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ICAR-Central Institute for Research on Buffaloes, Hisar

(ISO 9001:2015 certified institution for "Improved Buffalo Germplasm Production")



From The Director's Desk

Best wishes for a happy and prosperous New Year 2024! The January to June, 2023 edition of our official newsletter from the ICAR-Central Institute for Research on Buffaloes, Hisar is here.

At ICAR-Central Institute for Research on Buffaloes, we remain committed to evolve with the new knowledge translating into enhancing the productivity and profitability of buffalo rearing systems through cutting-edge research and innovations. ICAR-CIRB serves as a hub for multidisciplinary research encompassing various aspects of buffalo production systems, including breeding, genetics, nutrition, health management, and reproductive biotechnologies. As a new initiative the Institute is initiating research in the area of digital platforms for buffalo management using sensor and IoT based gadgets, which will help farmers and the researchers to monitor various production, reproduction and health parameters related to the performance level of buffaloes in real-time, enabling precise management. This targeted approach will not only optimize resource utilization but will also minimize wastage, thereby mitigating the negative impacts of climate change. The development and adoption of these buffalo specific systems by small holder buffalo units holds immense promise in ensuring sustainable buffalo production. ICAR-CIRB will be partnering with other organisations, nationally and internationally, for research and development. Ultimately, these concerted efforts will help empower millions of dairy farmers with the knowledge and tools needed to optimize buffalo productivity, genetic improvement and elevate their socio-economic status.

I extend my heartfelt gratitude to the Indian Council of Agricultural Research (ICAR), especially the Secretary (DARE) and Director General, Dr. Himanshu Phatak, and the Deputy Director General (Animal Sciences), Dr. R. Bhatta, for their unwavering support and guidance. Their visionary leadership has been instrumental in steering our institute towards excellence.

I also take this opportunity to applaud the dedication and achievements of our scientists, technical officers, and staff members who work tirelessly towards our shared mission. Their passion for research and commitment to serving the farming community are truly commendable.

As we navigate the dynamic landscape of dairy farming, we reaffirm our commitment to the welfare and prosperity of buffaloes, recognizing them not just as livestock but as symbols of resilience, sustenance, and rural prosperity.



Dr. TK Datta

Director

Research Insights

Infrared thermography of testicles: Evaluation of breeding soundness in bull

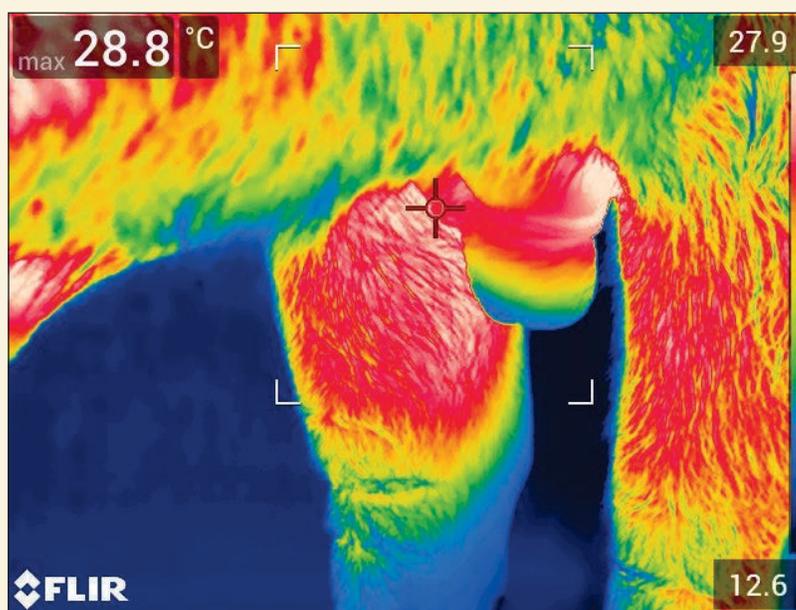
AK Balhara, P Kumar, SK Phulia, RK Sharma and S Singh

In testicular physiology, the artery carries an average blood flow of 12.4 ml/min, with arterial blood having higher temperature and hemoglobin saturation than the vein. By analyzing flow data and hemoglobin saturation differences, we estimated testicular oxygen uptake (1.2 ml/min) and metabolic heat production (5.8 calories/min). Conversely, considering blood flow and temperature differences, flow was estimated to contribute 28.3 calories/min, indicating blood flow as the primary heat source.

Thermoregulation in mammals involves heat exchange with the environment via conduction, convection, radiation, and evaporation. Maintaining an intratesticular temperature 2-6°C lower than core body temperature is crucial for normal spermatogenesis. The scrotum, attached to the body wall, exhibits a top-to-bottom temperature gradient due to vascularization. Testicular thermoregulation includes vascular (countercurrent heat exchange) and non-vascular mechanisms.

Infrared thermography (IRT) is effective for assessing animal pathophysiology. It accurately evaluates scrotal thermoregulation in bulls, correlating scrotal surface temperature with deep testicular temperature. Under normal

conditions, scrotal surface temperature is 5-6°C lower than abdominal temperature due to vascularization. IRT is used to evaluate natural mating fertility in bulls, identifying abnormal scrotal temperature patterns associated with lower pregnancy rates. It's a valuable tool for predicting reduced fertility in bulls with normal testicular size and semen quality.



Representative thermograph of a breeding buffalo bull indicating temperature gradients in testis

ICAR-CIRB produced Veer Gaurav through OPU-IVEP technology

A Jerome, RK Sharma, PS Yadav, D Kumar, M Punetha, R Kumar and R Rautela

Ovum pick up-In vitro embryo production (OPU-IVEP), in recent times has proven to be alternative method for propagate and disseminate superior germplasm, along with established techniques such as superovulation and embryo transfer. Factors which needs to be addressed for success of OPU-IVEP technology includes selection of donors, development/ refinement of culture conditions of oocytes and embryos. OPU-IVEP Technology, is an advanced reproductive technology for multiplication of superior female germplasm at much faster rate. Using MOET technology one can get 10-20 calf from a superior female animal in a year. Recently, ICAR-CIRB took a project

OPU-IVEP and was successfully in producing male buffalo calf out of semen of cloned bull and elite female. OPU-IVEP trials in buffalo carried out resulted oocyte recovery rate (55-65 %) with cleavage rate (25-52%) and blastocyst rate (25-30%). Use of OPU-IVEP technique resulted in birth of male calf (Veer Gaurav) using semen of cloned Bull (Hisar Gaurav). Trials are ongoing to standardise this technology in buffaloes. This has opened avenues to work on the OPU-IVEP technology and inclusion of sexing technology along with OPU-IVEP technology shall further enhance the genetic pool of bull mothers for future generations.



OPU-IVF Calf (Veer Gaurav)



Hisar Gaurav

One step delivery of CRISPR/Cas9 component into buffalo embryos

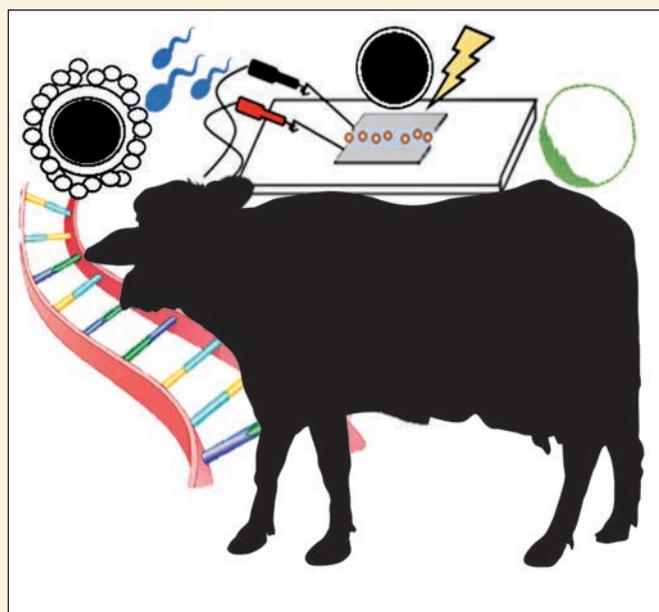
M Punetha, D Kumar, S Saini, S Chaudhary and PS Yadav

Genome editing is a rapidly advancing field in life sciences, involving targeted modifications in the genome using nucleases, also known as molecular scissors. The CRISPR/Cas system, discovered in 2012, is a notable addition to this toolkit, with constant improvements since its inception. Beyond its role in fundamental research, CRISPR/Cas9 is valuable for studying gene function and physiological processes and holds promise for enhancing livestock productivity, health, and biodiversity conservation.

Current methods for producing genome-edited animals include microinjection of CRISPR-Cas9 components into zygotes or editing somatic cells followed by somatic cell nuclear transfer (SCNT). However, SCNT is technically challenging and inefficient, with less than 10% of transferred embryos resulting in viable offspring. An alternative method involves microinjecting Cas9 mRNA/sgRNA into embryos, but this is labor-intensive and inefficient for larger animals like buffaloes.

Electroporation of zygotes has emerged as a promising delivery approach, creating temporary pores in cell membranes to facilitate the uptake of genetic material. This technique, termed CRISPR-EP, offers rapid action and greater editing efficiency. It is cost-effective and requires minimal equipment, making it suitable for large animals like buffaloes.

At ICAR-CIRB, optimized conditions for introducing the CRISPR/Cas9 system into buffalo zygotes via electroporation have been developed. Using specific parameters, such as voltage, pulse, and timing, increased membrane permeability and knockout efficiency were achieved without affecting embryonic developmental potential. Targeting the buffalo POU5F1 gene demonstrated consistent embryonic developmental competence, with significant alterations in pluripotent gene expression in POU5F1-KO-confirmed blastocysts.



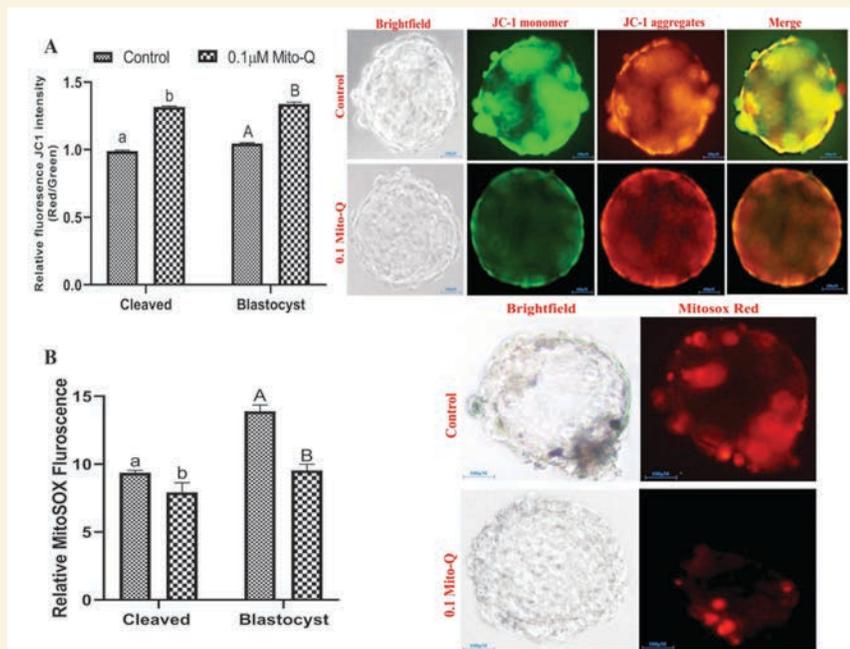
Mito-Q supplementation improves maturation and developmental competence of cloned embryos by reducing ROS production

M Sharma, M Punetha, S Saini, P Kumar, PS Yadav and D Kumar

Mitochondria play a crucial role in both oocyte maturation and cloned embryo production, exerting significant influence on the overall success of in vitro embryo production. During oocyte maturation, mitochondria orchestrate various metabolic processes and energy production required for the acquisition of oocyte developmental competence. The process of oocyte maturation necessitates large amounts of ATP, particularly for transcription, which increases ROS production by mitochondria. To compensate the excess production of ROS, mitochondrial-targeted antioxidants (Mito-Q) become a potent antioxidant due to having lipophilic triphenylphosphonium (TPP) cation, covalently linked to ubiquinol, which can cross the mitochondrial phospholipid bilayer and accumulate in hundreds of times higher concentrations inside mitochondria, as well as function as ROS inhibitors, reducing the production of mitochondria-specific ROS or superoxide.

To our knowledge, the effect of Mito-Q on buffalo oocyte maturation and developmental competency of cloned zygote has not been examined. To investigate the effects of Mito-Q on the in vitro maturation (IVM) of buffalo oocytes and the developmental competence of cloned zygotes, different concentration of Mito-Q were supplemented with IVM medium or in vitro culture (IVC) medium. Buffalo oocytes obtained from slaughterhouse ovaries were subjected to IVM and somatic cell nuclear transfer (SCNT). The IVM or IVC medium was supplemented with 0, 0.1, 0.5, 1, 2 μM or 0 and 0.1 μM concentrations of Mito-Q respectively. Supplementation of IVM medium with 0.1 μM Mito-Q significantly ($P < 0.05$) increased the cumulus expansion, nuclear maturation, mitochondrial membrane potential (MMP) and antioxidants genes (GPx and SOD) expression and effectively reduced ROS production during IVM, leading to a significant improvement in the maturation rate of buffalo oocytes. Further, the supplementation of 0.1

μM Mito-Q in IVC medium promotes the cleavage and blastocyst rate significantly over the control. Mito-Q supplementation also improves ($P < 0.05$) MMP, and reduced the ROS level and in cloned blastocysts. In conclusion, this study reveals that 0.1 μM Mito-Q supplementation in IVM or IVC media exerts a protective role against oxidative stress by decreased ROS production and improved MMP, fostering improved maturation of buffalo oocytes and enhanced developmental competence of cloned zygotes. These findings contribute valuable insights into the optimization of assisted reproductive technologies protocols for buffalo breeding and potentially offer novel strategies to enhance reproductive outcomes in other livestock species.



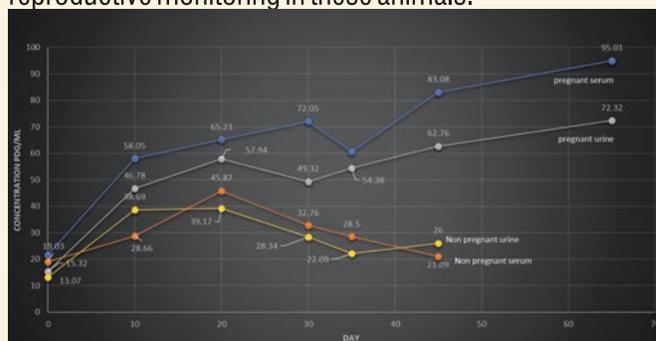
Effect of Mito-Q supplementation during IVC of cloned zygotes on mitochondrial membrane potential and ROS production. A) Evaluation of MMP levels in cloned zygotes through measurement of relative fluorescence intensity of JC1 in cloned embryo which showed that 0.1 μM Mito-Q supplementation improved MMP at both cleavage and blastocyst stage compare to control, B) Evaluation of mitochondrial ROS through quantitative analysis of MitoSOX red fluorescence intensity and 0.1 μM Mito-Q supplementation significantly reduced ROS level in cleaved and blastocyst of cloned embryos.

Pregnanediol-3 α -glucuronide (PdG) in urine as an indicator of serum progesterone levels in buffaloes

Suman, SK Phulia, RK Sharma, S Balhara, A Ganguly, S Kumar and AK Balhara

Pregnanediol-3 α -glucuronide (PdG) in urine can serve as an indicator of serum progesterone levels in buffaloes. An endocrinological study was conducted to establish a correlation between serum progesterone and its metabolite, Pregnanediol-3 α -glucuronide (PdG), in urine. Urine and serum samples were collected from a total of 40 buffaloes, comprising 20 heifers and 20 multiparous Murrah buffaloes. The multiparous group was further divided into 10 synchronized and 10 unsynchronized animals. Sampling was performed on critical days, including day 0 (day of estrus), 10, 20, 30, 45, and 65 post insemination. To quantify progesterone and PdG levels, both immunoassay and high-pressure liquid chromatography (HPLC) methods were used simultaneously. The experimental results, obtained through a rigorous quantitative comparison of immunoassay and chromatographic profiles, unveiled a

noteworthy correlation between progesterone and PdG levels in both serum and urine. This correlation suggests that PdG in urine could potentially serve as a reliable indicator for assessing serum progesterone levels in buffaloes, offering a valuable non-invasive approach for reproductive monitoring in these animals.



Variations in PdG concentration in pregnant and non-pregnant buffaloes

Assessment of pregnancy rate (conception rate) in field buffaloes bred with cloned buffalo bull semen in aspirational district of Haryana

PS Yadav, S Sajjan, H Tripathi, N Saxena, D Kumar, P Kumar, A Jerome and M Gururaj

The Department of Biotechnology (DBT) sponsored societal development project was implemented in Nuh, aspirational district of Haryana for duration of 2 years (Jan 2022 to Jan 2024). The district estimated human population was 11.70 lakhs (2019-20) and it has one of the lowest per capita income, literacy rate, under developed infrastructure, poor health facilities and less

developed in agriculture as well dairy sector. As fact, district has per capita income of Rs. 27,791/- as against the state average of Rs. 70,467/- in 2011-12. The literacy rate was 56.10% as compared to the state average of 76.64% (census, 2011). The district has 1.75 lakhs buffaloes (20th census) in which about 95 thousand are breedable which contribute about 69% of the total milk



Buffalo calves born in field from semen of clone buffalo bulls of ICAR-CIRB, Hisar



production in the district. District endowed with 25 veterinary hospitals, 61 veterinary dispensaries and 01 Disease diagnostic lab for livestock health & breed improvement. During 2018-19, district could perform only 1,30,000 Artificial Insemination (AIs) in bovines, which is much lower than AIs performed by the many districts in the state due to lack of development in infrastructure and other basis facilities. As a result the milk productivity of milch animals was much lower in the district. To address the

problem, under the project a total of 3768 AIs was performed using cloned buffalo bull semen. As on September, 2023 a total of 1322 animals were pregnant with conception rate 43.79%. About 186 Calving record were done. Among beneficiary farmers about 84% belongs to OBC, 12% general & 4% of SCST. During the project period, about 08 training programs were organized and about 343 dairy farmers were participated and in which about 65.01% are women dairy farmers.

Isolation and identification of Bubaline herpesvirus 1 from buffalo milk

S Yadav, A Boora, Nisha, P Devi and Nishu

Bubaline herpesvirus 1 (BuHV1) belongs to the Varicellovirus genus within the Alphaherpesvirinae, representing a cluster of ruminant alphaherpesviruses genetically related to the prototype bovine herpesvirus 1 (BoHV1). While the pathogenic mechanisms of BuHV1 in water buffalo remain unclear, it is primarily associated with inapparent infections, occasionally presenting as reproductive and respiratory clinical issues.

The first isolation of BuHV1 dates back to 1972 in Australia, from the prepuce or penis of buffalo bulls without visible lesions. However, recent clinical observations in May 2022 reported a suspected herpesvirus infection in a 5-year-old Murrah buffalo in Khoka village, Hisar, Haryana, presenting with generalized diffuse pustular skin lesions on the body and udder. Notably, other herd animals remained asymptomatic.

To identify the causative agent, skin lesion and milk samples were collected and initially screened using PCR. Although previous studies have isolated BoHV1 from milk, limited research exists on BuHV1 in milk. Considering the presence of leukocytes in milk akin to blood, it represents a

potentially valuable sample for viral shedding analysis.

This study aimed to evaluate milk's feasibility in detecting clinical BuHV1 in naturally infected buffaloes through further cell culture and molecular viral analysis. Following PCR confirmation, the virus was successfully isolated from the milk sample, inducing a cytopathic effect in MDBK cells. Whole genome sequence analysis identified the virus as BuHV1 S104_1 strain, closely related to BuHV1 strain b6 (accession no. NC_043054.1) from Australia, with a genome sequence identity of 97.95%. The Indian BuHV1 isolate's genomic sequence has been deposited in NCBI GenBank under accession no. OQ669138.1.

This study sheds light on milk as a valuable source for viral shedding analysis, offering easy sample collection compared to other sources, particularly in diagnosing clinical cases of suspected herpes infections. Furthermore, it unveils the presence of BuHV1 in buffalo milk for the first time, emphasizing milk's significance as an important sample collection source in suspected herpes infections.

Buffalo Health & Welfare

Effective mastitis control in dairy animals: the role of post-milking teat dipping and hygienic practices

AS Habbu, N Paul, MH Jan and FC Tuteja

Staphylococcus aureus, Streptococcus spp., and coliform bacteria are primary contributors to mastitis, leading to milk contamination and rendering it unsuitable for human consumption. The udder and the environment serve as the main sources of infection, with the teat canal being the primary route of transmission for mastitis. A management tool for mastitis prevention, teat dip, was integrated into the Nili Ravi herd at CIRB Sub Campus, Nabha, resulting in a significant reduction in clinical cases compared to the previous year.

Key points for an easy-to-implement mastitis prevention strategy are outlined below:

- *Hygiene practices during milking:* Ensure cleanliness and dryness during milking, irrespective of whether machines or hand milking is employed.
- *Cleaning and disinfection of milking equipment:* Regularly clean and disinfect milking machines using recommended chemicals to ensure hygienic milk production.
- *Post-milking teat dipping procedure:* Adopt post-milking teat dipping to prevent new udder infections. Use a teat dip solution with four parts of povidone iodine (5% w/v) and one part of glycerine. Understand

that the antimicrobial activity of the solution, combined with glycerine, helps prevent infections and ensures longer adherence.

- *Role of glycerine:* Recognize glycerine as an effective emollient, beneficial for treating fissured and dried teats, especially during winter.
- *Povidone iodine as an antiseptic agent:* Acknowledge the effectiveness of povidone iodine (0.5-1% available iodine) as an antiseptic agent, crucial in preventing causative agents like Staphylococcus aureus and Streptococcus uberis.
- *Teat dip for prevention of injuries:* Understand that the teat dip solution helps prevent injuries caused by fluctuations in vacuum pressure during milking, reducing the risk of intramammary infections.

A comprehensive mastitis control program, encompassing premises cleaning, udder hygiene, regular screening for subclinical mastitis, and timely detection and treatment of clinical cases, is a vital part of the strategy. Regularly monitor the effectiveness of the mastitis control program and remain open to adjustments for continuous improvement.



Postpartum care of buffaloes: Packages and practices

N Paul, M H Jan, AS Habbu, A Bhaladhare and FC Tuteja

The postpartum period in buffaloes is critical for their survival and productivity. Following calving, dams experience significant stress, rendering them vulnerable to life-threatening conditions. While the cervix seals tightly during pregnancy to protect against microbial invasion, the reproductive tract opens upon delivery, creating a window for bacterial colonization. Factors such as hygiene, nutritional status, and innate immunity influence the risk of infection. Failure to clear infections can lead to severe conditions like metritis and pyometra, impacting both economic and milk production aspects.

The postpartum period in buffaloes is characterized by physiological changes and increased susceptibility to reproductive disorders. Factors such as hygiene, nutritional status, and innate immunity play crucial roles in determining the risk of infection. Failure to address these factors can lead to severe conditions like metritis and pyometra, resulting in economic losses and decreased milk production.



Risk Factors:

1. **Prepartum Care of Dams:** The last trimester of gestation is characterized by rapid fetal development, leading to increased metabolic demands on the dam. Failure to meet these demands can result in negative energy balance and compromised immune function, increasing the risk of infection.
2. **Improper Vaccination:** Inadequate vaccination against pathogens like *Brucella* can lead to abortion and metritis in herds, posing a significant risk of transmission to unaffected animals.
3. **Overcrowding:** Close proximity to infected animals increases the risk of disease transmission, emphasizing the importance of proper animal housing and management practices.
4. **Poor Management Practices:** Inadequate management during pregnancy and calving can create unhygienic environments conducive to infection, leading to delayed ovarian rebound and increased calving intervals.
5. **Lack of Disease Screening Infrastructure:** Failure to screen breeding bulls for diseases can result in widespread transmission to females, highlighting the importance of regular screening protocols.

Management Practices:

1. **Transition Animal Management:** Providing additional nutrients and minerals during the peripartum period helps meet the increased metabolic demands of the dam.
2. **Hygiene and Environmental Management:** Regular cleaning and disinfection of calving areas, along with stress-free environments, reduce the risk of infection.
3. **On-Farm Disease Diagnosis:** Periodic screening for diseases like Brucellosis and mastitis helps identify and isolate infected animals promptly.
4. **Vaccination:** Vaccinating young females against Brucellosis minimizes the incidence of the disease in herds.
5. **Awareness and Education:** Educating farmers about proper management practices and disease prevention strategies is essential for minimizing postpartum reproductive disorders.

Sectorial Growth

Quality buffalo meat production: Raising broiler buffalo

S Yadav, A Boora, AK Balhara and S Balhara

Water buffaloes (*Bubalus bubalis*) are primarily raised for milk production, but they also possess a significant and untapped potential for meat production. Most of the buffalo meat comes from animals that are no longer economically viable due to a decline in milk production or draught power, with a smaller contribution from younger animals. Buffaloes outperform other bovine species in terms of converting poor-quality roughages, especially locally available crop residue biomass into high-quality animal protein sources like meat and milk. The superior feed conversion ratio in buffaloes is attributed to various factors, including high dry matter intake, extended retention time of feed in the rumen, a more favourable rumen microbiota for digesting coarse fibrous feed, and greater environmental stress tolerance compared to cattle. Despite the ample opportunity for development, limited research has been conducted on this species with regard to efficient meat production.

The water buffalo plays a significant role as a meat source in many countries, often obtained from advanced age spent buffalo or draft animals that are culled when the cost of maintenance exceeds their production value. Additionally, meat may come from animals culled from the herd due to infertility, mastitis, metritis, or other ailments as well as emergency slaughter. Another source is the young male buffalo calves, weaned off when they exhibit poor growth and are no longer required for dam's milk letdown. However, the meat derived from these spent buffalo tends to possess undesirable qualities such as being dark, tough, less tender, and lacking in flavour. Overall, it is generally of lower quality, influencing consumers' preferences when making meat purchasing decisions. Nevertheless, these limitations can be addressed by opting for high-quality meat sourced from properly raised and well-nourished young male buffalo calves, ideally around 18 months old which ensures a lean, tender, and highly palatable meat with lower collagen content in the muscles. The quality of buffalo meat is comparable to that of beef, exhibiting similar physical and chemical properties. Notably, buffalo meat stands out for its added positive characteristics, including low intramuscular fat / lean meat, reduced calorie and

cholesterol content, lower saturated fatty acids, elevated levels of conjugated linoleic acid (CLA), increased iron content, higher dry matter, and enhanced tenderness.

Despite the buffalo's notable advantages over cattle in terms of efficient conversion of poor-quality roughages and superior meat quality, it remains paradoxical that buffaloes have yet to be developed for commercial meat production, following the established steps and strategies applied in beef cattle production. An example of a promising meat breed is the Buffalypso of Trinidad and Tobago, which is obtained through the selective crossbreeding of Jafarabadi females with Bhadwari males. Other buffalo meat breeds include swamp buffaloes such as the Sulawesi breed from Indonesia and the Carabao breed from the Philippines.

To propel the development of buffaloes for meat production, scientific advances are essential. This includes selective breeding and crossbreeding of swamp buffaloes, aimed at enhancing meat-related traits and creating distinct meat breeds. Additionally, it is crucial to establish comprehensive rearing practices, incorporating scientific feeding methods for riverine male buffalo calves to ensure the production of high-quality meat. By addressing these key aspects, the buffalo industry can make strides in optimizing meat production and overcoming the current paradox in its development.

Every year, millions of male buffalo calves become available in Asia from breeding buffaloes, presenting considerable potential for meat production if managed scientifically. Unfortunately, resource-limited farming households often adopt suboptimal practices in calf management and feeding for male calves. These neglected and underfed male calves, primarily kept for milk letdown, either face high mortality rates within the first three months or are weaned and sold at a low price between 8-12 months with a body weight ranging from 70 to 100 kg. In contrast, there is a substantial opportunity for buffalo farmers to generate additional income and employment by appropriately fattening male calves. This can be achieved by utilizing locally available, cost-effective feed resources such as crop residues and agro-industrial by-products to

create a balanced ration. Subsequently, these well-fed male calves can be sold for meat production when they reach a body weight of 250 kg at 16-18 months, resulting in higher dressing yields. In India, intensive feeding in a commercial feedlot of 5000 male buffalo calves for meat production was started in 1999. Purchased male calves at 8-10 months age from farmers doorstep were reared with high energy high protein ration (20 % crude protein, 65% TDN) and good management practices in feedlot. Calves gained weight @ 900-1000 gm per day (additional weight gain of 120 Kg in 4 months), achieving slaughter weight of 250 kg at 14 months and dressing percentage of 65%.

Despite the ample opportunities for development, there has been minimal focus on enhancing efficient meat production in this species. Given the current preference for lean meat, buffaloes are well-suited to contribute to beef production to meet future demands. Existing facilities for buffalo meat processing are limited, mainly dealing with culled and aged animals. Despite the toughness and lack of flavour in such beef, there is still a market for it. Buffaloes raised with improved management and careful feeding can yield high-quality beef carcasses that are readily embraced by consumer.

Technology Interventions

Preg D : Urine based pregnancy diagnosis kit for dairy animals

A Suman, , M Ghosh, R Kumar, RK Sharma, SK Phulia, AK Mohanty and AK Balhara

The Preg-D kit is a urine based novel technique for pregnancy diagnosis in dairy animals. The kit utilizes a simple thermophilic biochemical colour reaction in urine to diagnose pregnancy and can be performed by any literate person. It does not require any instrumentation to read results - interpreted by seeing development of colour. The kit is a very effective alternate method for identifying non-pregnant animals in the herd. The kit can be used by the farmer himself, so very useful in rural areas where it is very difficult to have a veterinarian for pregnancy diagnosis.

The method is based on a colorimetric test of least six metabolites forming a red-violet lactone derivative coloured conjugate, which has been named as Preg-D Bovine pregnancy diagnosis kit. Colour intensity is highest at around day 150 till end of pregnancy and this has been confirmed by measuring intensity at wavelength 665nm. A patent has been filed for the method developed (Ref. Indian Patent 'Urine based pregnancy detection method for ruminant livestock animals' filed vide application no. 202011013074,

Dated March 25, 2020). The method and kit is suitable for pregnancy diagnosis after completion of estrous cycle in cows/buffaloes. Considering that the pregnancy establishment biological process in bovine is complete around day 40 post insemination, the method (and hence kit) gives high accuracy around days 30-40 of pregnancy.



Concept of area-specific mineral mixture in livestock ration

V Mudgal, N Saxena and M Gururaj

India has the largest bovine population in the world and ranks first in total milk production. However, per-animal productivity is very low in the country mainly due to poor genetic makeup along with compromised nutritional status of the dairy animals which leads to various metabolic disorders and reproductive inefficiencies such as anestrus, repeat breeding, and infertility. Low reproductive efficiency in cattle and buffaloes is one of the major problems in livestock production in India, resulting in poor economic returns to the livestock farmers.

Most of the animals in developing countries including India are fed on agricultural by-products and inferior quality crop residues, which have low nutritive value and digestibility. The productivity of dairy animals is dependent on balanced feeding by providing adequate quantities of all necessary nutrients to meet their requirements for a particular physiological function. Minerals are the essential nutrients bearing an important role in animal production and reproduction. Deficiency of single or multiple minerals or their imbalances leads to delayed puberty, delayed ovulation, lower conception rate, high embryonic/foetal losses, and prolonged postpartum anoestrus. Even in males, deficiencies of some trace minerals can reduce testicular development, sperm production, and viability. Mineral deficiency in dairy animals is the foremost cause of poor growth rate, suppressed body immunity, decreased milk yield, and various reproductive disorders. Dietary deficiency of macronutrients is well combated by commercially available feeds and hence optimum supplementation of all the minerals results as a stimulus for ovarian rebound and initiation of ovarian activity.

During the past decade, significant research has been conducted to understanding the effect of macro/micro mineral supplements on the production efficiency of dairy animals. Deficiencies of minerals like P, Cu, and Zn have been associated with subnormal fertility and anoestrus conditions. Zinc plays an important role in reproductive function of dairy cows, particularly by decreasing the incidence of abortion and abnormal oestrus and reducing days to first oestrus. Moreover, other researchers found that Cu improved the reproductive performances of cows and Mn improved bovine sperm activity. A fall in Zn level

was associated with a fall in steroid hormone concentrations which indicated that there was some correlation between plasma Zn levels and progesterone-oestrogen levels for proper reproductive processes. It is well established that minerals play an intermediate role in the action of hormones and enzymes at the cellular level and their deficiency ultimately affects the reproductive performance of females. The problem of mineral deficiency conditions in dairy animals has been reported regularly because of the low availability of some essential micro and macro minerals in different feedstuffs. Some trace minerals have important roles in maximizing reproductive performances. Even marginal deficiency of these minerals can impair reproduction without showing clinical signs of deficiency. Deficiency problems have been observed in cattle of different regions and in most cases they differ from one region to another region due to different soil composition, intensity of cropping, precipitation patterns, and soil erosion patterns. The assessment and prevention of mineral deficiency need a thorough understanding of the factors like age of animals, season, soil profile, plant materials and feeding practices. It is, therefore, important to understand the basis of mineral nutrition very clearly as also to meet the requirements of the animals to overcome the problem of deficiencies or excesses, so as to maintain productivity.

Animals cannot synthesize minerals inside their body and hence are supplied through feeds and fodders fed to the dairy animals and the rest of the amount which is unable to get supplied through feeds needs to be supplemented through mineral mixture. The mineral content of a feed depends on several factors including mineral content of the soil, soil type, fertilization, and hence mineral content of the same crop grown at different agro-climatic conditions differs. This variation in the mineral content of the same crop grown at different places in India creates a need for the use of area-specific mineral mixture (ASMM) because the mineral mixture of a standard composition (BIS recommended mineral mixture) may not be such effective and may not be going to address the deficiencies appropriately and efficiently.

Advantages of ASMM over BIS Mineral Mixture:

1. ASMM remains cheaper compared to the BISMM because it uses only deficient mineral elements in the feeds of the selected area.
2. The bioavailability of the minerals remains higher due to the presence of only required mineral elements and not having the minerals that remained already sufficient/excessive in the feeds.
3. Supplementation of area-specific minerals most deficient in that area or zone avoids antagonistic effects of excess levels of other minerals, thereby improving the utilization efficiency of micronutrients and could be a more practical and effective approach.

In India NDDB and other agencies have completed mineral

mapping for various states/ regions and accordingly, area-specific mineral mixture formulations have been developed for different states throughout the country. ASMM has to be fed at a specified recommended dose daily, depending on the physiological conditions of the animal.

The effect of supplementation of deficient minerals through ASMM was studied by a series of experiments. The results revealed increased growth, productive, and reproductive performances of dairy animals at different physiological stages, with reduced incidences of post-partum reproductive and metabolic disorders including mastitis.

Silage: A Sustainable Solution for Small Farmers' Livestock Feed Shortages

SK Kakraliya, FC Tuteja, S Singh and PC Lailer

Small and marginal farmers, facing limited fodder production, struggle during lean months like October-November and May-June, impacting livestock productivity and income. Reliance on dry roughage affects milk production. Silage, anaerobically fermented green fodder with 65-70% moisture, preserves nutrients and supports animal health. Ensiling, fermenting soluble carbohydrate-rich crops for 40-45 days, produces palatable silage. Jowar, Maize, Bajra, Oats, and Barley are suitable.

Silage making offers several benefits for farmers, including superior quality and maximized yield. Harvesting crops during the early flowering to mid-milk stage ensures high nutrient accumulation and minimal indigestible elements, resulting in high-quality silage. The size of the silage pit depends on available fodder and livestock needs, with a 1.0-meter pit capable of storing 500 kilograms of silage.

The ensiling process is akin to pickling, a familiar practice in Indian households. Farmers can easily grasp silage making techniques. It involves harvesting crops with 60-65% moisture and 30-35% dry matter, then chopping them into small pieces (2.5 to 4.0 cm) for better compaction. Choosing an elevated, well-drained location for the silo is crucial to prevent air and water infiltration.

Filling the silo within one to two days, tightly pressing the fodder, and covering it with plastic or dry crop residues and soil ensures proper fermentation. After 45-60 days, the

silo is ready for feeding, characterized by a vinegar-like smell and bright yellow colour. Well-made silage has a pH value of 4.5 and a moisture content of 65-75%, similar to green fodder in nutrient composition. Good silage contains 3-14% lactic acid and minimal butyric acid (<0.2%). To produce high-quality, balanced silage, proper treatment with additives at appropriate concentrations is essential. For example, treating 10 quintals of silage may require 2.0 kg of jaggery, 1 litre of whey, and 1.0 kg each of urea, common salt, and mineral mixture. These additives enhance nutrient content and ensure optimal silage production.



Screen shot: Silage making demonstration (adopted from CIRB You Tube channel <https://www.youtube.com/watch?app=desktop&v=igifXVthB2s>)

Production Performance

Animal farm

Institute maintains a high pedigree herd of over 500 Murrah and Nili-Ravi buffaloes at Hisar and Nabha, respectively. During the period (January to June, 2023), a total of 1,89,585 kg of milk was produced with wet average of 10.18 kg per day per buffalo and 7.06 kg per buffalo of herd average from Murrah at Hisar. Similarly, at Nabha campus a total of 1,77,055 kg of milk produced with 7.71 kg and 4.96 kg of wet and herd average of Nili-Ravi buffalo, respectively.

Agricultural farm

Institute at main campus HISAR has a total area of 780 acres of land, out of which about 50% is arable and unde fodder cultivation. During the period, about 23,582.85 quintals of green fodder and 793.15 quintals of grains was produced at Hisar. In sub campus Nabha about 27,326 and 6,929.65 quintals of green fodder and grains, respectively were harvested during the same period.

Elite Bulls of ICAR-CIRB, Hisar

The Semen Lab at the Institute has established itself as an important center for sourcing elite Murrah buffalo germplasm in the country. Two elite bulls of CIRB namely 4,354 and 5,723 are under semen production and donating excellent quality germplasm. The bull number 4,354 is a PT bull and has 1.67% superiority over contemporary daughters. The best standard lactation milk yield (SLMY) of its dam is 3,528 kg with highest peak yield of 20.1 kg. We have more than 25,000 doses of this bull in stock. The semen of this bull is in high demand among buffalo breeders.

The bull number 5,723 has recently started donating semen and being used as test bull. The best standard lactation milk yield (SLMY) of its dam is 5,170 kg with highest peak yield of 26.8 kg. At present, its semen is being used in field testing PT program. The pedigree and production records of both bulls are as follows:

Sr. no	Bull no.	D.O.B.	Dam no.	Sire no.	Dam's All SLMY (305 or less days)	Highest Yield/ Highest Peak	Dam's Calving Intervals	Bull Dam's Dam No.	Bull Dam's Dam All SLMY (kg)	Bull Dam's Dam CI	Bull Sire's Dam No.	Bull Sire's Dam All SLMY	Bull Sire's Dam CI
1.	4354 PT	05/09/11	4353	NK	NK/ NK/ 2829/ 3528/ 3058/ 1409 (211d)	3528/20.1	NK/ NK/ NK/ 369/ 720	NK	NK	NK	NK	NK	NK
2.	5723	07/10/20	5179	7227 Set 18	4073/5170	5170/26.8	560	4413	2341/2592/ 3048	451/389	6625	NK/ 3348/ 2247/ 3465 /3127	NK/452/ 341/415



Elite Murrah Bull 4354 PT



Elite Murrah Bull 5723

Breed in Focus

Nili Ravi

A Bhaladhare, N Paul, AS Habbu, MH Jan and FC Tuteja

- Origin and Evolution:
 - Nili Ravi, a prominent dairy buffalo breed, emerged in 1938 through meticulous cross-breeding of Nili and Ravi breeds.
 - Initially considered a unique breed, they were later distinguished as separate entities by the 1941 all-India cattle show.
 - The complexities of cross-breeding led to their amalgamation in 1960, forming the unified Nili-Ravi breed.
- Geographical Presence:
 - Nili-Ravi Buffaloes thrive in the fertile belt between the Sutlej and Ravi rivers in undivided Punjab.
 - Significant populations are found in bordering districts of Pakistan and Indian Punjab, including Amritsar, Gurdaspur, and Ferozepur.
- Distinctive Characteristics:
 - Unique physical features include bright walled eyes, wedge-shaped body in females, deep abdomen in males, and pink markings on the udder.
 - Recognizable by broad horns, a broad head with a slight depression, long teats, and distinctive white markings, earning them the name "Panch Kalyani."
- Population Statistics:
 - As per the 20th livestock census, pure Nili Ravi breeds number around 1.08 lakh, while crossbreds reach approximately 1.27 lakh.
 - It focuses on imparting knowledge and skills to ensure optimal care and management of Nili-Ravi buffaloes.

ICAR-CIRB Sub Campus Nabha

- A Genetic Resources Hub and a knowledge dissemination and training centre
- Established in 1987, houses elite genetic resources of Nili-Ravi breed of buffalo
- Plays a pivotal role in genetic improvement through progeny testing and disseminating semen and bulls to farmers and other semen stations
- Remarkable improvements (1992 to 2022)
 - Wet average rising from 5.86 kg to 8.28 kg
 - Herd average has grown from 3.42 to 5.64 kg
 - Standard lactation yield from 1813 kg to 2571 kg
- Actively engages in training programs, campaigns, and extension activities for farmers



INDIA_BUFFALO_1600_NILI RAVI_01002

Female Nili Ravi Buffalo



Male Nili Ravi Buffalo

Events at CIRB



Republic day celebrations (26th January 2023)



39th Foundation day celebrations (1st February 2023)



Visit of DDG (AS) Dr. B N Tripathi (10th January 2023)



Bill & Milinda Gate Foundation delegates interacting with scientists (4th February 2023)



Training on CRISPR mediated genome editing (18th January 2023)



MoU established with Uttarakhand Council for Biotechnology, Govt. of Uttarakhand (20th March 2023)



QRT meeting held under chairmanship of Dr. P Biswas (11-12 May, 2023)



26th RAC meeting held under chairmanship of Dr. U K Mishra (1-2 June, 2023)

CIRB Among Farmers

CIRB organise extension activities mainly under SCSP scheme, TSP scheme, MGMG program and aspirational district program to uplift the socio-economic status of the resource poor farmers. During the period, the institute has organised a total of 5 training programs on scientific buffalo husbandry practices at Hisar and Nabha campus. About 147 dairy farmers, rural youths and dairy women participated in the training. One day Kisan Gosthi was held

at Hisar campus on the occasion of 39th foundation day of the institute. Two Kisan Gosthis was organised at Kewalpur (V), Chittorgarh, and Roba (V), Udayapur (D), Rajasthan under TSP scheme. A total of 200 farmers were participated. Institute participated in pashu mela at Muzzafarnagar organised by the Uttar Pradesh State Government and dairy mela organised by ICAR-NDRI, Karnal.



Kisan Gosthi held on the occasion of 39th Foundation Day (1st February 2023)



Kisan Gosthi held at village Kewalpur, Distt. Chittorgarh, Rajasthan under TSP Scheme (23rd February 2023)



ICAR-CIRB in Pashu Mela organised by Govt. of Uttar Pradesh (6-7 April 2023)



ICAR-CIRB in Dairy Mela organised by ICAR-NDRI, Karnal (7-8 April 2023)



Training program for dairy farmers under SCSP scheme (13-17 March 2023)



Training program for dairy farmers (22-28 February 2023)