in buffaloes Abdulkareem, *et al.* (2011) (3.89 ± 0.53 ng/mL at 32-34 days); Paudel (2015) (3.8 ± 0.01 ng/mL at 4th weeks) by using same BioPRYN® assay and was slightly lower than EI-Battawy, *et al.* (2009) in Egyptian buffalo (4.48 ± 0.92 ng/ml) by using RIA. Increment of serum PSPB at day 27 was continue to reach peak at day 48 (30.46 ± 12.67 ng/mL), which may be associated to continuous development of cotyledons and placental development to this time. Level may decline slightly at day 69 (18.88 ± 4.57) and increased gradually upto 90 days (27.23 ± 8.23). The reason of fall in concentration was little understood it may due to necessary preparations regarding fetal physiology in uterus. The study of Khanam, (2012) in Black Bengal goat stated that the histological study of fetal connective tissue, maternal connective tissue, epithelium trophoblast increases during 50 days of pregnancy and decreases during 90 days of pregnancy. Thereafter concentration increased upto day 90.

Apart from stage of pregnancy, in the literature other effects on PAGs levels were mentioned, such as the number of fetuses (Dobson *et al.*, 1993; Patel *et al.*, 1995 and 1997), fetal weight (Amiri, *et al.*, 2013; Abdulkareem *et al.*, 2012), breed (Shahin, 2016) fetal sex (Zoli *et al.*, 1992), placental mass (Echternkamp *et al.*, 1993; Abdulkareem *et al.*, 2012), birth mass of calf, finding of early placental abnormalities, and embryonic and fetal mortality (Humblot, *et al.*, 1988; Heyman *et al.*, 2002; Szenci *et al.* 2003).

Comparisons of heifer and parous buffalo showed that there were somewhat variation in concentration of serum PSPB in heifer and parous buffaloes. This result was similar to Arnold, *et al.* (2006); describe that in heifer pregnancy was diagnosed earlier and increase serum PSPB level sharply than parous. We hypothesize that due to less sample size this study cannot predict that this variation was significantly different or not to each other. But meaning of variation on PSPB among heifer and Parous remains to be established.

In conclusion, the determination of serum PSPB profile by BioPRYN(®) test is useful to know the stage of pregnancy as well as further physiological investigation. Thus these findings become milestone in buffalo to improve the reproductive efficiency in future.

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