SAARC

Herd Health Management of Dairy

Buffalo

Inderjeet Singh | A Bharadwaj | SS Paul | KP Singh | RK Sharma

ICAR-Central Institute for Research on Buffaloes

भाकृअनुप
ICAR

Training Program the for SAARC Countries on

Herd Health Management of Dairy Buffalo :

Nutrition, Breeding, Reproduction, Diseases, Management and Record Keeping

(August 22-27, 2016)

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Sponsored by :

SAARC

SAARC Agriculture Centre, Dhaka, Bangladesh

Organized by :

ICAR-Central Institute for Research on Buffaloes

Hisar-125 001 (Haryana) India

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ICAR

Published by
Inderjeet Singh,
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Compiled and edited by
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Correct citation:
Inderjeet Singh, A Bhardwaj, SS Paul, KP Singh and RK Sharma, (Eds.) 2016. Herd Health Management of Dairy Buffalo : Nutrition, Breeding, Reproduction, Diseases, Management and Record Keeping. Compendium of Training for SAARC Countries-2016 pp 1-171.

Printed by
Radhey Krishna Offset Press, Hisar (94162-64604)

सत्यमेव जयते

त्रिलोचन महापात्र, पीएच.डी.
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सचिव एवं महानिदेशक

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Message

It gives me immense pleasure to know that the ICAR-Central Institute for Research on Buffaloes is organizing a SAARC sponsored International Training programme on 'Herd Health Management of Dairy Buffalo : Nutrition, Breeding, Reproduction, Diseases, Management and Record Keeping' in Hisar on 22-27 August, 2016.

Animal husbandry is an integral part of agriculture that is closely linked to livelihood and food security of people in SAARC countries. Thus, concerted efforts in improved animal breeding, balanced feeding, proper healthcare and making available latest technologies to the farmers are warranted. Amongst cattle, buffalo play a pivotal role in food security of most of these countries. Buffalo meat export from India is about Rs. 26000 Crores annually. Eventually, improvement in buffalo productivity is basic to sustain overall agricultural development in the country.

I hope, this training will provide an interesting forum for researchers and farm managers from different SAARC countries to appraise and appreciate the current state of the art in buffalo herd health management and exchange scientific knowledge in animal science to cope with common and emerging problems faced by the SAARC nations.

I wish the training a great success.

Dated the 10th August, 2016
New Delhi

10/08/16

(T. MOHAPATRA)

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Message

I am delighted to hear that a training compendium/manual to be published for the SAARC regional training programme on "**Herd Health Management of Dairy Buffalo: Nutrition, Breeding, Reproduction, Diseases, Management and Record Keeping**" jointly organized by SAARC Agriculture Centre (SAC), Bangladesh and ICAR- Central Institute for Research on Buffaloes (CIRB), Hisar 125001, Haryana, India.

SAARC Agriculture Centre (SAC), under the framework of SAARC has been working for strengthening agricultural research & development as well as technology transfer through regional networks among agricultural research/extension institutions and policy makers in the SAARC member countries. ICAR- CIRB is one of the premier research institute in India undertaking research and developmental activities for the promotion of buffalo industry. The regional training on "**Herd Health Management of Dairy Buffalo: Nutrition, Breeding, Reproduction, Diseases, Management and Record Keeping**" would provide hands-on and theoretical knowledge to the participants on modern concept of buffalo farming that may expedite buffalo production & reproductive research and development activities in their respective countries. I believe the contents of the compendium/ manual is certainly the store of information related to recent research and development of modern buffalo farming. This book is unique and surely a work to treasure for anyone who is interested in pursuing research on buffalo production and reproduction.

I wish all the grand success for this regional training programme and its endeavours.

(Dr. S.M. Bokhtiar)
Director, SAARC Agriculture Centre

भाकृअनुप
ICAR

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Message

I am happy to know that ICAR-Institute for Research on Buffaloes, Hisar, Haryana, India is organizing a SAARC sponsored International Training programme on **"Herd Health Management of Dairy Buffalo : Nutrition, Breeding, Reproduction, Diseases, Management and Record Keeping"** during 22-27 August, 2016.

SAARC nations experience a huge gap in demand and supply of food of animal origin. Amongst domesticated animals, buffalo play a very important role in food security of most of these countries as it is embedded in meeting the energy, food and nutrition requirement of farm families. Hence, health management of buffalo is essential to continue deriving all benefits for farming community.

I am sure, this training will give an opportunity to each participant to learn and share knowledge on various aspects of herd health management of dairy buffaloes and will provide a platform to address challenges facing by SAARC countries.

I extend my best wishes for the success of this training.

Place : New Delhi
Dated : 09/08/2016

(H. Rahman)

Preface

Late 1970s proposal of President Ziaur Rahman of Bangladesh for regional cooperation in South Asian region ultimately lead to the formation of SAARC in 1985. Ever since its inception, SAARC - comprising Afghanistan, Bangladesh, Bhutan, India, Nepal, Maldives, Pakistan and Sri Lanka, started cooperation and further diversified it in varied fields including agriculture and rural development.

Thickly populated (1710 million), the association occupies 3% of the world's area in habitated by 21% of the world's population. The population in SAARC countries is predominantly dependent on agriculture - ranging from about 40% in Bangladesh and Pakistan to as high as 80% in Afghanistan and Bhutan. Contribution of agriculture in national GDP ranges from 14% (India) to 36% (Bhutan). Yet, the average land holding of farmers is no more than two hectares. Increasing cost of cultivation and sub-dividing land-holdings are forcing greater dependence of the farming families on livestock and fisheries, hitherto supplementary activities. Thus livestock are going to play a pivotal role in sustainability of livelihood for the rural masses in SAARC region, particularly in the mainland countries.

Increasing dependence of large population in SAARC countries on livestock requires gearing up the sector to meet the livelihood security. Fortunately, the region treasures huge livestock population and equally large biodiversity in domestic livestock species. However, the productivity is by and large low, except a few very valuable exceptions particularly the buffaloes. Yet, the productivity is not at par with well-established exotic cattle breeds. At the same time, the resource poor livestock owners can ill afford to properly manage highly productive livestock. Thus, a low input – low output system is in place. This needs to be upgraded with improved inputs through improved germplasm, cost-effective nutritional strategies and management practices. There is also a need for improved vigilance for preventing diseases in view of greater mobility and international trade.

The ICAR-Central Institute for Research on Buffaloes has established elite germplasm resource units of six important buffalo breeds viz. Murrah, Nili-Ravi, Jaffarabadi, Pandharpuri, Surti and Bhadawari in their respective home-tracts across India, coordinated through the Network Project on Buffalo Improvement since 1993. From these centres, including main campus of the institute at Hisar (Haryana) and Sub-campus at Nabha (Punjab), the institute research focuses on buffalo genetics, nutrition, physiology and reproduction. Several technologies have been developed for application in farmers' place viz. highly pedigreed bulls; progeny tested frozen semen; area specific mineral mixture; heat-stress alleviation protocol; fortification of poor quality roughages; Ovsynch Plus protocol for induction of estrus in anestrus buffaloes; ultrasonography for assessment of ovarian activity, pregnancy status with fetal age and sex; field application of embryo transfer technology and cloning; nutrient requirements of various categories of buffaloes, besides others. These technologies are transferred to the farmers across globe through 'Buffalopedia' – internet based information media, organizing trainings to farmers in villages, at institute campus and its Network centres and through awareness programs / agricultural fairs. Technologies relevant to field veterinarians are transferred to field vets through special hands-on trainings on ultrasonography, semen technology and reproductive biotechnology.

It is a matter of pride for the institute to host the SAARC sponsored training on 'Herd Health Management of Dairy Buffaloes' for SAARC member country experts. The deliberations in the program are going to be mutually beneficial to the participants as well as to the host faculty in view of the professional profile of the participants. My best wishes for a fruitful outcome that will help in substantiating the efforts of respective governments in improving the lot of the farming communities.

Inderjeet Singh
Director, ICAR-Central Institute for Research on Buffaloes

Contents

1 Role of ICAR-CIRB in buffalo farming production research

Inderjeet Singh
ICAR - Central Institute for Research on Buffaloes, Hisar- India

Buffalo holds great hope for food security and poverty alleviation in India, because of the largest population comprising diverse and the best buffalo germplasm of the world. Of the 216 million (FAO 2012) World buffalo population, 97% is located in Asia and 58% in India alone. India's buffalo germplasm treasures include the world renowned breeds viz. Murrah, Nili Ravi, Jaffarabadi, Mehsana, Banni and Surti besides seven other recognized breeds and several distinctive population groups dotting different regions across the country in the form of a necklace of 'Black Gold'. The contribution of buffalo milk to the total milk production in the world is about 13% and to the milk produced in India, it ranges from 51 to 55% during different years, making India the highest milk producer in the world since nearly one and a half decade. This has helped the country achieve the targeted daily per capita availability of milk for large human population, as recommended by the WHO. Majority contribution of buffalo to milk pool, makes this daily milk allowance even more nutritious for the masses. At the same time, annual increase ranging from 14 to 25% in export of buffalo meat in recent years has made India largest beef (buffalo meat) exporting country in the world. In 2014-15, India exported 1.476 million tonnes of buffalo meat worth US $ 4.78 billion, which also attained the distinction of being the largest exported agricultural commodity for the country.

India's milk production increased by almost 4.33 per cent between 2007 and 2012, or 3.5 million tonnes per annum but the consumption was increasing much faster @ 6 million tonnes p.a. due to increasing population, higher income of the masses, increased health consciousness and education. Per capita average monthly expenditure of Indian rural and urban household on milk and milk products stood at about ₹ 80 and ₹140, respectively, in 2009-10, which evidenced an increase of over 66% within five years. The increase is anticipated to be much faster during the coming years. In comparison, milk production in the country reached 146.31 million tonnes in 2014-15, with an increase of 6.2 per cent over last years' 137.7 million tonnes (2013-14). To keep pace with the increasing demand, milk production has to increase by over 36 per cent in the coming years to reach the targeted 200 million tonnes by 2021-22. Buffalo is geared-up to remain at the centre-stage in meeting this challenge.

The National Dairy Plan-I - initiated in 2012 with a funding of US$ 352 million from the International Development Association, besides Govt. of India and the National Dairy

Development Board with a total outlay of over Rs. 20 billion - is pivotal for enhanced livestock productivity through improved germplasm production and dissemination to the door-step of farmers, together with emphasis on balanced ration and fodder production. It also undertakes to improve milk procurement and processing capabilities at the grass-root level.

To address the growing improved germplasm need for cattle and buffaloes, breeding bull production is being implemented through progeny testing, pedigree selection as well as ONBS. The country presently has 44 'A' and 'B' graded accredited frozen semen stations, producing around 85 million frozen semen doses and targeted to produce around 140 million doses by 2021-22. The increasing awareness of farmers regarding benefits of artificial insemination together with improved infrastructure support are helping spread of AI in the field. The country has embarked upon an important disease control program, especially for Foot and Mouth Disease and Haemorrhagic Septicaemia. Official data from the department of Animal Husbandry, Dairying and Fisheries, Govt. of India, indicate that during the first nine months of the year 2014-15, vaccinations in livestock registered an increase of almost 40 per cent from 552 million in 2013-14, resulting in a simultaneous drop in outbreaks from 377 in 2013 to 238 in 2014 and much less (46) in the first six months of 2015. These programs are directed at meeting the livestock health and productivity targets for addressing the growing requirement of animal proteins for largely vegetarian Indian population.

ICAR- Central Institute for Research on Buffaloes

Realizing the importance of buffalo as a milch animal, with added advantage as a meat animal without the religious taboos attached to its slaughter and its inherent draft qualities especially for small farmers, the Government of India, through the Indian Council of Agricultural Research (ICAR), acquired the Progeny Testing Bull Farm from the Haryana State Government to establish the Central Institute for Research on Buffaloes (CIRB) at Hisar in February, 1985. A sub-campus was established in December 1987 near a small town of Nabha in Patiala District of Punjab, with the transfer of Nili-Ravi Buffalo Farm from the Punjab State Animal Husbandry Department. The Institute is mandated to promote and undertake research on all aspects of buffalo production with national and international collaboration for buffalo development and to establish nucleus breeding herds of important buffalo breeds of the country. It has approved cadre strength of 40 scientists in the production specializations, including genetics, breeding, nutrition, reproduction and management.

Soon after its establishment, the institute came to lime-light in the 'buffalo world' by successfully hosting the II World Buffalo Congress during 1988. Subsequently, it was the host to IV Asian Buffalo Congress in 2003 and an International Buffalo Conference in 2010. It has remained in contact with buffalo research and development fraternity across the globe through organization and participation in various national and international seminars, symposia, visits and as invited speakers at various such fora. In September 2013, the Institute obtained ISO 9001:2008 certification for 'Improved Buffalo Germplasm

Production'. More recently, Institute nutrition laboratory was certified by FAO-IAG for proficiency in feeding stuff evaluation.

Various farm management operations to manage the two herds – Murrah at Hisar campus and Nili-Ravi at Nabha campus are carried out through qualified technical and skilled support staff and mechanized automation of various farm operations. In addition to these two breeds, the institute is also addressing other buffalo breeds through its all India coordinated 'Network Project on Buffalo Improvement', starting in 1993. These breeds include Surti, Jaffarabadi, Bhadawari and Pandharpuri buffaloes in their respective breeding tracts located in different regions of the country. Herds with approximately 50 to 150 breedable females are maintained at these centres for production of superior germplasm with pedigree information and testing. In addition, a herd of Swamp buffaloes is maintained in the North-Eastern part of the country as a distinct germplasm resource. Progeny test evaluation of bulls is done through associated herds and field units to have a large population of daughters to be recorded for each bull put to test. In Murrah, each set of 15 bulls is used over a period of 18 months and currently sixteenth set is in use and first ten sets have already been analyzed for identification of proven sires. Upto December 2015, 203 Murrah bulls have been put to progeny testing in 15 sets and 26 top-ranking progeny tested bulls were identified for nominated mating with elite buffaloes to produce future breeding bulls. Percent superiority of proven bulls, on the basis of 305 or less day lactation milk yield of daughters, ranged from 3.53 to 24.89 per cent over their contemporary daughters in the first nine sets and per cent superiority over least square means was 1.23% for proven bulls in the tenth set.

The ICAR-Central Institute for Research on Buffaloes, Hisar is the only source of progeny tested Murrah buffalo bull semen. Current stock of frozen semen from progeny tested Murrah bulls is over 80,000 doses. Since 1992, a total of approximately 0.93 million frozen semen doses have been produced as part of the progeny testing program, out of these, nearly 0.23 million doses were used for progeny testing and over half (0.52 million) were sold to the farmers and other developmental agencies for breed improvement. Current stock of frozen semen from all breeds maintained at respective centres is almost 500,000 doses. Over 1000 breeding bulls of various breeds, predominantly Murrah, have been disseminated to various semen stations, state departments, developmental agencies, non-governmental organizations and farmers / breeders. With the field progeny testing program, institute has associated over 45,000 farmers from about 225 villages adjacent to respective centres. The conception rate in Murrah field units is ranging between 40 to 52 per cent during different recent years.

As the young and enthusiastic scientists joined its ranks, the institute embarked upon active research programmes to unravel the physiological, nutritional and genetic virtues and distinctiveness of this species through numerous research projects including outside funded projects and with international cooperation as well. The institute carries out research on

various aspects of buffalo improvement including development, conservation, evaluation and propagation of superior germplasm; development of optimum balanced diets for various categories of buffaloes, enhancing nutritive value of poor quality fodders alongwith strategies for reduction in methane emissions and formulation of economic feeding regimens; enhancement of reproductive efficiency and health management practices, besides application of reproductive biotechnologies, genomics, proteomics and other technologies for augmenting milk, meat and draught performance of the species.

One of the important areas of research is improvement of reproductive efficiency in the species, which is often blamed for poor fertility. The underlying hormonal profiles in fertile and infertile animals were revealed, the dynamics of ovarian structures of follicles and corpus luteum were monitored and their correlation with manifested behavioural changes was outlined. Based on these findings, critical stage progesterone supplementation lead to improved conception rates in normal and repeat-breeding buffaloes. Ovarian follicular dynamics in seasonal anestrus buffaloes and pubertal heifers paved the way for strategic Ovsynch Plus protocol so as to ensure the uniform presence of at least a large follicle at the time of first GnRH to respond with ovulation for higher fertility at fixed time AI after second GnRH. Altered management practices and application of supplements and hormones have been attempted for improved fertility with induction and synchronization of estrus. Non-invasive technique of ultrasonography has been used extensively in the study of genitalia, infertility, conceptus development and fetal sex determination. Recent forays into proteomics have helped identify the embryo-specific bio-molecules in maternal circulation and excretions, which holds the premise to the development of early pregnancy detection pen-side kit. In pregnancy, serum levels of MX2 and OAS1 increase significantly during 14-28 days post AI and MX2 protein was also detectable in urine. Research in polymorphism of important genes related to poor postpartum reproductive function lead to detection of polymorphism associated with post partum anestrus, which can be useful in developing molecular methods for identification of buffaloes prone to such infertile conditions.

Semen freezing activity, initiated in early 1990s, witnessed significant improvement from 35 per cent post-thaw motility during initial years to over 55 per cent currently. The peculiar issue of apparent backward motility, due to sharp bending of mid-piece of sperm, was tackled with standardization of glycerol level and stage of glycerolization, leading to reduced ejaculate rejection rates by almost 20 per cent, thus improving efficiency of frozen semen production. Sperm dose per insemination straw was also reduced from traditional 25 to 20 million, although 15 million has also been found suitable without any significant reduction in conception rates. Scrotal circumference, as an indicator of total testicular volume and sperm production capacity, was correlated with body weight and age in Murrah bulls to be considered as an important criterion in bull selection. The CASA values for buffalo semen were standardised and used in various experimentation for determining its application in prediction of bull fertility, which in turn was correlated with seminal plasma

biochemical profiles and genetic polymorphism. The use of fluorescent probes to evaluate integrity of plasma and acrosome membranes, as well as mitochondrial membrane potential and DNA status of sperm cells, increased the accuracy of semen analyses.

Reproductive biotechnology work on super-ovulation and embryo transfer remained constrained due to poor superovulatory response of the species as well as inadequacy of inherent mechanisms or techniques to harvest all ovulated oocytes through non-surgical uterine flushing. The ultrasound guided follicular ablation only partially improved superovulatory response but the ultimate transferable embryo recovery has remained as low as 1.5 to 2 embryos per flush. Nevertheless, the institute has so far produced 36 calves of high genetic merit using the embryo transfer technology. Trans-vaginal ultrasound guided ovum pickup technique was made use of in the study of genomic regulation of early follicular growth. Follicular development was retarded during summer months, resulting in lower recovery of oocytes per ovary from slaughter-house ovaries during the season as compared to favourable breeding season. Even during the later period, it remained as low as one oocyte per ovary, pointing to inherently low population of graffian follicles and their development in buffalo ovaries. However, high in vitro fertilization rates were consistently obtained and improvement in development of in vitro fertilized oocytes with co-cultures, additives and media was studied. The IVF technology was also applied in the study of fertility of bulls through homologous oocyte penetration assay and also with CASA assessment of sperm parameters.

Stem cells of fetal origin – placental, amniotic fluid and amnion – were characterised for stemness properties with molecular markers and their differentiation was attempted into different lineages as per specific niche provided. Stem cells as well as other somatic cells from high merit male and female buffaloes were used for production of clones with hand-made cloning technique. So far one cloned buffalo male calf 'Hisar Gaurav' was born on December 11, 2015 through application this technology. This calf is a clone of the superior Murrah bull no. 4354 of the Institute herd. In addition, three buffaloes are currently pregnant with clones of elite Murrah females.

With respect to nutrition of dairy animals that accounts for major expenses in the enterprise, research efforts aimed at reducing the cost of feeding through balanced diet as per need, incorporation of cheaper substitutes, fortification of roughages and improving digestibility while also making the diets environment friendly through methane mitigation. Nutrient requirements of various categories of buffaloes viz. growing males, females, lactating and pregnant females were determined. Also determined nutrients needed to ward-off heat and humidity stress. Economic feeding regimes utilizing locally available ingredients and crop residues for rearing surplus male buffaloes were formulated. Complete feed ration formulations have been developed using locally available agro-industrial byproducts, which resulted in 23-26 per cent higher dry matter intake with reduction in cost of feeding (12 per cent) in growing calves without affecting growth rate and nutrient

utilization. Lower transportation and storage costs add additional value to the technology. Various agro-industrial by-products, including spent-wash and filter press mud waste, were evaluated for replacing costly ingredients in the ration of different categories of buffaloes.

Control of rumen metabolism - aimed at better digestibility and nutrient utilization, included identification of effective defaunating agents to remove protozoa from rumen for improving the growth performance of calves. In this regard, Enterolobium and Neem leaves were tested. A protocol for encapsulation of superior fibre degrading anaerobic fungal isolates for delivery of their inoculums as feed additives was developed. Novel sulphate reducing bacteria (SRBs) were isolated from buffalo GI tract, which reduced methane emission by as high as 40 per cent in vitro. In vivo experiments are in progress. Potent methane mitigating mixture with little or no adverse effect on fibre digestion was developed. Protein sources containing limiting amino acid (LAA) were identified and successfully tested to increase buffalo milk production by 10 per cent. Cotton seed cake was found to be a source of less degradable protein leading to higher milk production (10 per cent) in buffaloes as well as growth (approx. 20 per cent) and semen quality in buffalo bulls. At the same time, experiments on feed safety included development and standardisation of methods for detoxification of anti-nutritional factors such as aflatoxins, gossypol, mowrin, tannins, terpenoids and neem bitter, besides development of multi-residue method for estimation of 29 pesticides as well as for simultaneous estimation of synthetic pesticides i.e. 3 neonicotinoids and 5 pyrethroids in animal feed and milk.

A popular contribution of the institute is the development of area specific mineral mixture based on the feed and fodder analysis and its beneficial impact on reproductive and productive performance has been demonstrated through initiation of cyclicity in almost 70 per cent anestrus buffaloes within 4-5 weeks of supplementation. Based on mineral status of the region and Nili-Ravi buffaloes, another area specific mineral mixture formulation was made for Patiala district. Similarly, in tribal areas of Rajasthan state, mapping of soil, vegetation and livestock sera samples revealed deficiencies of several minerals, for which appropriate supplementation was developed and made available to the farmers. Specific supplementation of Vitamin E and Selenium during late gestation and early lactation significantly reduced somatic cell count (SCC) in milk, indicative of improved udder health. Ultrasonographic observations on buffalo mammary gland and teats indicated almost three times longer teat canal of buffalo in comparison to cattle, which endows the species with greater protection against mastitis.

On the socio-economic aspects, the institute has increased its penetration into the rural masses for adopting scientific buffalo husbandry practices related to breeding, nutrition, reproduction and management, for enhanced productivity and profitability. Buffalo farming is becoming vital for sustainability of small-holder farmers with squeezing land-holdings and increasing cost of cultivation of major crops. The dairy cooperatives have played a pivotal role in making dairy farming a livelihood enterprise for large number of farmers. The

role of institute is being recognized as the centre of excellence for elite germplasm production, testing and dissemination across the country. Recognizing the contribution of farm women in animal husbandry activities, special trainings and awareness camps are arranged for them in their own or adjacent villages. Calf rallies, infertility treatment camps, health camps, breed championships and milking competitions are other fora which provide opportunity to our scientists to interact with farmers and emphasize the need for adopting modern farm management practices. Demonstrations of novel technologies are also arranged frequently. Proper use of mass media viz. newspapers, television and radio is done in accessing the stakeholders. More recently, initiatives using information and communication technology for spreading across the region, country and globe are put in place and include 'Buffalopedia' – an internet based buffalo information centre; 'e-Bhains Gyan Kendra' (e-buffalo knowledge centre) – portal for making farmer friendly videos, documentaries, films etc.; toll-free telephony; WhatsApp groups to instantly disseminate tips and information to users as well as a Facebook group in the name of 'Central Institute for Research on Buffaloes'.

The institute became birth-place of 'Murrah Buffalo Breeders Welfare Association', on 21st June, 2013, which aims at coordination and collaboration in Murrah buffalo development activities through public-private partnership. Scientists of the Institute are also entrusted with diverse responsibilities to manage the affairs of 'Indian Society for Buffalo Development', which periodically organizes scientific and developmental activities across the country. Interactions with field functionaries/veterinarians through field visits and organizing special trainings for them in areas like ultrasonography, allows two-way interactions for research and development of the species. Expertise of the institute scientists is shared at national and international fora through trainings, workshops, conferences, symposia and other similar events.

The Central Institute for Research on Buffaloes is relentlessly striving to take the 'black gold' to its pristine glory. It looks forward to greater collaboration with national and international developmental agencies in the areas of buffalo research and development for making this virtuous species 'Dairy Animal of the 21st Century'.

2 Performance recording in the field for enhancing the productivity of dairy buffaloes

Kamlesh Trivedi
National Dairy Development Board, Anand, Gujarat, India

Performance recording (PR) can be defined as a framework that collects data at the individual animal and herd level, transmits, processes and stores the data centrally, and generates relevant information for traits of economic importance related to growth, reproduction, production, health, inputs used, product quality, longitivity, adaptability, etc. primarily to enable farmers to make management decisions leading to increase in profitability of their farm operations, and secondarily to aid breeding companies to evaluate sires and cows for improving quality of genetics they supply, service providers to improve efficiency of services they extend, and governments to have sector level data for planning and investment decisions they make. The central to any Performance Recording System (PRS) are the animal and the herd helping producers to improve productivity and health of their animals, enhance quality of their produce, and increase profit of their farm operations.

The paper provides a conceptual framework for developing a PRS and offers guideline for implementing it for dairy buffaloes under low-medium input production systems in developing countries. Identifying uniquely an individual animal by appropriate identification devices and developing an Information and Telecommunication (IT) infrastructure are prerequisites to developing any PRS. The aim of this paper is to help decision makers decide on an appropriate PRS for a given situation and implement it successfully achieving the goals set for the PRS.

Conceptual frame work of PRS

For developing a PRS one has to define objectives and scope of the PRS, identify stakeholders and their information needs, identify data sources and persons to collect data, define data collection procedures, and define IT infrastructure.

Objectives and scope

The main objective of developing a PRS is to make available information to producers for decision making to improve productivity and health of their animals, enhance quality of their produce, and increase overall profit of their farm operations. Other objectives may include:

- Make the use of data collected through PRS for genetic evaluation of bulls and cows to achieve faster genetic progress in the target population;

- Make the use of data for identifying constraints and developing strategies in improving productivity, quality of produce and overall profit of farm operations;
- Develop a national level database on productivity and health of animals;
- Document benefits of PRS;
- Achieve maximum participation of farmers, service providing organizations, organisations implementing herd health programmes, private veterinarians, farm consultants,etc. in data collection and encouraging them to use the information produced through PRS;
- Make the use of data for research and capacity building.

Defining scope of PRS

As information needs of producers vary depending on traits of interest, it is important to define first the scope of PRS in terms of traits. Traits of economic importance for dairy buffaloes could be grouped into five broad groups: growth, fertility, production, product quality, body conformation and health. The International Committee for Animal Recording (ICAR) has been setting rules, standards and guidelines for measurement of different traits in various species (www.icar.org). The latest document of ICAR on recording practices published in 2012 provides elaborate guidelines on measurement of various traits in different species. The ICAR guidelines could be used as a base document for selecting traits of interest and developing procedures for measurement of those selected traits for buffaloes. Table 1 provides a list of measurable traits for dairy buffaloes.

Table 1 : List of measurable traits for dairy buffaloes

Growth	Fertility	Production	Quality	Health	Conformation
• Birth weight • Body weight at 3 months, 6 months • Body gain in first 6 months • Age and weight at first calving • Weight at calving	• CR on non-return basis • CR on first service • CR on all services • Services per conception • Service period • Dry period • Inter calving period • Calving ease	• First lactation yield • Yield lactation wise • Life time yield • Yield per day • Milking speed • Length of productive life	• Fat% • Protein% • Lactose% • Milk Urea nitrogen • SCC	• Fat% • Protein% • Lactose% • Milk Urea nitrogen • SCC	• Udder health: - Mastitis - SCC - Udder conformation traits • Reproductive disorders: - Retained placenta - Metritis - Cystic ovary • Digestive & metabolic disorders - Milk Fever - Ketosis - Digestive - ruminal, abomasal and intestine disorders • Leg and Feet disorders • Recording of infectious and parasitic diseases • Disease resistance • Adaptation to stress factors

Identify stakeholders and their information needs

Once the scope of the PRS is defined, a detailed exercise should be done to identify the stakeholders, who are willing to participate in the PRS. Of course, the primarily stakeholders are producers in any PRS. But apart from producers, there would be many who would benefit from the PRS. It would be important to identify all potential stakeholders and have dialogue with them for their interest in the PRS. Potential stakeholder for a PRS for dairy buffaloes for example could be: Producers, AI Service providers, breeding companies, milk component laboratories, breed associations, feed and fodder Seed suppliers, feed testing laboratories, provincial health authorities, disease testing laboratories, private veterinarians, farm consultants, milk processors, policy makers, etc.

After identifying stakeholders for the PRS, the next step will be to identify their information needs. Workshops inviting the identified stakeholders can be organised to list down specific information needs of each stakeholder along with frequency and specific content of each of their information needs. As an illustration, probable information needs of dairy buffalo producers could be as listed below:

1. Pedigree details of their animals
2. Animals that are: due for pregnancy diagnosis, to be dried off, due for calving, to be served after calving, inseminated more than three times, having sub-clinical mastitis, etc.
3. Lactation performance of their animals with respect to total milk yield, fat%, fat yield, protein %, protein yield, lactose %, etc.
4. Reproductive performance of their animals in terms of number of services per conception, calving ease, service period, dry period, inter calving period, etc.
5. Body conformation scores of their animals
6. Performance of their animals compared to average performance of animals in the area - village, district, state, etc. - on key performance parameters
7. Bulls to be used for breeding their animals
8. Quantity of different feeds, feed supplements, forages to be mixed to feed to their animals to exploit their full genetic potential
9. Due date for vaccinations against specific diseases
10. Status on vaccination of their animals
11. Due date for de-worming
12. Status on de-worming of their animals
13. Animals having sub-clinical mastitis
14. Animals having foot problems
15. Animals having chronic disease problems - TB, JD, brucellosis, milk fever, ketosis, etc.

Identify data sources and persons to collect data

In any PRS, the major sources of data could be producers, but who collect data depend on herd size and education levels of producers. If animal holdings are large and producers are

educated, producers may themselves collect and supply data on paper or electronically. Often smallholders educated or not, may not participate in data collection, as they may not see any benefit in collecting data. This may also be true in cases of large herds with farmers not having adequate education or resources. Most of developing countries fall under latter two categories. In such situations, the responsibility of collecting and supplying data from producers on paper or electronically will have to be entrusted to service providers.

Defining data collection procedures

Once the traits to be measured and persons who collect data have been decided, the next step is to decide on both the data elements for each trait and the detailed procedures for measurement of each element. For example, for artificial insemination for the trait conception rate, a detailed standard operating procedure for carrying out AI is to be spelled out and the data elements that need to be recorded after each AI is to be specified. The data to be recorded for AI could be: identification number of female animal, date and time of insemination, and details of the bull whose semen dose is used. The ICAR Guidelines referred earlier (www.ICAR.org) may be used in writing down detailed procedures for measurement of data elements of each trait selected.

Defining IT infrastructure

Defining IT infrastructure means specifying : central infrastructure to store data and provide web-based services, and distributed infrastructure for capturing data and receiving information.

A central infrastructure needs to be set up to store data and provide web-based services. The main resources required for a central infrastructure include: a database server to host data; a web server to host web-based applications; a data warehouse server to produce specific reports and have dashboards, and high speed connectivity. It should also have data backup and recovery services and should be well secured.

As regard capturing data there are two options: One, collecting data on paper and entering data through desktops/laptops, andSecond, entering data directly on smart phones by field workers and synchronizing local database periodically with the central database. In the first option, field workers collect data on specified formats and dispatch them to a nearby workstation, where data entry operators enter data using a web-based application and transfer data to a central server. In the second option, field workers are provided with smartphones. The smartphone would have a client application to enter and validate data and update the local database embedded in the smartphone, and a synchronization middleware to interact with the synchronization component of web server via wireless connectivity (GSM/ GPRS/ CDMA).

A suitable software application need to be developed then that provides facilities to capture and validate data through different platforms such as smart phones, net books, tablets, laptops, desktops, etc. It should also generate and transmit the required information

to various users – producers, service providing organisations - all personnel from the lowest level in an organisation say field technicians to the top level in the organisation say policy makers – through various ways such as print output, email, PDF files, HTML pages, smartphones, mobiles, etc. in various forms such as alert messages, operational reports, review reports, graphs, analytical reports, statistical summary reports, etc.

Preparing a strategic plan for PRS

For preparing a strategic plan for any PRS, it would be necessary to access the current situation with a purpose to define objectives and a scope of PRS, identify stakeholders and their information needs, identify data sources and the persons who collect data, definedata collection procedures, specifying IT infrastructure, etc. Incorporating, in the beginning itself, all traits may not work out feasible not only due to constraints of time and financial resources, but also due to its manageability. Hence, it would be necessary to establish priorities so that those areas most critical to the success of establishing PRS are undertaken and completed first and others are included later in a phased manner.

Implementation of PRS

Having prepared a strategic plan, an implementation strategy would need to be prepared. No PRS can be initiated without having an IT infrastructure and a suitable application to capture and process data and generate information for the stakeholders.The following points may be considered while implementing the PRS:

1. Initiate the activities identified in a small area first and test all functionalities of the application. Having satisfied with the functioning of the application, organise information campaign and encourage more producers to join the network.
2. Select a service provider in the selected area and sign an agreement with them.
3. Supply training manuals and organise training programmes for field workers including training in software usage.
4. Work with the service providers and assist them to get reqsuired equipment and consumables - ear tags, applicators, handheld devices, netbooks, laptops, desktops, measure jars, measuring taps, etc.
5. Monitor the field operations: ear tagging, registering of herds and farmers,capturing of data, use of information, supervisory systems, etc.
6. Take corrective actions whenever and wherever required.
7. Expand activities in more geographical areas
8. Prepare short movies, pamphlets, posters on PRS and deploy them effectively to encourage producers to join the network.
9. Organise workshops for producers on the PRS.
10. Give TV interviews and contribute through writing on the PRS in press media and technical journals.
11. Prepare documents on SOP (Standard Operating Procedures) for measurement of different traits as well as on interpreting computerised outputs sent by the application

for different target groups such as producers, field workers of service providers, supervisors and managers of service providers, policy makers, etc.

12. Organise training programmes for producers and personnel of service providers.

An example of integrated PRS

National Dairy Development Board of India has developed an integrated IT System referred to as "Information Network for Animal Productivity and Health (INAPH). INAPH covers all areas of productivity enhancement including animal registration and identification, traceability, artificial insemination, milk recording, milk component analysis, body typing, genetic evaluation, ration balancing, health (treatment, diagnosis, testing, vaccination and outbreaks), and advisory services. The idea is to bring together all service providing organizations in the country and develop an industry level database on productivity of animals.

INAPH is based on field force automation using mobile technology (GSM or CDMA). The field force is provided with handheld devices (Smartphones/ Netbooks/ laptops) to record activities in real time with proper validation and to generate information for monitoring and control of their daily activities at the village level. Field level workers synchronize their data with the INAPH central server at Anand in the state of Gujarat using GPRS/CDMA services. The Web-based version of the network is available on the desktop/laptop for entering data and generating the required information. The desktop version is developed using .net framework. A DBMS Microsoft SQL server is set up at Anand to host data. A separate web server is maintained to host all web-based applications. The Microsoft ASP .net framework is used in developing the applications.

Different components of the network that are operated independently include: (i) Admin Application; (ii) PDA Application; (iii) Desktop Main application; (iv) Laboratory Application; (v) Analytical Reporting Application; (vi) SMS Application; (vii) INAPH MIS Tools, and (viii) Database Synchronization Middleware. The INAPH application produces a variety of reports for each service. The various reports have been developed considering the requirement of different users; the reports generated by INAPH could be grouped into seven classes: Operational Reports, Performance Review Reports, Analytical Reports, Graphs, Alerts, SMS Messages, and Data Extraction Tools. The system also has facility to send SMS messages to farmers on their cell phones to alert them on what is due on animal(s) they have (For details visit: www.NDDB.coop)

As on 30th April, 2016, INAPH had records of 5.88 million registered animals, 3.15 million registered farmers, 4.80 million AIs, 0.97 million calving, 1.40 million test day records, 0.12 million vaccinations, 0.55 million treatment cases, and 7.6 million ration balancing transactions. These records were spread over about 39,000 villages in 254 districts and there were about 30,123 users of INAPH.

3 Molecular and genomic selection tools for superior cattle and buffaloes

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Enhancing genetic gains by reducing generation interval has been the basis of selection programmes for overall genetic improvement and higher productivity in dairy animals. Need of the day is to infuse more precision and accuracy in breeding plans through genome based technologies. The study of enzymatic polymorphisms to investigate the genetic variation of animal populations as of blood groups and mutants of color as the unique genes of known inheritance through electrophoresis opened new ways, in 1960s (Neimann-Sorensen and Robertson, 1961). These studies revealed an increasing genetic variability of genes independent of their phenotypic variability. However, only a handful of genetic variants were detected due to the limitations of the technique.

The QTL explosion and deception since 1990s: Analysing genomic DNA information through sequencing. Taking advantage of high pic markers, microsatellites, spread throughout the genome, researchers were able to build genetic maps of domestic species to search genomic regions harbouring genes which affect the performance for economically important traits. Linkage disequilibrium between markers and QTL generated by crossing highly divergent breeds for the traits of interest, or within-family linkage disequilibrium, due to available large half-sib families in some species [http://www.animalgenome. org/QTLdb] where, the number of reported QTLs are 9862, affecting 653 traits as in pigs; 8305, affecting 467 traits in cattle; 3919, for 297 traits in chicken and 789, for 219 traits in sheep.

Gene Detection

One QTL in a chromosome as a region of about 20–40 cM (probably harbouring 200–400 genes) made it difficult to identify the underlying genes responsible. It necessitated either to increase then number of individuals to carry- out fine mapping or to try the 'candidate gene approach' to refine their position. Latter approaches are difficult, expensive in terms of time, money and no guaranteed success, proving gene location a formidable task. Lessons are learnt by three successful stories :DGAT1and ABCG2 that affect milk composition in cattle and IGF2 and MSTN influencing muscle mass in pigs and sheep, respectively, (Georges, 2007). Not with standing, the difficulties for finding the causal mutation scan be illustrated. The first QTL reported in livestock was FAT1 located in swine chromosome 4 (Andersson *et al.,* 1994); however, its causal mutation is still unknown. Also, not more than a dozen of causative mutations are firmly established out of 9000 QTLs reported in pigs.

Marker-assisted selection for early evaluation of germplasm

Identification of markers linked to genes with large or medium effects in animals, allowing the possibility of assisting early selection programs has been the race over several species.

One of the main motivations for QTL detection in domestic animals is Marker Assisted Selection (MAS) which included a three-step process first, detect one or several QTLs., second, find the gene responsible (causal mutation) and third, increase the frequency of the favourable allele either by selection or by introgression. There are some examples as the halothane gene in pigs or the Booroola gene in sheep. This strategy should better be called Gene Assisted Selection. Another approach is to use markers that are in linkage disequilibrium or linkage equilibrium with QTLs (Dekkers, 2004). If the phenotype and the true QTL s for a trait were known, the advantage of QTL-selection response with respect to phenotypic selection would be 1/h, where h is the square root of the heritability. Thus for heritability's of 0.10, 0.25 and 0.50 the advantage would be huge : 316%, 200% and 140%, respectively. If markers explain just p percent of the additive variance the advantage would simply be p/h. They also developed selection in dices that combined individual and family phenotypic in formation and molecular scores. Linkage disequilibrium among markers and QTLs is the key factor for the success of MAS, therefore, considering across populations more appropriate approach. Genetic gains are generally calculated as:

G = Intensity of selection X Accuracy of selection X Genetic standard deviation / Generation interval

Information on genetic markers affect only the accuracy of selection. Breeding schemes running under progeny testing are running with high selection accuracy. Hence, MAS will specifically be useful for traits with low conventional selection accuracy as:

- Traits with low heritability
- Traits with a few recordings
- Traits measured late in life
- Slaughter quality traits
- Disease resistance traits

Despite of high selection accuracy, progeny testing schemes have disadvantage of high generation interval which reduces genetic gains. Use of marker information at early age can reduce this interval by making statistically significant QTLs based selection. It skips several small genes to be covered which do have polygenic effect on selected QTLs but remain unidentified and un-measured. Therefore, total genetic variance of the function which is constituted by polygenes is not measured completely. MAS require the trait records, hence awaiting trait records with complete generation is only option . Hence credibility of MAS lies in prediction of total or nearly total genetic valueincluding all large and small genes associated with ratio. Genetic gains are expected to be higher for early generations, where

time period for selection programme is shorter (as positive QTL alleles are fixed over the time period) and for the traits which are recordable earlier. MAS has been useful where QTL detection remains difficult as low heritability traits with low pedigree index.

Genomic selection Meuwissen *et al.*, (2001) : Fact associated with QTL mapping that a limited fraction of genetic variation can be explained by QTL due to stringency of statistical testing for its presence to avoid false positive, where genomic selection estimates the effects of all genes or chromosomal positions simultaneously thus making direct selection. SNPs emerged as simple and inexpensive promising markers, since they did not need the presence of genes of large or medium effect as in case of QTLs due to the scanning of the whole genome. Use of information of a large amount of SNPs permits having a prediction of the breeding value at birth which is accurate enough to be used in dairy cattle. It reduces generation interval. Genomic Selection (G S) typically consists of two steps:

(i) Estimation of the marker effects in a dataset with marker genotyped and phenotyped individuals (called the training set)

(ii) Using estimated marker effects to predict the breeding value of individuals that are not phenotyped (called the test set)

In GS, since the number of individuals (*n)* genotyped is lesser than the number of markers/SNPs (*p) (problem of low degree of freedom),* Thus,

1. The multiple linear regression models have failed due to the problem of over fitting
2. Selection operator, best linear unbiased predictor (Fit allelic effect as random effects, no degree of freedom, all genes under total variance), but difference due to small and large effect genesremained un-detected.
3. Statistical techniques such as parametric (Bayesian estimations) least absolute shrinkage
4. Bayesian ridge regression, ridge regression,
5. Elastic net and non-parametric (support vector machine, neural network) & Semi-parametric (reproducing kernel Hilbert space) have been used for the estimation of SNP effects and genomic prediction (Goddard et al. 2009; Heslot et al. 2012).

Based on many available markers at an affordable cost genomic selection has been conceptualized on two assumptions:

1. panels with tens of thousands of markers will be available together with cost-effective genotyping procedures [sequencing or subsequent re-sequencing for SNPs]
2. Marker-density will be sufficient for all responsible genes of a trait to be in linkage disequilibrium with flanking markers.

Today, commercially available 'SNP chips' comprising of 750,000 SNPs for cattle and

90,000 SNPs for buffalo are available in comparison with 250,000SNPs for dogs, 56,000SNPs for sheep, 60,000SNPsfor pigs, 55,000SNPs for horses and chickens (600,000SNPs), far from latest human SNP chips covering over 3000,000 SNPs.

Estimation of the effects of markers (450,000SNPs) in a reference (training) population that has been phenotyped and genotyped and prediction of breeding value of candidates in test population under evaluation, which is genotyped for markers ; is **Genomic selection**

The predictive ability using the whole genome (2,5 million SNPs) was the same as using 150,000 SNPs (Ober et al. 2012) as showed in Drosophila. In dairy cattle, Van Raden et al. (2011) obtained a gain in reliability of only 1.6% when using 500,000 markers instead of 50,000, and using imputation techniques even low- density marker panels (3,000 SNPs) can produce a similar predictive ability in dairy cattle (Berry and Kearney, 2011).

Advantage in using the whole sequence:

1. Inclusion of all QTL alleviating deterioration of linkage disequili- brium along generations [Meuwissen and Goddard, 2010]
2. Precised evaluation of multi-breed crosses by evaluating causal mutations, expected to be originated in a breed, but not in other breed when genomes sequences are studied at greater depth (Pérez-Enciso, 2014).
3. Another advantage of using the whole sequence is avoiding the ascertainment bias originated by marker pre-selection. Markers are preselected with the aim of segregating, which produces an overestimation of variability, affecting the estimated relationship between individuals. When the whole sequence is used, this problem vanishes.

Thus, using genomic selection for predicting the breeding values, selection of candidates at birth is possible with a higher accuracy than the classical pedigree index. Consequently, selection of animals at an early age, expectedly doubling the rate of genetic improvement per year.

Difficulties in implementing GS:

1. Today there is a need of large training population to relate SNPs with phenotypic information.
2. Large training populations are required to obtain acceptable accuracies for breeding values.
3. Training populations can be composed of several thousand animals in dairy cattle (Wensch-Dorendorf *et al.*, 2011),e.g. effective population number for reproductive traits may be very small because low heritability traits require larger training populations.
4. Lacking information on minimum number of animals in a training population in cattle/buffaloes.

5. The need of continuous phenotyping

GS is used in traits that are expensive or difficult to measure, for example meat quality traits. Selection produces LD between the markers and the trait affecting QTLs. GS is based on using these associations to avoid measuring the expensive traits. However, some meat quality traits are scarcely related to traits that are selected. Moreover, estimated LD is lost generation after generation and new training populations are required (Sonesson and Meuwissen 2009; Ibañez and Blasco 2011).Therefore, requirement of continuous phenotyping of large training populations makes GS a less attractive proposal for traits that are expensive to be measured.

6. Problems for genetic evaluation

Genetic evaluation in commercial programs is nowadays widely based in BLUP, ensuring unbiased estimates if the full relationship matrix and all data used in selection are included in the evaluation. Thus -

- Preselecting bulls in dairy cattle using genomic information can lead to biased predictions with lower accuracy (Patry and Ducrocq, 2011), leading to a decrease in genetic progress and distorting international dairy bulls comparisons (Patry et al., 2013)
- Integrating genomic and phenotypic information for predicting breeding values in a single step has been proposed by Legarra et al. (2009), but the computing cost is much higher and requires specific strategies for solving the equations (Legarra and Ducrocq, 2012). Including non-additive effects in the model or nonlinear traits as longevity which produces further complications.

Probable favourable frames for genomic selection

Optimization of genetic parameters to study the model for generating the data and its analysis are the same. For example, often an additive model generates the data and an additive model analyses the results. Generating data with non-additive genetic effects, common environment not considered, interactions genotype_ environment etc., and analyse results with the usual additive model.

Non additive interactions in- breeding, the coefficient of dominance cannot be estimated with biallelic markers such as SNPscan be difficult to analyse (García-Cortésetal, 2014). Also, if epistatic effects are large, then the accuracy of genomic breeding values may never reach 0.75 (Schaeffer, 2006).

Implanting GS in current breeding schemes

a. Cost of Genotyping

Cost of genotyping has been dramatically reduced in the last few years, allowing the implantation of genomic selection in dairy cattle in many countries at a reasonable cost by

using 45,000 SNPs in bulls and low- density 3000 SNPs chips for genotyping cows, heifers, and calves on commercial dairy farms for less than $50 per animal (Van Eenennaam et al., 2014).

Standard solutions for buffaloes are far to be clearly established and research is still needed on implementation of commercially available chips as 90000 SNP chip and or, developing SNP panels for Indian dairy buffalo.

The need is of large buffalo training populations that should be constituted breed-wise. Network project on buffalo genetic improvement has been running for more than two decades (since 1993) conserving seven (Murrah, Nili-ravi, Badhawari, Surti, Jaffrabadi, Pabdharpuri and swamp) out of 13 registered buffalo breeds in respective breeding tracts and different climatic zones, nation-wide. Herds of 50 to 150 breedable females are maintained at each of these centres. Genetic improvement through progeny testing for bull evaluation is done in associated herds of Murrah, Surti,Jaffrabadiand Pandharpuribreeds through with performance and pedigree records and Field progeny testing units (Murrah, Pandharpuri and Jaffrabadi breeds) to have large population of daughters for each selected bull under testing. For Murrah, each set of 15 bulls is used for period of 18 months. Sixteenth set is currently in use. Data of 10 sets of bull evaluation is available for selection of proven sires.

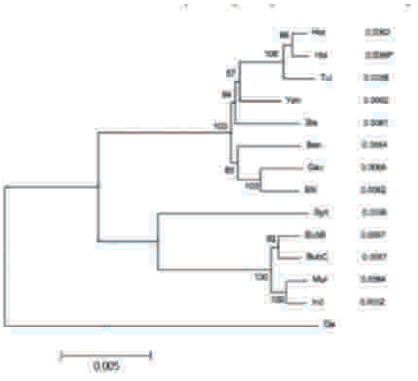

Fig. 1. Phylogeny of the Bovini tribe using neighbor joining analysis (As : the ancient Ban : Banteng, Bis: Bison: BubB & BubC: Asiam buffalo, water and swamp type, Ela: Eland Gau: Gaur, Her : Hereford, Ind & Mur: Indian water buffalo, Mit Mithan, Hol : Holstein, Syn : African buffalo, Tul: Tuli, and Yak) [Mac Eachern *et al* 2009]

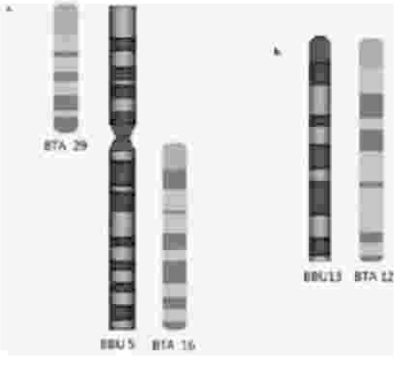

Fig. 2. At the cytogenetic level, water buffalo chromosomes can be matched to bovine chromosomes arm for arm. Each biarmed water buffalo chromosome is derived from the fusion of two bovine acrocentrics (a) shows similar banding patterns for bovine chromosomes 29 and 16 to water buffalo chromosome 5; (b) shows similar banding patterns for bovine chromosome 12 and water buffalo chromosome 13 [Michelizzi *et. al.* 2010]

Need is to test 90000 SNP chip for buffalo genotyping to make it usable for animal selection programme. The implementation of genomic selection is therefore, less clear and the way in which it can be useful under existing breeding plans needs to be carefully studied considering:

- The need for large populations for associating phenotypic data and markers
- The need for repeating the process continuously, overcoming all complicationsin its application

- Dependence on complex statistical tools to implement information provided by the SNPs in Currentgenetic programs
- Lacking efforts in joining groups working on biological, factorial and biometrical aspects

Genome-wide association studies (GWAS): A new impetus to the gene detection area

Although in some sense the genomic selection is related with the GWAS, there is a difference in the focus. In GWAS the aim is to decipher the genetic base of quantitative traits whereas in genomic selection the objective is to predict the genetic values of candidates to selection to choose the parents of the next generation. The GWAS strategies are now being implemented in livestock species with very limited success in traits controlled by one or few genes as MITF causing white spots in dogs or the SLC65 and ABCA12 that cause congenital muscular dystocia in cattle. Excessive expectations of GWAS arise due to lack of major genes determining most of the traits of interest; it seems that most traits are determined by many genes of small effects and large effect genes are usually fixed in selected populations. Misinterpretation of the amount of evidence provided by statistical tests is another limitation (Johnson, 2013), showing that in order to obtain an evidence of 95% of probability, the P-value needed is about ($p< 0.005$). Many SNPs would disappear, if multiple test techniques are applied for individual. Balance between LD of QTLs and probability of gene frequencies is important to estimate accurate genetic variance w.r.t. specific locus or group of genes.

By 2050 the need to feed 2 more billion people will require 50–70% more food production, and there will be a significant increase in demand for animal sourced foods. Limitations in land and water and climate change issues will challenge livestock producers worldwide. The biological sciences in the 21st century already have been transformed by genomics and their applications to the fields of medicine and agriculture. Employing genomic solutions to increase livestock production efficiency in the developing world to meet these demands will be required. Delivery of many of the genomic solutions in enhancing production employing improved breeding programs involving improved livestock, areyet to be developed with institutional capacities development.

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4 Selection and genetic improvement of dairy buffaloes

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The basis of increase buffalo productivity is the "only one way to select and to keep the best and cull the poorest". This is a two-stage operation in which the superior individuals are first identified / selected and then used as parents / breeding stock for future generations. Genetic progress improvement in buffalo productivity is largely determined by the genetic merit of bulls used as sires, so selection of young dairy bulls is an important step in any breeding programme. The merit of these sires is impacted by the pedigree records (merit of parents), herd size, accuracy and intensity of selection and maximum use of the best animals.From a genetic standpoint, the main purpose of selection is to change the mean value of a given population by increasing the frequency of desirable genes and genotypes. Therefore, selection of bulls / dairy animals is based upon individual performance, pedigree records and progeny performance as discussed below:

1. Selection based on breed characters and own performance
2. Selection based on pedigree records
3. Selection based on progeny performance
4. Show ring selection

Before considering the above basis of selection, some important points are considered for general selection of dairy animals (male and female) as:

i. Birth weight (should be above breed average)
ii. Animal should be free from any genetic abnormalities and
iii. Growth rate (growth rate should be optimum @ 500 to 600 gm /day in buffalo)

1. Selection based on breed characters and own performance

Selection based on individual own performance is a measure of the phenotypic value of the individual for selection. Since the phenotypic value is determined by both genetic and environmental influences, the performance test is an estimate, not a measure of the genetic value. The occurrence of this estimate depends upon the heritability of the trait. The other points considered for selection are:

a. General confirmation: The buffalo / animal should be in conformation to its breed characteristics.

b. Health Status: Animal should be free from all contagious diseases like- JD, TB, Brucellosis, IBR and Free from genetic abnormalities

c. Animal gait should be normal. The hooves should be strong and firm. There should not be any knuckling of the hind quarter, bad hooves and pasterns should be strong.

d. Reproductive organs should be check for any abnormalities.

e. Based on the production performance, body weight and body confirmation traits like body structure, udder conformation, placement of teats, prominent milk veins, thin neck region, wedge shape body, soft and stretchable (loose skin) are important traits considered for the selection of dairy animals.

Advantages

- Simple procedures, the performance test are the most accurate.
- Environmental influences can be minimised by testing candidates for selection in similar environmental conditions.
- The measure is direct, not on a relative basis.
- Generation intervals are usually short.
- Testing can usually be done under normal management conditions.

Disadvantages

- Accuracy becomes low when heritability is low.
- Phenotypes are not available for one sex for the sex limited traits such as milk yield.
- Traits which are not expressed until maturity may become expensive or difficult to manage by performance tests since most selection decisions must be made before maturity.
- Performance tests should be the backbone of most selection programmes. Although much publicity has been given to other selection methods, it remains a fact that most of the progress in livestock improvement to date has been due to selection on the individual's own phenotype i.e. performance test.

2. Selection based on pedigree records

A pedigree is a record of an individual's ancestors including its parents. This information is valuable because each individual possesses a sample half of the genes from each parent. If we can precisely know an individual's phenotype, little is gained by considering pedigree in selection. Pedigree considerations are useful when we do not have sufficient accurate records of production of the individual. Also, it is useful in the early selection when the traits in question might not have expressed themselves. It is also useful for selection of males when the traits selected for are expressed only by the female (sex limited traits) such as milk.

Advantages

- It provides information when own performance data are not available for the buffalo.
- It provides information to supplement performance data information.

- It allows selection to be completed at an early age. Pedigree records may be used to select animals for performance or progeny testing in multi-stage selection scheme.
- It allows selection of bulls can be selected on the milk records of their female relatives.

Disadvantages

- Accuracy is usually low
- Too much emphasis on relatives, especially remote relatives, greatly reduces genetic progress and improvement
- Progeny of favoured parents are often environmentally favoured.
- Relatives often make records under quite different environments, thus introducing non random bases into the selection system.

3. Selection based on progeny performance

Under this method we evaluate the breeding value by a study of the expression of the trait in its offspring's. Individuality tells us what an animal seems to be, his pedigree tells us what he have / must be, but the performance of his progeny tells us what he is.

Progeny testing is, of course, a two-stage selection system because some preliminary selection determines which animals first produce progeny followed by further culling of these which produce poor progeny.

Advantages of progeny testing

a. High accuracy when evaluated based on large number of progeny
b. When traits are sex-limited

Disadvantages of progeny testing

a. Long generation interval
b. Requires high reproductive rate
c. Low selection intensity

4. Show ring selection

Selection on the basis of show ring performance occupied considerable value in the past. Essentially this selection has been directed towards bringing the conformation of the animal to some ideal conformation.

Improvement of conformation has economic value because a part of the sale price is determined by the conformation of the individual. The type of selection was most likely to give importance to the traits which are correlated with animal productivity i.e. udder size and type, teat confirmation and placementetc significantly.

With the introduction of record keeping it was found that direct selection for performance traits resulted in much faster progress than selection through correlated conformation traits under show ring selection.

Advantages of show ring selection

- It enables breeders / farmers / scientists to exchange ideas and experience.
- It allows comparisons among superior animals.
- It allows new breeders / farmers to make contact with established breeders / farmers.

Disadvantages of show ring selection

- Emphasis is usually placed on traits of little economic importance.
- Clever fitting and showmanship can mask defects of various kinds.
- Differences between exhibited animals are usually small.
- Conformation and production traits usually have low genetic correlations.

Importance and contribution of sire and dam for genetic improvement

The genetic improvement in dairy animals significantly affected by four factors

a. Increasing the genetic variation in the population, for which we have little control
b. Decreasing the generation interval by selecting younger animals as parents of the next generation
c. Increasing the accuracy of selection, which is reflected by the accuracy of the genetic evaluation methods and
d. Increasing the intensity of selection – most important factor.

The intensity of selection refers to the percentage / degree to which the best animals are used as parents. In facts, there are four pathways to selection and each has a varying level of impact on genetic improvement in population as given below:

Sr. No.	Pathways	Contribution in genetic Improvement
1	Selection of dams of heifers **(Dams to Daughters path)**	3%
2	Selection of dams of young bulls for A I **(Dam to Young Bull path)**	29%
3	Selection of Sires of daughters **(Sire to Daughter path)**	27%
4	Selection sires of young bulls for A I **(Sire to Young Bull path)**	41%

Genetic improvement / progress in different pathways clearly indicate the importance of selecting the elite sires (41%) and dams (29%) for young bull testing under A I programs, representing a total influence of 70% on genetic progress / improvement.

5 Digital imaging: a tool for buffalo performance evaluation

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A digital image is an electronic file that forms into square picture elements (pixels) when displayed on a viewing device (e.g., a computer monitor). The displayed image is a two-dimensional matrix of thousands or millions of pixels each of which has its own address, size, and colour representation. It appears to think of pixels as serving a role similar to the grains in a photograph. Digitizing a photograph means converting or capturing its image electronically through a scanner or digital camera. Digital image processing software allows magnifying an image to see the pixels, and to sometimes measure the numeric colour values for each pixel – like a sophisticated, computer generated, and paint-by-number matrix.

The current discussion introduces the buffalo body structure using a digital image and explains the measures through digital image characterisation. The basic concentration is on images created by 2D digital photography using digital camera. Understanding digital image steps can assist in planning effective scanning services. The process of capturing a genuine digital image requires good practice, standards in the categories noted here depends upon a variety of factors including i.e. quality of the equipment, attached software, quality control processes, operator skill, technical support throughout the process including management of the digital image and its metadata when the digital image is created.

Due to difficulties in weighing large sized buffaloes, feasibility goes to devise image digital methods, based on correlations between some quantifiable morphological measurements and live weight in addition to other production parameters. To the best of our knowledge, basic researches are limited on the determination of the variation in body shapes in specific species of interest like buffaloes using opto-informatic techniques. To overcome the difficulties connected with biometric evaluations, in recent years, studies have involved the use of new measuring instruments, including optical tools based on the analysis of computerized images.

Such methodologies present advantages from a technical, economic and ergonomic point of view since they allow morphological measurements to be obtained at a distance. In recent years, buffalo species have attracted growing attention, not only in Asian countries, where it is principally found, but also in countries in the Mediterranean basin. Consequently, the application of opto-informatic methodologies to this species would be of considerable interest.

Body condition scoring as designed numerically from 1 to 9 with an adaptive automated system that calculates the Body weight within seconds using Template Matching by Bayesian Sequential Hypothesis method - an image processing technique. A network camera was used to capture the image of the animal, which were then processed and analysed by the Template Matching method to calculate the Body Condition Score and Body weight. The body measurements on animals analysed through stepwise regression revealed that in buffaloes up to 24 months of age, body height was the most significant variable for predicting body weight. In heifers above 24 months of age and in adult buffaloes, heart girth was the most significant variable. Addition of body length and height in the basic equation slightly increased the R^2 values. The skeletal check points are identifiable to develop a BCS system for buffaloes using anatomical features. Using ordinary two dimensional image analysis, it is confirmed that computerized image analysis is an effective measuring system for developing body condition score . The automatic measurements of the angle between the back and the hook bones, together with measurements of the surface area behind the hook bones, observed as significantly correlated to the body condition score. The results confirm that, for buffalo species, computerized image analysis is an effective measuring system for the indirect determination of live weight.

A study on body morphometric digital imaging has been conducted at CIRB, Hisar with basic expected output as…

i. Database of digital images.
ii. Recording of observed Body Weight and Milk Yield.
iii. Results based on statistical analysis.
iv. Prediction equations for Body Weight and Milk Yield.
v. Depictions based on graphics and tabulated representations.

The applications of the techniques can outrightly assist the clientele and stake holders in terms of economic and socio aspects through buffalo judging, configurations of breed types and their distribution and migration in different habitats. To know the Importance of different body surface views of digital images which creates precised linear measurements at object level and their relation to predict performance characters using coordinates of pixels location and their intensity addresses. Referential devices used in connection with digital image pixels are comparable with depth dimensions and other world space characteristics. Techniques of measuring body surface area with specific precautions are helpful in determining true body weight in relation to body shape and size. Recognition and identification of dairy buffaloes and bulls with advance techniques depicts the developing ideas in trading, culling and selecting individuals under breeding system.

The biometry of body shape and body condition score (BCS) system is a quick, inexpensive, non-invasive and universally accepted method to estimate the degree of body structural surface and its deviations from normal conformation measures in relation with

production traits. Related extension activities are being disseminated to the farmers in the form of training programme. The identification and judging of calibrated buffaloes would probably be approached to evaluate the principal individual's comparative status. The body state of the buffalo can immediately be appraised and readily incorporated into operational decision making. In order to derive the maximum potential from buffaloes, the body condition scoring system would be useful in suggesting the feeding and management practices. Under long objectives the invention can easily be employed in large herds with high number of animals on a daily basis. The trend of body condition score can thus be traced and tracked frequently. The hardware tools and accessories are digital camera of large memory, high resolution, self made devises,computers peripherals and softwares for analysis.

The generated data sets are converted to the informative databases under repository system in relation with production and reproduction records under database management system. The statistical analysis for drawn of inferences under various frequencies and probabilities distribution to check the usability and applicability of fitness of collected records. Constructions of mathematical models using dynamic deterministic and stochastic system are necessary for the development of appropriate predictors.

Expected Benefits in Economic Terms Techniques will be evolved to speed up the measurement traits of body condition score and their relation with production and reproduction parameters.

For insertion of referential object lay out, an add-on constant diameter Digit Marker Ball (DMB) was attached with the previously used Digit Marker Plates (DMP) of arbitrary rectangular dimension were got utilised in getting images in five views of body surface of buffaloes. During measurements, the "F" type self-designed instrument, as per convenience, got assembled / fabricated from local market of Hisar. The functioning of aforesaid instrument was superimposed to maintain the linearity in measurement over the previously used process where it was hard to measure the linearity by using the ordinary flexible measuring tape. Adult Nili-Ravi buffaloes from Institute's herd maintained at CIRB sub campus Nabha (Pb), were classified under various categories and measured physically and linearly with their PS-Length (PSL) (cms/inch), Height at Wither(HW) (cms/inch) and Heart Girth(HG)(cms/inch) for comparing the estimated values depicted from digital images. Observations on Body Weight (BW) (Kg) were also recorded. To increase the accuracy, additional eight measurements i.e. (i) Height At Hook Bones (ii)Height At PIN bones (iii)Height At Paunch (iv)Width between Hook Bones (v)Width between Pin Bones (vi)Width Between Horns, (vii)Width between Eyes and (viii)Width between Front to Muzzle with the assumptions of lateral bi-symmetry or similarity of continuous characters in both right and left side (Figure 1-4).

The comparison in characters of both right and left sides was carried out showing a small difference due to standing posture of buffalo during shooting event. For insertion of

referential object lay out, an add on constant diameter ball were attached with the previously used Digit Marker Plates (DMP) of arbitrary rectangular dimension were got fabricated from local market, which were used in getting images in five views of body surface of buffaloes. The digital images of adult buffaloes of Nili-Ravi breed were obtained with five sketches at (i) right side (ii) left side (iii) Back side (iv) Front and (v) Upper views using 2D camera of Polaroid make. Each view was further replicated into four to ten images to collect a total of 423 digital images. The downloaded data of Exif (Exchangeable Image File) Format of 2D digital images of Nili-Ravi adult buffaloes were checked and screened against their image event specifications. In image layout, the diameter of ball attached to DMP was digitized in all five views to be used specifically as referential dimensions. There were 12,11,34,31 and 31 characters identified in 2D digital images for Back, Front, Upper, Right and Left respectively.

All the characters of each view were further compared with results obtained from two referential points. Novel designed tool under the name of 'Kalrumpscale' were designed for measuring 3D angular and linear orientation of buffalo external rump/ pelvic surface with the assumption of quantification of dairy characters. Murrah and Nili-Ravi breeds currently with high milk yield are identifiable for taking 2D digital images. The major proportion of population of both Murrah and Nili-Ravi breed in their native tract have been distributed and configured into two types (Type-C with Curled horns and Type-D with Drooped horns along the other distinguished characters) of sustainable groups other than non-descripts (Figure-6).

Quantification of both magnitude and shape of lactation curves in a consistent and repeatable way, based on the simple observation of milk records and looking into various influence occurred by genetics, environment, and health were carried out. Main data were formatted as per required JSON-1 Data Format (Java Scripts Object Notations) represented as text field names separated by a colon from values or arrays of values nested in complicated structures. The partitions of lactation curve i.e. scale, ramp, decay and offset were estimated from online open source on website of Dairysight. The quantification of persistence was also obtained mathematically as the rate of decline in production after peak milk.

Morphometric data, as observed from the digital images, were statistically analysed. The normality and trends of data of both breeds were checked for precision and biasness. The descriptive statistics of different measurements under all views i.e. Back, Front, Upper, Right and Left view. The precision of data was tested the mean values from both breeds i.e. SE, Median, SD, Variances, Kurtosis, Skewness, Ranges and finally compared with confidence level (95%). The multiple correlations among all measurements were estimated along with their level of significance.

The measurable and productive characters have been depicted. The correlated values were used to screen out independent variables applied for estimation of predictors. Adult body weights of Murrah and Nili-Ravi were plotted against Body length, Height at wither

and Heart Girth. The body weight have shown curvilinear with increasing trend of PS length, Height at wither and Heart Girth. These results have been applied for developing prediction equations. Trends between predicted and observed body weight in both the breeds were observed and analysed to create prediction equations measurable independent variables using multiple regression techniques.

An application for provisional patent entitled "KALRUMPSCALE -- A DEVICE TO MEASURE BUFFALO RUMP ANGULARITY FOR IDENTIFICATION OF DAIRY CHARACTERS" has been submitted during this period. Buffalo Judging for dairy appraisal is a comparative evaluation on rank based and their closeness to "ideal" dairy conformation. Desirable dairy conformation involves functional traits associated with high milk production over a long, trouble free productive life. In addition, good judges of dairy buffaloes require a definite mental image of the ideal animal for the breed being judged. Such judging indicates that the parameters measured above may be used as good indicators of buffalo's dairy characteristics with large pelvic area. Traditional with old technology, still continued, functioning at approximation scoring of body conformation characters based on individual's visual observations, accompanying 4-5 judges, where these erroneous techniques generally comprise average passing of approximation idea showing unmatched scoring differed under troublesome processing. Efforts have been made to find a solution on troublesome approximation to precision using following creative and novel idea where the whole invented and typical final designed device called "Kalrumpscale" in the form of assembled apparatus mounted on rump surface area of buffalo, in a real fabrication, to measure the vertical and horizontal angles with all possible linear measurements under 3D orientation.

Advanced models and predictors are applicable for culling and selection purpose at early age. Reconstruction of linear and curvilinear trend, to enhance the knowledge of inheritance pattern, is probably connected to phenomics and usable for further molecular studies. Application of Kalrumps cale in measuring and ranking of buffaloes on performance evaluation through 3D rump shape analysis will prove the basic tool in the hand of stakeholder. The research work outcome basics are best fit for application in automation of evaluation system through CCTV digital cameras connected with multi-viewers orientation. Continued efforts on application of research outcomes as body weight and milk yield predictors and its increased database volume will certainly assist linear and multi-curvilinear modelling experiments. The available models evolved through digitised morphometric algorithmically written on electronic chip can be inserted into sophisticated devices applied non-invasively to furnished accurate and precised and immediate results on performance traits. To carry out future line of research to go ahead as transformed from 2D to 3D image analysis will precisely assist to measure body depth, size and shape in relation to production characters.

Fig. 1 : Surface orientation (3D shape) of rump dimensions in Murrah and Nili-Ravi buffaloes.

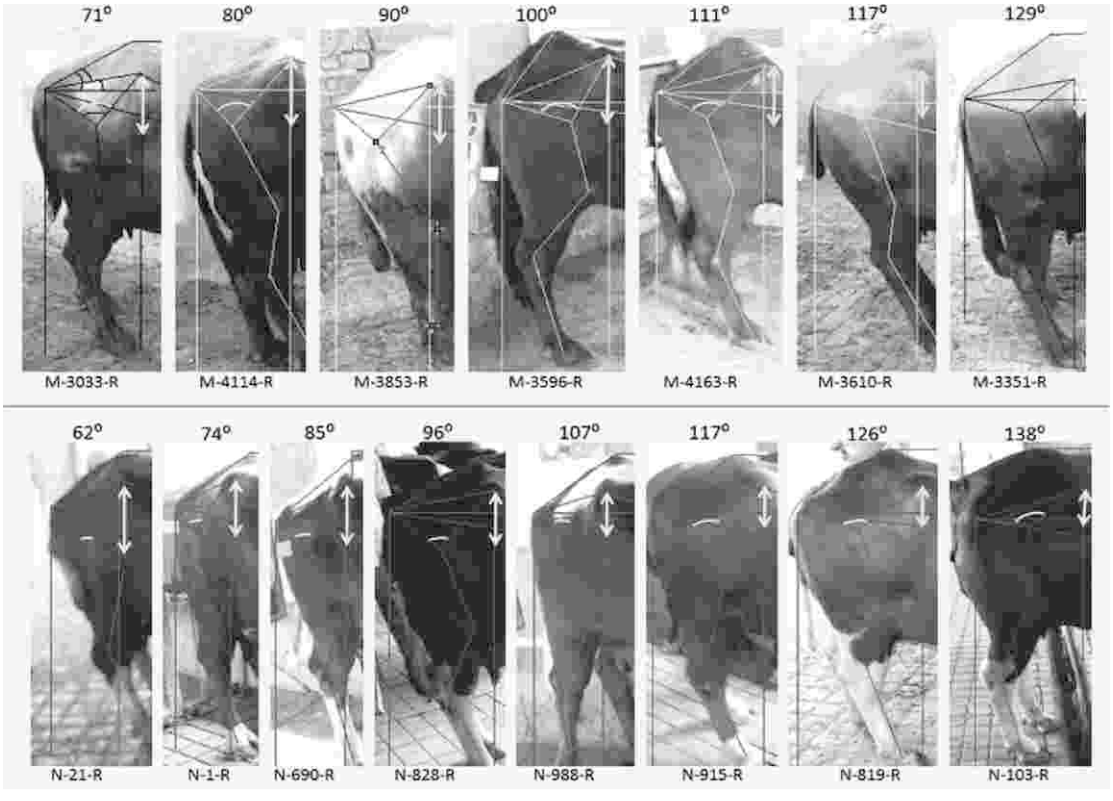


Fig. 2 : Back view (Right) and Front view (Left) of Nili Ravi buffalo showing dimensions as observed using digital image.

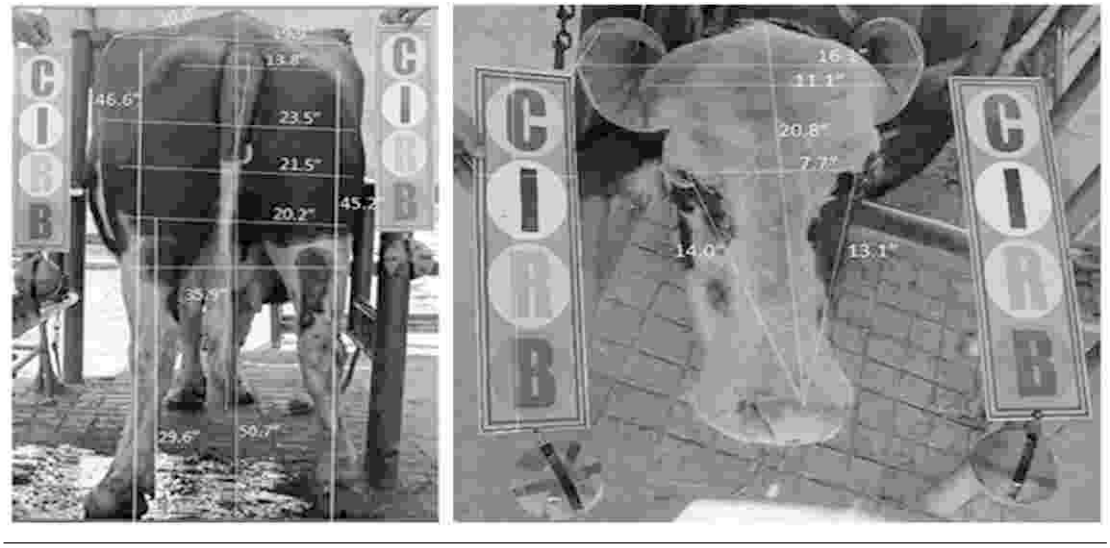


Fig. 3 : Right view of Nili Ravi buffalo showing dimensions as observed using digital image.

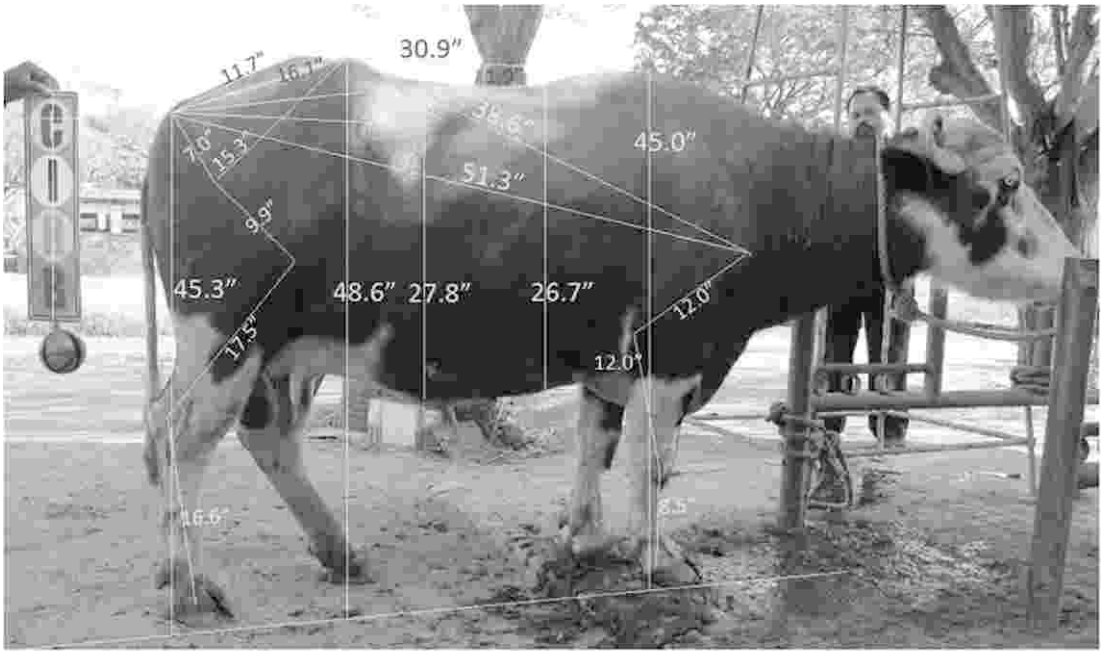


Fig. 4 : Upper views of Nili Ravi buffalo showing dimensions as observed using digital image.

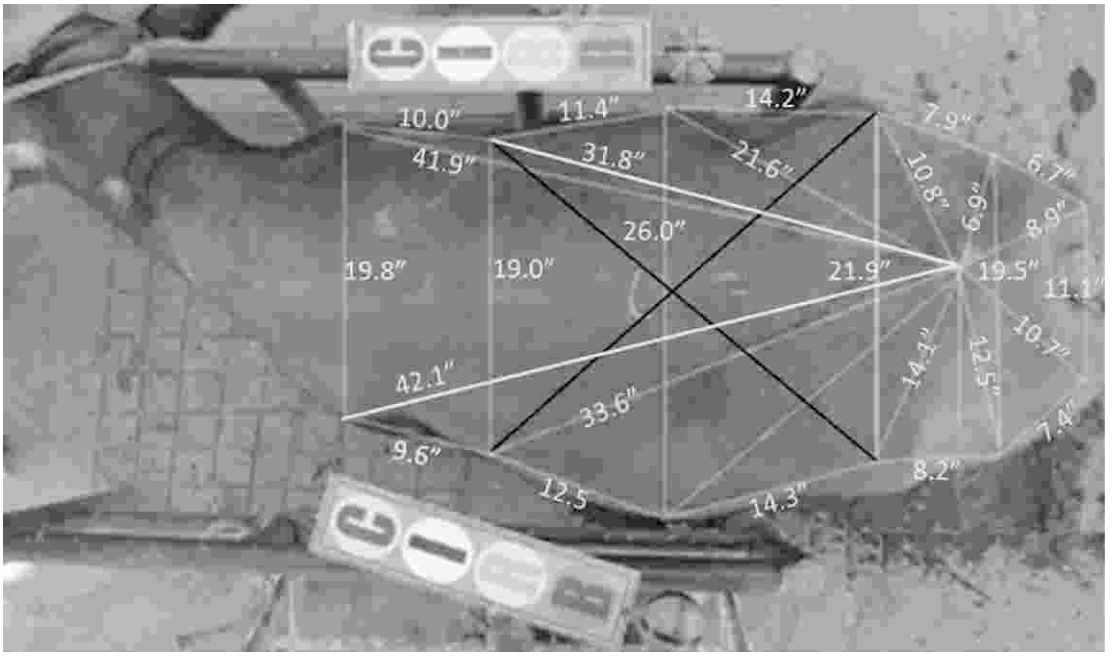
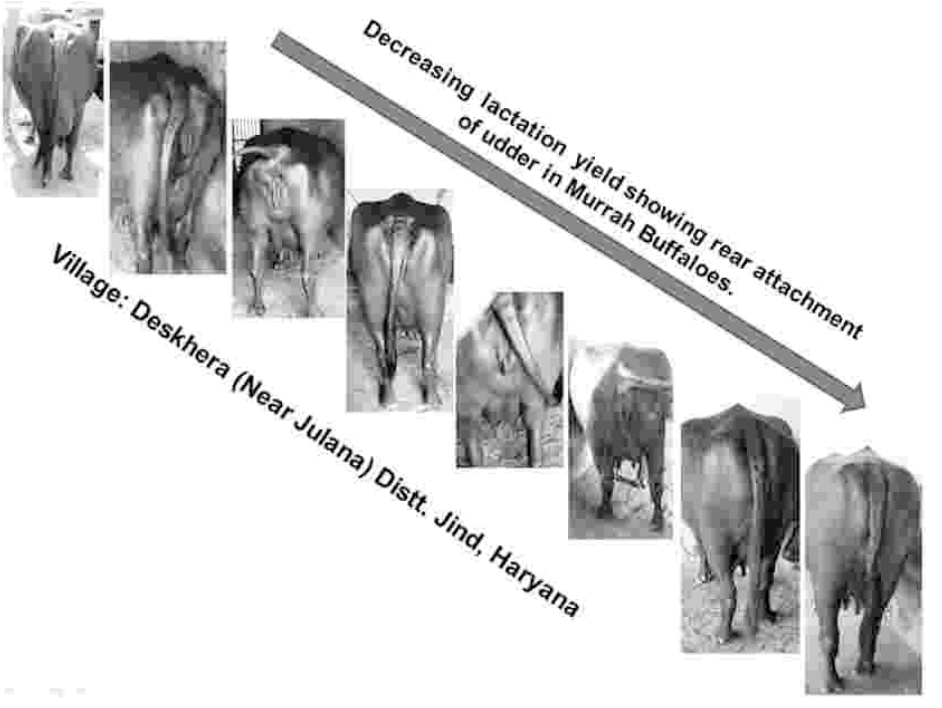


Fig. 5 : Showing rear attachment of udder,



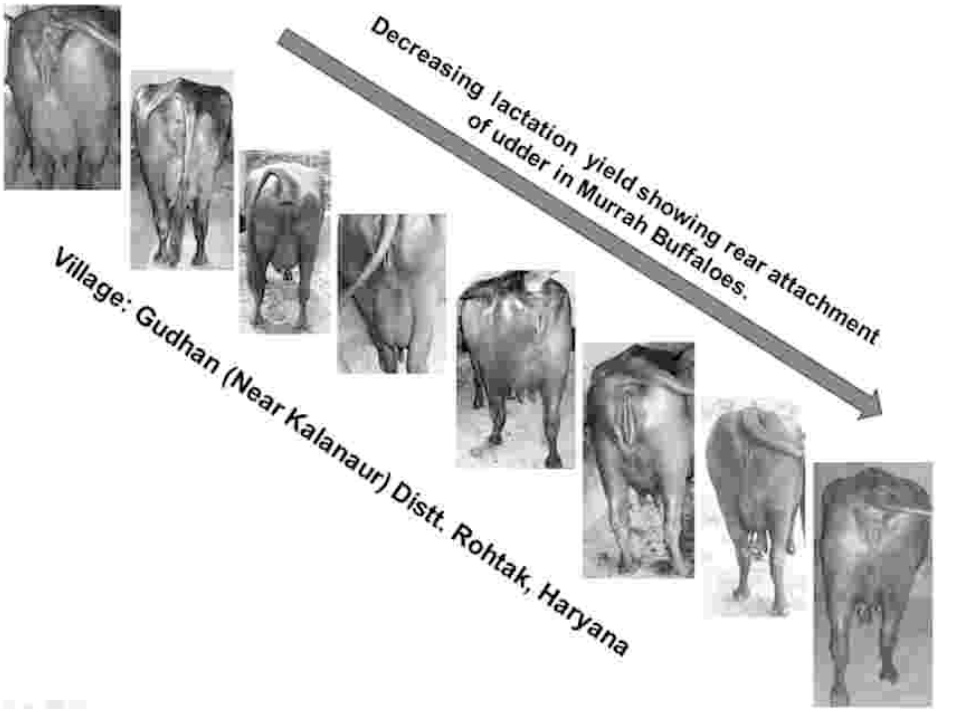


Fig. 6 : Showing Murrah (Above) and Nili Ravi (Below) breed types based on horn shape.

	Murrah - Sustainable Types	
	Murrah-C	**Murrah-D**
Male		
Female		

	Nili Ravi Breed - Sustainable Types	
	Nili Ravi-C	**Nili Ravi-D**
Male		
Female		

6 Buffalo herd management and farm records

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Livestock offers immense livelihood opportunities considering the growing demand of livestock products. The economic well being of a nation, particularly the developing one depends to large extent upon a healthy and productive livestock industry. The livestock enterprise not only provides the income stability through hidden rural employment, but also utilizes the crop residues by efficiently converting them into the animal products. Therefore, dairy farming is being practiced on commercial lines and which acts as a specialized dairy farm/ dairy enterprise. Indeed buffaloes are considered as the backbone of commercial dairying due to their fat rich, low cholesterol milk production potential and play an important role in the rural household economy. With dairying becoming an organized activity and being looked upon as a tool for economic development, the producer may now look to know whether he is getting the amount of returns from the milk as expected. Proper organization is essential for the success of any enterprise and buffalo farming is no exception. In fact, manager of a buffalo farm or the dairy farmer of small buffalo unit has to integrate several operations to make his buffalo an economical producer. An ideal dairy buffalo is one which starts producing at an early age of about 3 years; calves regularly at 12-15 months interval; remains in milk for 300 days after each calving and have a capacity to produce more than 2500 kg milk in every lactation. To achieve optimum results, efforts are to be directed towards attaining early maturity, high conception rate and low intercalving period using semen of bulls of high genetic merit.

Management of the herd

Management of calves : The main objective of good management and balanced feeding of calves is to obtain optimum growth rate in keeping with their genetic potential so that they can attain early maturity weight. The success of any dairy farm depends upon fast rearing of calves to a breedable age with a minimum mortality so as to have replacement stock. The half of the deaths among calves occurs in the first month of life and majority in rainy and winter seasons, mostly from digestive (diarrhoea and dysentery) and respiratory (pneumonia) disorders. The first 10 day of calf life is crucial and if given preventive treatment, their chances of survival is good. It has been established that feeding of at least one litre of colostrum within an hour of birth of the calf is necessary to

build up sufficient resistance against infection. Prenatal care of dam and postnatal management of calf is very important to overcome buffalo calf mortality. Calves should be dewormed once in a month till 3 months of age.

Management of growing buffalo heifers: The growth potential of buffalo calf is best up to 2½ years of age and half of the mature body weight is attained by 1½ years of age. Therefore, this growth has to be exploited by appropriate feeding. Unfortunately at this age they are most neglected on farms due to scarcity of green fodder as available fodder is mostly offered to milking buffaloes and growth rate of heifers decline due to negative nutrient balance. Care of heifers especially during summer is important as animals grow poorly or even loose weight during this period largely due to inadequate feeding. The heifers should be protected from thermal stress by providing foggers or splashing water on animals 2-3 times a day. Adequate nutrition and provision of thermal amelioration are effective to overcome summer slumps in growth of buffalo heifers. The winter slump in growth can effectively checked by shifting them in covered sheds.

Management of lactating buffaloes: The quantity of feeding of concentrate mixture/ dry roughages will depend upon the availability and type of green fodder. For higher milk production the animal should be fed daily at least 30 to 40 kg green fodder beside 4 to 5 kg wheat straw and concentrate mixture. An animal needs 1 kg concentrate for its maintenance. Additional ½ kg concentrate ration for every 1 kg milk produced is recommended. For example animal producing 10 litres of milk should be offered 6 kg concentrate. A good concentrate ration has 30% grains, 35% cake and 35 % bran all locally available. Besides recommended quantities of minerals, salts and vitamins should be given for optimum growth, production and reproduction. Usually the peak production is reached 8 weeks after calving and higher the output at this time, the higher will be the potential production curve for the remainder of the lactation. A good dairy buffalo may produce 15 to 18 litres of milk during the peak period. If feed limits the actual nutrients intake, the animal starts using body reserves as a feed supplement to meet the production level. For this reason it is normal for high producing buffaloes to lose weight following calving. Adequate feeding for the first two to three months will minimize the weight loss and ensure that buffaloes are in good condition for further mating. It is recommended that at least 40-50 days post partum rest should be given and mating during this period may be missed. Animal should be bred after this period as soon as it comes in heat. Dairy manager should keep a close watch on the animals and observe symptoms for the animals in heat. As a thumb rule, buffaloes observed in heat in the morning may be inseminated in the afternoon and vice versa. Animal must be inseminated within 50-90 days. In case the heat is delayed beyond 90 days it should be properly tested and brought into cyclicity and inseminated so that inter calving period is

minimized. If the animal continues to repeat regularly, the breeding be stopped and treatment given immediately. It has been observed that keeping the buffalo's body cool either by keeping it under good shade or by splashing cold water on the body helps in expression of heat symptoms and better conception rate. Each animal must be got checked for pregnancy after 2 months of insemination. Once the pregnancy is established, special care for feeding must be ensured and expected date of calving should be noted. During the last three months of lactation the production decline steadily even if good quality feed is available. This is the period when buffalo can readily replenish the already depleted body reserve and gain weight. It should be ensured that buffaloes are adequately but not excessively fed. A dry period of 60-90 days is necessary in order to provide sufficient time for the development of the foetus and mammary tissue in the udder before calving.

Clean milk production: Clean milk which has a normal composition, possesses a natural milk flavour with low bacterial count is safe for human consumption. The animal itself is one of the most significant sources of contamination. Health of the animal is, therefore, the starting point for clean milk production. Mastitis is the most common ailment in milch animals, leaving the milk unfit for human consumption. Regular screening of lactating buffaloes with CMT to identify subclinical mastitis and subsequent culture of the positive animals and their relevant treatment will help to nip the evil in the bud. Tuberculosis and brucellosis are the other two important diseases, which can infect human beings and can be transmitted via milk. Buffaloes suffering from any contagious disease should be segregated from the healthy ones. The animal shed is one of the main sources of contamination. Mud, urine, faeces and feed residues should regularly be removed from the shed. The shed should have sufficient light and ventilation. Washing and grooming of animals should be practiced to keep the dust and hair away from milk. The udder needs to be washed with antiseptic solution before each milking and dried with a clean cloth. The empty milk vessels have to be washed and cleaned immediately before and after milking. The milking area should be thoroughly cleaned after each milking. Knuckling and thumb method is a faulty method of milking. Putting thumb against the teat at the time of milking is wrong from the teat's point of view. There is great reduction in persistency and total lactation milk yield of buffaloes if they are not milked completely, therefore, milking should be done with full hand (Fisting) method followed by stripping for complete milking.

Management of breeding bulls: For sustained and rapid genetic improvement of buffalo productivity, optimum use of outstanding buffalo sire of good pedigree and proven transmitting ability has to be assured. The buffalo bulls are known for their overall weak and erratic libido. The relatively smaller testicular size, lower daily sperm

production rate and epididymal sperm reserve in buffalo bulls compared to cattle are some of the natural inbuilt constraints of this species which may not permit maximization of breeding potential of an outstanding and promising buffalo sire, similar to that possible in case of cow bull. The wide individual variation in service ability and semen production make it critical for proper selection of buffalo bull for breed improvement. Systematic culling of young buffalo bulls for low sperm count, sperm freezability and fertilizing ability will improve the performance of breeding bulls for artificial insemination. Optimum feeding right from birth not only helps in early maturity but also in production of quality semen. Bull exercise also improves the sexual behaviour and semen quality of buffalo bulls.

Healthcare: Both morbidity and mortality need to be minimized. While mortality causes direct losses, morbidity causes indirect losses to decreased production and slower growth rate. Prophylactic vaccination against prevalent diseases and administration of anthelmintics at regular intervals, timely identification & segregation of sick animals and their treatment are quite beneficial.

Culling: Even in best managed herd, there may always be some problem cases including uneconomical animals because of their age, low production or reproductive efficiency. There may be some animals suffering from chronic diseases which are either incurable or hard or expensive to treat. Similarly, some animals may give birth to progenies which are either incurable or hard or expensive to treat. Similarly, some animals may give birth to progenies with undesirable traits. Timely disposal of these animals is essential for economic health of any dairy unit. However, a prerequisite for this exercise is the proper maintenance of records regarding production, reproduction and health of each animal.

Labour management : For any organization, good workers are the best asset for a dairy farm. The expenditure on labour accounts for only one fifth of the cost of production, one cannot under estimate the importance of labour just on the basis of cost alone, and hence the returns depend on the proper utilization of labour and their efficiency. Efficient utilization of labour would also help in bringing down the price of milk. It is essential to provide suitable equipment to perform the farm operations quickly and efficiently. It is also important that worker is not over burdened and jobs are to be assigned to individual depending upon their physical strength, skill and experience.

Farm records and calender for dairy farm operations

Farm records : It is not possible to run any business profitability if we don't have a sound planning for synchronization of all the inputs and all the factors that are chain linked to each other. Unless the records are kept the best buffalo in the herd is likely to have equally treated with the poorest and thus, the full potential of the best animal is not exploited.

These are the farm records which help the dairyman making future planning such as knowing total feed and fodder requirements, labour requirements, health programmes and other such requirement during various period of the year. The kind of records includes: Daily diary; Livestock register; History sheet; Insemination register; calving register; Treatment register; Mortality register; Disease testing register; Fodder register; Milk production register; Disposal register and Labour register. Besides, body weight, feed consumption, vaccination, deworming, milk disposal and other records as per need and convenience may also be maintained.

Dairy Farm operations : The Dairy Farm operations run 365 days a year and almost 24 hours a day. Depending upon the nature and urgency of individual operations, these can be grouped into four categories i.e. Daily operations; weekly operations; monthly operations and annual operations

Daily operations: Some of the operations need to be performed on daily basis and cannot be postponed. Major daily operations are enlisted below:

i. Milking
ii. Sale and disposal of milk
iii. Cleanliness and disposal of dung
iv. Supply and chaffing of fodder, pasture grazing, concentrate ration, feeding of calves with milk substitutes etc.
v. Grooming of animals
vi. Wallowing
vii. Heat detection/teaser bull parade, sexual health check-ups/heat expectancy charts and insemination.
viii. Healthcare
ix. Calving line - feeding of colostrum, naval disinfection, ear tagging, disposal of placenta etc.
x. Transfer of freshly calved animals to milking herd after 4 days
xi. Postmortem and disposal of carcass
xii. Watch and ward staff and labour distribution
xiii. Emergencies - dystocia, bloat, tympany, poisoning etc.
xiv. Agricultural farm operations
xv. Record-keeping

Weekly operations: The operations which can preferably be undertaken on weekly basis are as below:

i. Milk recording - complete stripping/Mastitis test
ii. Drying off of animals

iii. Calving forecast
iv. Agricultural Farm Operations
v. Roster of weekends and holidays
vi. Review of weekly progress

Monthly operations: Some of the operations can be planned on monthly basis. These include:

i. Culling and auction of animals
ii. Deworming
iii. Cleaning and change of water of wallowing tank
iv. Vaccinations
v. Calving forecast
vi. Purchase of medicines, chemicals and other consumables
vii. Fodder purchase/preservation - silage, karbi, wheat straw etc.
viii. Agricultural Farm operations
ix. Review of monthly progress report

Annual operations : Some of the operations are better performed on annual basis and these are:

I. Testing against T.B., J.D., Brucellosis and other listed diseases
ii. Replacement and purchase of equipment/machinery
iii. Addition/replacement of animals and review of herd strength
iv. Review of human resources and training needs
v. Evaluation of economic health
vi. Critical review of annual progress

In summary, it is evident that some of the operations cannot be postponed or delayed even by a few hours. Operations like milking, feeding, watering and cleaning have to be done on right time. Emergencies such as dystocia and acute health problems need to be attended without any loss of time. It is essential that a qualified veterinarian and paraveterinary staff are available 24 hours a day preferably on the farm. An effective roster of work force as well as supervisory staff for weekends, holidays and off hours is a must. Above all, critical review of progress at regular intervals is of utmost importance to know the problems and finding out their solutions in time.

Livestock Register

S. No.	Animal No.	D.O.B./ Purchase	Sex	Dam No.	Sire No.	Remarks Died/Sold/Auction

Daily Artificial Insemination Register

S. No.	Animal No.	D.O.B.	D.O.C.	Heifer/ Parity	D.O. Last A.I.	D.O. A.I./ No. of A.I.	Bull No.	Pregnancy Status	Remarks

Calving/Birth Register

Calving for the month of _____ 20 _____

S. No.	Animal No.	Date of Calving	Calf No.	Sex	Weight of Calf	Sire No.	Date of A.I.	Gestation Period	Remarks

History Sheet

Animal No.				D.O.B.				Dam No.			Sire No.		
Partiy	Bull No.	Date of A.I.	Date of Calving	Calf No.	Sex	Date of Dry	Number of teats	Lactation Length (days)	Total Lactation Yield (kg)	305 days Lactation yield (kg)	Peak Yield (kg)	Date Peak Yield	Remarks

Treatment Register

Yearly No.	Monthly No.	Animal No.	Sex/ Type	Shed No.	Clinical Observation	Diagnosis	Treatment Given	Remarks

Treatment Register

S. No.	Animal No.	D.O.B.	D.O.D.	Age	Sex/Type	Clinical Observation/History	Post Mortem Findings	Remarks

Mortality Register

For the month of _____

Date	Green Fodder			Silage				Dry fodder				
	Received	Fed	Silage made	OB	G. Total	Fed	Balance	OB	Received	G. Total	Fed	Balance

Livestock Disposal Register

S. No.	Animal No.	Date of Sale/Auction	D.O.B.	Sex	Type of Animal	Age	Cost	Remarks

Disease Testing Register

S. No.	Animal No.	D.O.B.	Sex/Type	Date of testing	Type of test T.B., J.D., Brucella	Result	Remarks

Milk Production Register

For the month of _____

S. No.	Anim. No.	D.O.C.	Peak yield (Kg)	Date of PY	Date												Total of the month (kg)	Previous Cumulative (kg)	Grand total (kg)
					1		2		3		4		5		& so on				
					M	F	M	F	M	F	M	F	M	F	M	F			

7 Nutrient requirements and feeding of buffaloes at different stages of life

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There is considerable physiological difference between cattle and buffaloes. Differences exist in ruminal movements, fluid volume in reticulorumen, rate of passage of digesta, efficiency of conversion of carotene into vitamin A and above all, the milk composition between the two species. Result of an meta-analysis on comparative nutrient utilization efficiency in cattle and buffaloes has been presented in Table 1. The data suggest substantial difference in feed efficiency in these two species. Hence, it is appropriate to use buffalo specific feeding standards for calculation of ration for buffaloes.

Table 1 Comparative nutrient utilization efficiencies in lactating cattle and lactating buffaloes at different levels of production (Paul et al., 2003)

Parameter	Cattle	Buffalo	Sig
Gross Energy Efficiency	23.17±0.49(n=77)	25.19±0.55(n=54)	**
Gross Protein Efficiency	38.45±0.93(n=43)	45.72±1.05(n=49)	***
Net Energy Efficiency	52.79±1.18(n=75)	60.70±1.57(n=53)	***
Net Protein Efficiency	59.86±1.33(n=40)	72.56±1.77(n=44)	***
DM Intake, kg/kgFCM	1.243±0.03(n=71)	1.175±.02 (n=53)	NS
DCP Intake g/kgFCM	93.88±2.33(n=40)	80.36±2.1(n=52)	***
TDN Intake g/kgFCM	774.83±17(n=74)	707.6±19(n=54)	**

P<0.05;P<0.01;***P<0.001; NS=Not significant*

Dry matter intake of lactating buffaloes

The biggest inaccuracy in scientific feeding of any species occurs due to inability to predict DM intake. The statistical analysis of results of almost all of the experimental feeding trials conducted so far on lactating cattle and buffaloes in India indicated that the voluntary consumption (expressed as % of body weight) is significantly less (2.56 vs. 3.09 %) in buffaloes than in cattle of similar production level (Paul *et al*., 2003). However, regression analysis and Pearson correlation coefficients computed on the data showed that FCM (4% fat corrected milk yield) alone accounted for 44.8 % of the variance in DMI (dry matter intake). Similarly, on single factor regression analysis, MBS (metaboilic body size, $W^{0.75}$ kg), WC

(weight changes, g/d), TDNP (total digestible nutrient percent in diet), DCPP (digestible crude protein percent in diet), RP (roughage percent) and species (individually) explained 34.3, 5.5, 16.8, 11.5,11.6 and 19.6 % of variation in DMI, respectively (n=80, P<0.05). The final DMI prediction model obtained by stepwise multiple regression method is as follows: DMI (kg d^{-1}) = 4.469 (±1.466) + 0.589 (±0.045) X FCM (kg d^{-1}) - 0.751 (±0.082) X DCPP + 0.0032 (±0.000) X WC (g d^{-1}) + 0.0589 (±0.015) X MBS (kg) +1.037 (±0.334) X Si (Si = i th species. Si=1 for cattle & 0 for buffaloes); R^2 =0.853 (P<0.001); Adjusted R^2 = 0.844; SE estimate = 0.7085 kg; CV of the model, 6.38 % of mean DMI; N = 80 (46 from buffalo and 34 from cattle). The value of partial regression coefficient for the species effect in the model was 1.037-kg d^{-1}, which implies that out of the observed difference in DMI between cattle and buffaloes in the database (average DMI in a cattle of 400 kg body weight was about 2 kg higher than that of a buffalo of same body weight) only a difference of 1.037-kg d^{-1} was attributable to species effect and rest of the difference is due to difference in FCM, WC, DCPP and other unaccounted factors which affect DMI.

Expected dry matter intake capacity of buffaloes of less than six month age

Dry matter intake capacity of new born calves fed whole milk or milk replacer.

From birth to 1 month of age is 30-35 g per Kg $W^{0.75}$
From 1 month to 2 months is 35-40 g per Kg $W^{0.75}$
From 2 months to 3 months is 35-45 g per Kg $W^{0.75}$

Dry matter intake capacity of new born calves fed restricted whole milk alone or partly mixed with skim milk in combination with calf starter and fodder

From 1 month to 2 months of age is 50-60 g per Kg $W^{0.75}$
From 2 months to 3 months is 60-75 g per Kg $W^{0.75}$

Dry matter intake capacity in early life of 3-6 months

High concentrate diet, 75-90 g per Kg $W^{0.75}$
High roughage diet of good quality leguminous or cereal -legume mixed fodder is 65-80 g per Kg $W^{0.75}$

DM intake capacity of working buffaloes

Dry matter intake increases with the increase in workload and may be similar to that in lactating buffaloes. The dry matter intake may be 2, 2.5 and 3 Kg per 100 Kg body weight in animals engaged in light, medium and heavy work.

Nutrient requirements of buffaloes

Method of estimation of nutrient requirement also influence estimates substantially. The feeding standards developed by Mandal *et al.* (2003), Paul and Lal (2010) are based on analysis of pooled primary data on nutrient intake, body weight and production from large number of experiments conducted on buffaloes in different research institutes across India

and hence, can be considered as broad based and adjusted to the diversified feeding situation.

Nutrient requirements of buffaloes estimated primarily by regression method on long term feeding trial data are presented below (Table 2A).

Table 2A. Energy and protein requirements of buffaloes

Stages of life cycle	Maintenance (g/kg $W^{0.75}$)	Growth (g/g ADG)	Lactation (g/kg 6% FCM)	Reference
Energy requirement (TDN)				
Growing	35.4-39.3	1.4-2.23		Paul and Patil, 2007; Paul *et al.,* 2004
Lactating	35.3	1.97	406	Paul *et al.,* 2002
Protein requirement (CP)				
Growing	5.98-6.74	0.44-0.51		Udeybir and Mandal, 2001
Lactating	5.43	0.33	90	Paul *et al.,* 2002

Effect of environmental stress on nutrient requirements

Without accurate accounting of the effect of environment on nutrient requirements, it is not possible to diagnose performance problems and to develop quantitative feeding strategy to counter adverse effects of environmental stress. A study was undertaken to estimate requirements of energy and protein for temperature and humidity stress employing regression method of partitioning of nutrient intake from feeding trial data and the results have been presented in Table 2B.

Table 2B. The regression equations [a] of TDN, and CP requirements for maintenance, temperature stress, humidity stress and body weight gain

Nutrient	b_1 ±SE	b_2 ±SE	b_3 ±SE	b_4 ±SE	R^2	Model CV (%)
TDN	27.0*1.74	1.06*0.110	0.312*0.050	1.77*0.080	0.99*	9.43
CP	6.60*0.601	0.113*0.038	0.046*0.001	0.246*0.030	0.97*	17.14

[a] *Model: Nutrient Intake (g/d)= b_1MBW (kg) + b_2 RSTS X MBW + b_3 X RHS X MBW + b_4 ADG (g/d); MBW, metabolic body weight; RSTS, root square temperature stress =((av. temp inC -20)^2); RHS, relative humidity stress=RH-60; CV, coefficient of variation= (residual standard deviation /Mean of dependent variable) X 100; * = significant (P<0.01).*

Partitioning of energy intake data indicated that about 13.6% of energy (TDN) intake was utilized to offset temperature stress and 9.3% of energy intake was utilized to offset humidity stress and only 31% of energy intake was utilized for growth and 46.1% for maintenance. Similarly proportion of CP intake utilized for maintenance, growth, temperature stress and humidity stress were 61.7, 23.1, 7.8, and 7.31%, respectively.

Importance of feeding protein in optimum quantity and quality

Protein is vital to the maintenance, reproduction, growth, and lactation of animals. Low level of dietary protein severally affect rumen microbial growth and fermentation resulting in increased retention time of nutrients, decreased capacity to digest organic matter and depressed feed intake which affect animal`s performance. Low protein intakes also depresses growth rate as well as milk production, the latter being through decreased lactose synthesis and reduced mobilization of body fat. When fed in excess, protein is utilized as source of energy especially in cases of energy shortage, since deposition of protein in reserve tissues of ruminants is limited to the extent of 8-22 % of total body protein (Huber, 1976). Inefficiencies arise from elimination of surplus urea, which in turn increases energy requirement and may affect health and reproduction of the animals. Quality of protein in term of its ruminal degradability is also important for ruminants. When bypass protein is fed to ruminants, protein remains mostly undegraded in rumen thus lowering production of ammonia in rumen, which otherwise is produced in large quantities and excess is absorbed and converted to urea in liver (involves energy expenditure) and is mostly excreted through urine. By feeding bypass protein, at least 30% of the dietary amino acids are saved from getting excreted as urea. The excess amino acids absorbed from the lower tract are converted to glucose in liver and serve as precursor for lactose synthesis in mammary gland, which regulates osmotic pressure of milk and resulting in larger volume of milk produced.

Research conducted in the 1960s showed that the rumen of a cow was capable of supplying all of the protein required by cows producing up to 4500 kg of milk per lactation. In India, for many years, CP and DCP content were used in formulating diets for ruminant animals considering the fact that most of the Indian animals are low producing. However, with introduction of selective breeding milk yield per buffaloes has increased during the last 15-20 years. Now-a-days buffaloes, in India, reaching a peak milk yield of 25 kg per day are not unusual at farmer's level. However, most of the research farms do not have high yielder buffaloes. Hence, very limited data are available from controlled experiments utilizing RDP and RUP based feeding systems in buffaloes and results are not consistent. However, under rural condition where ruminants are mainly fed on crop residues and have little access to concentrate, response of feeding bypass protein is good. Crop residues are deficient in crude protein and mineral. In the absence of supplementary sources of ammonia and minerals, microbial growth is slow as a result proportion of MP in the ME is low. Thus, MP becoming limiting for animal growth or milk production. Hence, animals fed only straw respond by a logarithmic function when supplemented with bypass protein. Supplementation with a bypass protein source increases the efficiency of use of absorbed nutrient and energy. The

levels of production achieved when proportion of MP in the ME is increased over an initial small range, is many fold than that predicted from the ME content of the feed (Leng, 1990). This is probably due to lower heat production associated with improved protein energy balance even where feed intake is not increased.

Recent research has also demonstrated important role of UDP in modifying disease course and on performance of animals under stress. Feeding bypass protein to stressed calves resulted in increased performance (Philips, 1984).

In a study involving Nili Ravi buffalo heifers the CP and MP requirements for maintenance at 125-150, 151-200, 201-250, 251-300, 301-350 and 351-400 kg body weights were worked out to be 6.96 and 4.03; 7.43 and 4.47; 7.27 and 4.07; 6.19 and 3.57; 9.48 and 4.65 and 9.48 and 6.30 KJ or g / kg $W^{0.75}$. The respective values for ADG were 0.30 and 0.21; 0.27 and 0.18; 0.24 and 0.20; 0.45 and 0.31; 0.42 and 0.24 and 0.26 and 0.24 KJ or g/ g ADG (Paul and Patil, 2007).

Balanced rations for various categories of buffaloes

Feeding of calves up to 3 month age

At this stage calves require high plane of nutrition and good quality easily digestible feed. However, to speed up development of rumen and early initiation of microbial fermentation the calves should be offered calf starter and green grass from second week of life. As intake of calf starter and green grass increases milk has to be reduced gradually as per following schedule (Table 3)

Table 3. Feeding schedule for calves from birth up to 3 months of age

Age (Days) roughage (kg)	Whole milk (Kg)	Calf starter (Kg)	Green
1 to 7	2.50	nil	nil
8 to 14	2.50	0.05	0.25
15 to 21	3.00	0.10	0.35
22 to 28	3.50	0.20	0.50
29 to 35	3.50	0.40	0.55
36 to 42	3.00	0.60	0.60
43 to 49	3.00	0.70	0.70
50 to 56	2.00	0.80	0.80
57 to 63	1.50	1.00	1.00
64 to 70	1.50	1.20	1.10
71 to 77	1.00	1.30	1.20
78 to 84	0.50	1.40	1.40
85 to 90	Nil	1.70	1.90

Calf starters : Calf starter should contain 22% CP and 70-75% TDN and it should be prepared from good quality feeds i.e. easily digestible low fibre feed and has to be free from any kind of toxins or antimetabolites. Components of calf starters should be finely crushed. 10 g vitamin supplement (A, B2, D3) should be added in 1 quintal of the mixture if green fodders are not fed.

Feeding of calves from 3 to 6 month of age

After 3 months of age, the calves attain about 60-70 Kg body weight under normal feeding condition. In this phase rumen developed substantially and microbial digestion in rumen become functional and beyond 3 months of age suckling is usually not allowed except for very short period to facilitate milk let down. Hence, the major portion of nutrient requirements has to be met from concentrate or roughage source. Generally, at this phase protein and energy level of diet are kept similar to those fed upto 3 month age. However, there is reasonable ability of compensatory feed intake if calves are given low plane of energy and protein. Thus a palatable diet containing 13-14 % crude protein and 60-62% total digestible nutrient may support 500-600 g average daily growth rate. Any of the following feeding schedules presented in table 4 can be followed.

Table 4. Feeding schedule for calves from 3 to 6 months of age

Schedule	Green fodder	Straw	Concentrate mixture#
1	10 Kg cereal fodder	*ad libitum*	1.0 Kg
2	15 Kg leguminous fodder	*ad libitum*	1.0 Kg
3	1-3 Kg green fodder	*ad libitum*	2.0 Kg
4	7.5 Kg cereal fodder & 2.5 Kg leguminous fodder	---	1.0 Kg

#A concentrate mixture can be prepared by mixing crushed cereal grain (maize, jowar, barley, oat, etc.), groundnut cake or cottonseed cake or soybean cake, mustard cake, wheat bran, mineral mixture and salt in the ratio of 30: 27: 10: 20: 2:1 or 15:22:5:55:2:1 or 30:20:0:45:2:1 or 0:20:0:77:2:1.

Feeding of buffalo calves from 6 month of age

At this phase requirement for high plane of nutrition and good quality feed is not essential. Calves can be reared on coarse fodder and straw based diet. Quality of feed or amount of ration will largely depend on target growth rate. Generally, 12% CP and 58-60% TDN in ration will support 450-550g average daily growth rate at this phase. In buffalo heifers puberty is achieved when animals attain threshold body weight that is about 50-70%

of mature body weight. Hence scientific feeding at rearing stage to support optimum growth rate is required to attain threshold body weight at early age. With proper feeding puberty is attained at age of 17-21 months at body weight of 270-300 Kg. Heifers can be served when they attain 300-325 Kg body weight. However, very high plane of energy nutrition inhibits development of milk secretary tissue in mammary gland, which in turn reduces lifetime milk production ability. Generally, a growth rate of 500-600 g/d between 100 and 300 Kg body weight is considered optimum growth rate for Indian buffalo heifers. Underfeeding that reduces growth rates during the rearing phase to 50% of the animal's potential delays puberty significantly. For attaining 450- 500 g daily weight gain a concentrate mixture containing 20% CP and 63% TDN may be fed @ 1.5, 2.0, 2.5 and 3.0 Kg per head per day at 100, 150, 200 and 250 Kg body weight or above along with 10 Kg green fodder and *ad libitum* straw. The concentrate mixture can be prepared by mixing crushed cereal grain, wheat bran, rice bran, mustard cake, soybean cake or groundnut cake, mineral mixture and salt in the ratio of 35: 15:25:10:10: 2:1 or 30:20:17:15:15: 2:1. When green fodder is not available, additional 1 Kg concentrate mixture should be fed as replacement of 10 Kg green fodder. For faster growth rate additional 1 Kg concentrate may be fed daily.

Feeding for maintenance of adult nonproducing or nonworking buffaloes

Dry adult non-pregnant buffaloes or nonworking bullocks can meet their nutrient requirement for maintenance from 6-7 hr grazing with *ad libitum* feeding of straw during off grazing hours. For stall-feeding of a 450 Kg buffalo, a) 9.5 Kg straw + 0.7 Kg groundnut cake/soyabean cake or b) 7.0 Kg straw +10 Kg berseem (15% DM) or c) 28 Kg cereal fodder (20% DM) will generally meet requirement. For every 50 Kg higher body weight than 450 Kg, a) 0.7 Kg straw+55 g groundnut cake/soyabean cake or b) 3 Kg green berseem or c) 2.5 Kg cereal fodder has to be added and vice versa.

Feeding of pregnant buffaloes

Appropriate intake of nutrients is essential to achieve optimal fetal growth and neonatal survival. In early pregnancy, buffaloes should be fed on maintenance level or restricted level of feeding to increase conception rate. In general, until the last one third of gestation, nutrient requirements for intra-uterine growth are very small relative to mother's maintenance. Only about one-third of the total products of conceptus are produced during the first 7 months of gestation period. Subsequently, there is rapid acceleration in fetal development during the last 3 months of gestation period. Generally, buffaloes should be fed to support 750-900 g average daily weight gain during last 2 month of pregnancy and about 700g average daily weight gain during the last 3 month of pregnancy. In pregnancy of adult buffaloes, CP requirement increases by 3, 8.4, 16, 26, 43 and 64% of maintenance requirement on 5^{th} 6^{th}, 7^{th}, 8^{th}, 9^{th} and 10^{th} month of pregnancy, respectively. The corresponding increases in TDN requirements are 4.3, 7.2, 18.8, 22.2, 39.0 and 67.4 % of maintenance requirement, respectively. During pregnancy substantial extra uterine growth

in the mother takes place especially during early pregnancy. This is necessary in immature animals that are still growing i.e. those in first and second pregnancy and hence 20 and 10% of maintenance requirement of energy and protein should be additionally fed to the heifers in first and second pregnancy, respectively. In case of adult buffaloes maternal growth is considered as non-essential for pregnancy. However, in high yielding buffaloes additional allowance during early pregnancy may be given to facilitate building up of extra body reserve which can be utilized to meet out energy deficiency in early lactation when animals are invariably in negative energy balance due to limited DM intake capacity. Pregnant buffaloes should be dried at least 2 month before expected date of calving. In pregnancy, DM intake is low (about 1.7- 2% of BW). Pregnant dry buffaloes (at > 5 month of pregnancy) should be fed with 30 Kg green fodder and 2 Kg concentrate mixture (20% CP & 70% TDN) and *ad libitum* wheat straw. With decrease in availability of green fodder 1 Kg concentrate mixture should be additionally fed to replace every 10 Kg green fodder. This ration will meet protein requirement for entire pregnancy and energy requirement upto 9.5 month of pregnancy but will fall short of energy requirement on the last 2 weeks of pregnancy when additionally 1-1.5 Kg grain has to be fed. For pregnant immature buffaloes in first pregnancy, additional 1 Kg grain or 5.5 Kg cereal fodder or 7.5 Kg legume fodder should be fed to support 300-350 g average daily maternal growth. Similarly, buffaloes in their 2nd pregnancy should be fed additional 0.5 Kg grain or 2.7 Kg cereal fodder or 3.7 Kg legume fodder to support 120-200 g average daily maternal growth. Challenge feeding of buffaloes with good quality fodder and concentrate mixture during last three weeks of pregnancy helps in priming the rumen for increased concentrate feeding in early lactation and build up body reserve for lactation .

Feeding of breeding bulls

Breeding bulls should attain body weight of 350-400 Kg body weight at 30 month of age and at this age they are ready to be used for breeding. As in case of females, in males also low plane of nutrition delay puberty. The adverse effects of malnutrition are more pronounced if they occur in early life than post weaning. In controlled experiment, low protein feeding delayed puberty of bulls by 5 months and such bulls had poor testicular development and small ejaculate volume as compared to their normal counterpart. Vitamin A and Zn deficiency can also delay puberty, reduce libido and may affect integrity "of testicular tissue and hence special care need to be taken to prevent deficiency of these two critical nutrients. About 40 to 60% restriction of energy and protein during growing phase causes retardation of testicular growth and the effect persists throughout the life. On the other hand feeding high concentrate diets (80% concentrate in DM) to growing bulls reduced testicular sperm reserve and also reduced semen quality as compared to total roughage diet. It has been recommended that breeding bulls should be given 100% higher CP and 20% higher energy than maintenance requirement for mature female buffaloes.

Breeding bulls should be fed with good quality balanced ration. However, care should be taken to avoid overfeeding as fatness lead to reduced libido and reduced reproductive performance. For a 700 Kg buffalo bull following feeding schedule can be followed: a) 40 Kg cereal fodder + 0.8 Kg deoiled ground nut cake/deoiledsoyabean cake or b) 10 Kg berseem + 10 Kg straw + 1.2 Kg deoiled ground nut cake/deoiledsoyabean cake or c) 8 Kg straw + 2.0 Kg concentrate mixture + 2-3 Kg green fodder + 1 Kg deoiled ground nut cake/deoiledsoyabean cake. For every 50 Kg increase or decrease in body weight from 700 Kg, a) 0.6 Kg straw +100 g deoiled groundnut cake/deoiledsoyabean cake or b) 3 Kg green berseem or c) 3 Kg green cereal fodder should be added/deducted from the ration suggested for 700 Kg body weight.

Feeding of working buffalo bullocks

For working animals, nutritional requirements depend on duration of work, speed of work and load carried.

For light work (4h/d)

Buffalo bullocks of 550 Kg body weight should be fed as follows: a) 4.0 Kg concentrate mixture + 7 Kg straw or b) 1.5 Kg concentrate mixture + 35 Kg cereal fodder or c) 20 Kg berseem+8 Kg straw + 0.5 Kg deoiled ground nut cake/ deoiledsoyabean cake or d) 2.0 Kg concentrate mixture+ 10.0 Kg berseem + 8.0 Kg straw + 0.4 Kg oil cake. For every 50 Kg increase/ decrease in body weight from 550 Kg , a) 0.65 Kg straw + 180 g deoiled ground nut cake/deoiledsoyabean cake or b) 4.0 Kg green berseem or c) 2.5 Kg cereal fodder + 100 g deoiled groundnut cake/deoiled soybean cake should be increased/decreased from the quantity suggested for 550 Kg body weight.

For heavy work (8h/d)

Buffalo bullocks of 550 Kg body weight should be fed as follows: a) 5 Kg concentrate mixture + 8 Kg straw or b) 3 Kg concentrate mixture + 40 Kg cereal fodder or c) 40 Kg berseem + 7 Kg straw or d) 3 Kg concentrate mixture + 14 Kg berseem + 8 Kg straw.

For every 50 Kg increase or decrease in body weight from 550 Kg body weight a) 1 Kg straw +180 g deoiled ground nut cake/deoiledsoyabean cake or b) 4 Kg berseem + 0.35 Kg straw or c) 4 Kg cereal fodder + 50 g deoiled ground nut cake/deoiledsoyabean cake should be increased/decreased from the quantity suggested for 550 Kg body weight.

Feeding of lactating buffaloes

The lactating animals must receive sufficient nutrients to supply the nutrient secreted in their milk, and for maintenance. If their nutrient needs are not met, they will not reach their optimum milk production capacity. Dietary energy is the most limiting factor in milk production. Milk production increases gradually, reaches peak at 42- 56 days after calving, and the peak is maintained for next 70 days. It declines gradually thereafter from 126 to 305 days. If the level of milk production is reduced during the early part of lactation below the

potential level, the yield during the remainder period will also be adversely affected. In high yielding buffaloes usually there is high drainage of energy in milk and dry matter intake capacity is limited in early lactation. Inadequate energy intake in early lactation leads to loss of body weight and delay in initiation of post calving estrus cycle. Generally, ovarian cycle ceases when buffaloes loose 15 to 24% of body weight. Thus utmost care should be taken so that they are not underfed during early part of their lactation. The lactating buffaloes in their first and second lactation continue to grow and thus additional 20 &10% of maintenance requirement should also be provided in first and second lactation, respectively.

The lactating buffaloes of 450 Kg body weight can be fed following any of the four type of ration presented on Table 5 depending on availability of feed and fodders.

Table 5. Some calculated rations for a 450 Kg lactating buffalo (Kg/d)

Milk yeild (kg/d; 7% fat)	Ration	Conc. mix/ cake/grain (Kg/d; 90% DM)	straw/ kadbi (Kg/d; 90% D)	legume fodder (Kg/d; 15% DM)	Cerea fodder (Kg/d; 20% DM)
6	a	CM, 2.7	8	15	—
	b	CM,5.5	6.4	---	---
	c	CM, 2.2	---	---	40
	d	---	8.5	30	---
8	a	CM, 3.7	8	15	---
	b	CM,6.4	7.5	---	
	c	CM, 3.3	---	---	40
	d	---	8.0	40	---
10	a	CM, 4.8	7	20	---
	b	CM, 7.3	7.6	---	---
	c	CM, 4.4	---	---	40
	d	---	7.6	50	---
12	a	CM, 5.6	8.3	20	---
	b	CM, 8.5	8.3	---	---
	c	CM, 5	---	---	45
	d	G, 1.0	7.6	50	---
14	a	CM, 6.5	7	20	---
	b	CM, 9.8	8.2	---	---
	c	CM, 6.2	---	---	45
	d	G, 1.0	7.60	60	---
16	a	CM, 7+G, 1.7	7	20	---
	b	CM, 10.9	9.3	---	---
	c	CM, 7.5	---	---	45
	d	G, 2.1	8.2	60	---

CM, concentrate mixture containing 20% CP and 70 % TDN; G, grains like maize or jowar.

For every 50 Kg increase or decrease in body weight from 450 Kg a) 350 g grain or b) 1 Kg straw or c) 3 Kg berseem or d) 2.5 Kg cereal fodder has to be increased or reduced accordingly. For buffaloes in first lactation additional 1 Kg grain or 5.5 Kg cereal fodder or 7.5 Kg legume fodder should be fed to support 300-350 g average daily growth. Similarly, buffaloes in their 2nd lactation should be fed additional 0.5 Kg grain or 2.7 Kg cereal fodder or 3.7 Kg legume fodder to support 120-200 g average daily growth.

Example of some standard concentrate mixture containing about 20% CP and 70% TDN are presented in Table 5.

Some common guidelines to be followed for all categories of buffaloes

While using the feeding schedule suggested in this article for any category of buffaloes following points has to be taken care of.

a) The amount of green fodder was calculated assuming DM% of cereal fodder (maize, oat, jowar, bajra, etc.) as 20% and that of leguminous fodders (berseem, buffaloes pea, Lucerne, etc.) as 15%. For much deviation from these values amounts of fodder need to adjusted.

b) In term of nutritive value, 1 Kg standard concentrate mixture (20% CP & 70% TDN) is equivalent to a) 7.5 Kg leguminous fodder (15% DM) or b) 5.0 Kg cereal fodder + 0.25 Kg groundnut/soyabean cake or c) 1 Kg straw + 0.45 Kg groundnut/soyabean cake. These values can be utilized to further modify the suggested ration depending on availability of feed and fodders.

c) All animals have to be supplemented with good quality mineral mixture. Mineral mixture (without salt type) should be fed @ 0.6% of total dry matter intake and salt @ 0.3% of total dry matter intake. When BIS type I mineral mixture is used, mineral mixture should be fed @ 0.9% of dry matter intake. Thus, young calves should be fed @ 10-20 g BIS type II mineral mixture and 5-10 g salt daily and lactating and adult buffaloes should be fed with 60-80 g mineral mixture and 30-40 g common salt daily depending on body weight and milk production. Mineral mixture of reputed companies and bearing BIS or ISI mark should only be purchased. Many spurious mineral mixtures are available in the market where costly ingredients like phosphorus, manganese, zinc, iodine, etc. are very low or nil and excess of cheaper ingredients like calcium carbonate are present. Many such mineral mixtures are being sold with 'as per ISI' level which is not ISI marked/certified. Feeding of such mineral mixture can be very dangerous because of a possible disturbance of the overall dietary mineral balance and the consequent adverse effect on the absorption and utilization of certain minerals by the animals. Special care has to be taken to meet P requirement of animals fed berseem-based diet. Phosphorus requirement can be met by feeding of 20 g mono ammonium phosphate or 50-70 g mineral mixture per head per day.

d) Buffaloes require vitamin A or its precursor beta- carotene in its diet. Daily feeding of 2-3 Kg green generally meets the requirement of vitamin A. When no green fodder is available, milch buffaloes should be supplemented with vitamin A (20000 to 45000 IU/d) and growing buffaloes should be fed 2000 to 8000 IU/d. Generally, there is no need for supplementing other vitamins to adult buffaloes.

e) The high yielding animals should be fed at regular interval, say twice daily, to maintain a continuous fermentation in their rumen. High yielding lactating buffaloes should preferably be offered four times a day by diving total ration into four equal meals. When high level of concentrate is fed, it should be mixed with the roughage before feeding. Such mixed feeding improves feed utilization. The feed ingredients should be offered after reduction in particle size, i.e. forage components should be chaffed properly and grains should be coarsely ground.

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8 Assessment of nutritional quality of ruminant feeds

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Nutritional quality assessment of feed ingredient is of paramount important for practical ration formulation to optimize overall livestock production efficiency. Feed ingredients are analyzed to get information about their value as a source of nutrients. Where possible feeding trials are conducted to obtain such information, but feeding trials require large quantities of feed, time consuming and expensive to conduct. In practice, feeding trials are conducted to evaluate complex diets and alternative feeding options. Although, certain chemical assays have long been used to give a general picture of the nutrient compositions of feeds, laboratory methods have been refined and extended in recent years. Crude fibre (CF) assays have been replaced by Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) assays. More recent developments have been included *in vitro* gas production, near infra-red spectroscopy (NIRS).

Sampling of feed ingredients

Sampling is the most critical step for quality assessment of livestock feed ingredients. Representative sample should be taken to avoid misleading analysis, with the consequences being unnecessary rejection of an ingredient or acceptance of an ingredient that will lead to losses in livestock production efficiency. Sampling of bulk loads must be performed methodically and meticulously if a representative sample is to be obtained. Sample should be taken at regular intervals from different portions of the bulk. It should not be taken from the first and last part of the load, as they are not representative. More than two samples should be collected, mixed and sub-sample should be stored for analysis.

Before sampling, the bulk should be checked for presence of foreign materials, adulterants, insects, and moulds. The commodity should be free from any foreign materials like sand, stones etc. They should be free from any adulterants like saw dust, mixture with other feed stuffs and weed seeds. It should be free from insect infestation and moulds and mycotoxins.

A. *Chemical analysis of feed stuffs*

The proximate composition (moisture/dry matter, crude protein, crude fibre, fat and total ash) of feed stuffs has been used for evaluation of feed stuffs since long time. The feed ingredients are analyzed as per the methods of Association of Official Analytical Chemists (AOAC). Before starting analysis, the sample should be ground properly to get

representative sample. The proximate principles are analyzed as follows.

1. Dry matter (DM)

A known quantity of sample is taken in pre-weighed moisture cups; and these cups are placed in a hot air oven at 100 ± 2°C for 24 hours. The loss in moisture content after drying is estimated and dry matter is calculated as follows:

$$\text{DM (\%)} = \frac{\text{Weight of sample after drying}}{\text{Weight of sample taken}} \times 100$$

1. Organic matter (OM)

It is determined by subtracting the total ash content from 100.

$$\text{OM (\%)} = 100 - \text{Total ash}$$

2. Crude protein (CP)

Crude protein is estimated as per Kjeldahl's method. A known quantity of sample is taken in Kjeldahl flask and digested with concentrated H_2SO_4 and 2-3 g of digestion mixture (K_2SO_4 and $CuSO_4$ in the ratio of 9:1) till the solution becomes bluish to colourless. After digestion, the contents are cooled and volume is made to 250 ml in volumetric flask. Ten ml of diluted sample is transferred into a micro-Kjeldahl's distillation apparatus and 15 ml of 40 percent NaOH is added to make the content alkaline. About 100 ml distillate is to be collected into a conical flask containing 10 ml of 2 percent boric acid solution having mixed indicator (0.1% methyl red and 0.1% bromocresol green in the ratio of 2:1 in absolute alcohol). The distillate is then titrated against standard sulphuric acid solution (N/100).

$$\text{N (g/100 g sample)} = \frac{0.014 \times \text{Titre vol.} \times \text{Normality} \times \text{Vol. made}}{\text{Aliquot taken} \times \text{Sample taken (g)}} \times 100$$

The crude protein of sample is calculated by multiplying the N-content with factor 6.25.

1. Total ash

A known quantity of sample is taken in pre-weighed China crucible. After charring the sample till the smoke disappears, the crucibles are kept in muffle furnace for ignition at 550°C for 3-4 hours. The ash content is calculated as:

$$\text{Total ash (g/100 g sample)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

1. Ether extract (EE)

A known quantity of sample is taken in Whatman's thimble and extracted for 16 h with petroleum ether (40-60°C) in Soxhlet's extraction apparatus having a pre-weighed oil flask. The oil flask is removed after evaporating the excess of ether and dried in oven for overnight. Again, these flasks are cooled in a desiccator and their weight is taken. The difference in two weights gives the amount of ether extract in the sample.

$$\text{EE (g/100 g sample)} = \frac{\text{Weight of ether extract}}{\text{Weight of sample taken}} \times 100$$

1. Crude fibre (CF)

A known quantity of feed sample is taken in spoutless beaker of 1 L capacity previously marked to 200 ml. Then, 25 ml of 10 percent H_2SO_4 (w/v) is added and volume is made up to 200 ml with water from the sides of the beaker to have 1.25 percent acid solution in the beaker. The contents of beaker are refluxed for 30 minutes. The beaker contents are filtered through muslin cloth with the help of buchner funnel with suction arrangements. After repeated hot water washing, the residue left on muslin cloth is transferred to the same spoutless beaker with smooth steel spatula, followed by little washing of muslin cloth.

Again 25 ml 10 percent NaOH (w/v) is added and volume is made up to 200 ml with water to have 1.25 percent alkali solution. Contents are refluxed for 30 minutes and filtered through the muslin cloth again and washed with hot water. The residue left on muslin cloth is transferred to a clean silica crucible with the help of steel spatula and little water washing. The content is dried in hot air oven at 100 ± 2°C and weighed. Silica crucible containing dried residue is kept in a muffle furnace at 550°C for 4 h for ashing and again is weighed on next day. The crude fiber content of sample is estimated as follows:

$$\text{Crude fibre (g/100 g sample)} = \frac{\text{Wt. of dried residue} - \text{Wt. of ash}}{\text{Wt. of sample taken}} \times 100$$

1. Nitrogen free extracts (NFE)

The nitrogen free extracts (NFE) of sample is calculated as follows:

NFE (g/100 g sample) = 100 – [CP% + EE% + CF% + %Total ash]

A. *Estimation of Fibre fractions*

Proximate analysis divides carbohydrates into two components, the crude fibre fraction which was originally intended to represent the indigestible component and nitrogen free extracts which includes soluble sugars. However, ruminants are capable for digesting some of the fibre components. Modification aimed at characterizing the carbohydrate more

effectively by partitioning it into poorly digested cell-wall component and a readily digested non-structural carbohydrate (Goering and VanSoest, 1970; Vansoest et al., 1991). The assays include neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). NDF is a good indicator of intake and ADF is highly correlated with the digestibility. These assays are now widely used for forage analysis.

Neutral detergent fiber (NDF)

Preparation of neutral detergent solution

Reagents Sodium lauryl sulphate30 gDisodium ethylene diamino tetra acetate (EDTA) dehydrate18.61 gSodium borate decahydrate6.81 gDisodium hydrogen phosphate (anhydrous)4.56 g2 Ethoxy ethanol10 mlDistilled water1 L

EDTA and sodium borate decahydrate are put together in a large beaker with some distilled water and heated on hot plate until dissolved. The solution containing sodium lauryl sulphate and 2 ethoxy ethanol is added. Sodium hydrogen phosphate is taken in another beaker and some amount of distilled water is added and the contents are heated until dissolved. Then, it is added to solution containing other ingredients and volume is made up to one litre.

Determination Procedure

0.5 to 1.0 g of sample is taken in 1 L spoutless beaker. To this, 10 ml neutral detergent solution and 0.5 g sodium sulphite are added. Decalin is avoided in low fat samples. The content of spoutless beaker is refluxed for one hour. After refluxing, the sample is filtered through pre-weighed 50 ml capacity sintered glass crucible grade-I using oil-free vacuum pump. Material is washed with hot boiling water and then acetone to remove all salts. The sintered crucible containing residue is dried in hot air oven (100 ± 2°C) and weighed. It is then kept in a muffle furnace at 550°C for 3 h for ashing to get ash free NDF.

The NDF is calculated on ash-free basis as follows :

$$\text{NDF (\%)} = \frac{\text{Weight of dry residue} - \text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

1. **Cell contents**

   It is determined by subtracting the NDF contents from 100.

   Cell contents (%) = 100 – NDF (%)

2. **Acid detergent fibre (ADF)**

Acid detergent solution: 20 g cetyl-trimethyl-ammonium bromide (CTAB) is added to one litre of 1 N H_2SO_4 (26.7 ml H_2SO_4 in 1 litre distilled water).

Determination Procedure

Approximately 1 g of sample is taken in a spoutless beaker of 1 L capacity. To this, 100 ml acid detergent solution is added and the contents are refluxed for exactly 1 hour. After refluxing the residue is filtered through pre-weighed sintered glass crucible grade-I using vacuum pump, washed with hot water and then acetone is given to remove all salts. Then the residue is dried in hot air oven at 100 ± 2°C and weighed.

The ADF is determined on ash-free basis as follows :

$$\text{ADL (\%)} = \frac{\text{Weight of lignin} - \text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

1. Hemicellulose

Hemicellulose is calculated by subtraction of ADF from NDF as follows:

Hemicellulose (%) = NDF (%) – ADF (%)

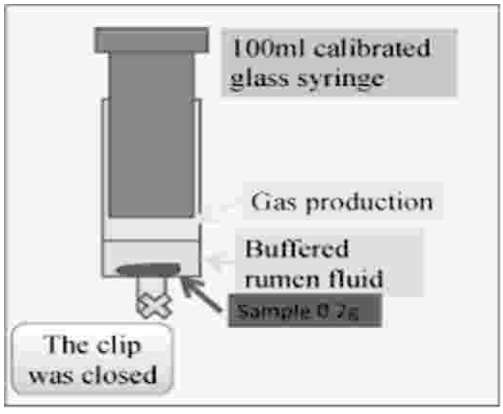
2. Cellulose

Cellulose is calculated as follows :

Cellulose (%) = ADF (%) – (Lignin + Ash) (%)

A. *In vitro gas production test*

This is relatively a new technique, studies the fermentation characteristics of the feed stuffs. This technique enables selection of feed or feed constituents for high efficiency of microbial protein synthesis in the rumen along with high dry matter digestibility, and provides a basis for development of feeding strategies to maximize substrate fixation into microbial cells. This could lead to increase in the supply of protein to intestine and reduce methane production from ruminants. In addition, this technique provides an easy tool to study the effects of various plant bio-active moieties or synthetic compounds for their adverse or beneficial effects on partitioning of nutrients to fermentative gases, short chain fatty acids, and microbial mass production and also methane inhibition potential. The major biological digestion technique currently available to determine the nutritive value of ruminant feeds digestion is with rumen micro organisms as in Tilley and Terry (1963) or using a gas method (Menke *et al*., 1979). The *in vitro* gas method based on syringes (Menke *et al*., 1979; Blümmel *et al*., 1997) appears to be the most suitable for use in developing countries. In the gas method, kinetics of fermentation can be studied on a single sample and therefore a relatively small amount of sample is required or a larger number of samples can be evaluated at a time. In addition, the *in vitro* gas method can better monitor nutrient-antinutrient and antinutrient- antinutrient interactions.



In this method, fermentations are conducted in 100 ml capacity calibrated glass syringes containing 200mg feedstuff and 30 ml buffered rumen fluid (buffer solution and rumen liquor, 2:1 ratio). The syringes are then generally incubated at 39°C for 24 h. For kinetics study, the syringes are incubated for 72 h. After incubation, the total gas produced is measured by the displacement of the piston. Head-space gas (200 l) is sampled and injected into gas chromatograph to know the methane concentration and total methane production is then calculated by taking into account of total gas produced. Liquid sample (2 ml or more) from syringes after completion of fermentation is taken for estimation of volatile fatty acids (VFA) and enzyme activity after processing. The contents of the syringes are then transferred to 500 ml spoutless beakers by repeated washings with NDS. The contents were then refluxed for 1 h and the residue was recovered in pre-tarred filter crucibles. After drying the crucibles (with residue) to constant weight, ashing was done at 500°C for 2 h. Truly degradable dry matter (TDDM), truly degradable organic matter (TDOM), organic matter digestibility (OMD) and microbial biomass production (MBP) are calculated as follows:

TDDM = Feed (DM) incubated - residue (DM)

TDOM = Feed (OM) incubated - residue (OM)

MBP = TDOM - (2.2 x net gas volume)

The gas produced after 24 h of incubation are also used to predict digestibility of organic matter determined *in vivo* and metabolizable energy.

For roughages, the relationships are: ME (MJ/Kg DM) = 2.20 + 0.136 Gp + 0.057 CP, R2= 0.94 OMD (%) = 14.88 + 0.889 Gp + 0.45 CP + 0.0651 XA, R2=0.92

Where ME is the metabolizable energy; DM dry matter, OMD organic matter digestibility; CP, crude protein in percent; XA, ash in percent; and Gp, the net gas production in ml from 200 mg dry sample after 24 h of incubation and after correction for the day-to-day variation in the activity of rumen liquor using blank.

The parameters in the above equation also allow the calculation of a partitioning factor (PF). The PF is defined as the ratio of substrate truly degraded *in vitro* (mg) to the volume of gas (ml) produced by it. A feed with higher PF means that proportionally more of the degraded matter is incorporated into microbial mass, i.e., the efficiency of microbial protein synthesis is higher and lower methane output. Roughages with higher PF have been shown to have higher dry matter intake. Therefore, the PF calculated *in vitro* provides meaningful information for predicting the dry matter intake, the microbial mass production in the rumen, and the methane emission of the whole ruminant animal.

The in vitro gas production technique is very useful in predicting voluntary dry matter intake as well as evaluation of different feed additives especially plant bio-active compounds for their effect on rumen fermentation and methane emission.

A. Near infra-red spectroscopy (NIRS)

Near infra-red spectroscopy is a new technique which is being used increasingly for quality control and feed analysis. NIRS is based on the irradiation with infra-red lights of the organic materials which selectively absorb the energy in the near infra-red wavelength region. The intensity of absorbance is proportional to the concentration of specific chemical bonding or nutrient in the sample. The key advantage of the technique is its speed, as no sample preparation is required. NIRS has been used for measuring chemical composition, *in vitro* digestibility, *in vivo* digestibility and metabolizable energy. The major disadvantage of this technique is high cost of NIR spectrometers and it requires detailed calibration before data produced can be interpreted. This technique is, therefore, suited to the limited ranges of feed, where sufficient reference data are available to calibrate the system.

Conclusion

All the techniques have their own advantages and limitations. Still we are depended on the nineteenth century chemical analysis techniques which are trusted but have considerable limitations. More research is required to further develop the science for tropical feed evaluation particularly for developing countries.

9 Fodder resource management for round the year green fodder supply including silage making

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In India, an estimated 50 million tonnes of concentrate feed ingredients are available annually which yield about 10 million tonnes of crude protein (CP) and 32.5 million tonnes of total digestible nutrients (TDN). In comparison, the annual production of green fodder is estimated at nearly 500 million tonnes, yielding around 12 million tonnes of crude protein and 55 million tonnes of total digestible nutrients. Thus, green fodder is a vital source of nutrients, especially vitamins, for livestock. Green fodder is primarily obtained through cultivation. Presently, 9.38 million hectares of cultivated land is under fodder crops.

The trend of decreasing land holding size and source of regular daily income of the agricultural farmers by cultivating seasonal crops are the key factors for developing more interests towards the dairy farming. Milk production and profit in dairy farming depends upon the feeding and management practices. During recent years, prices of various concentrate feed ingredients like maize grain, soybean meal, groundnut cake, rice polish etc, have increased, which is a major constraint for profitable milk production as the feed cost alone constitutes about 75% of the total cost of production.

Long term economical milk production along with the maintenance of better health and fertility of the dairy animals can only be achieved through feeding of quality green fodder. Thus, the need of the hour is not only to enhance the fodder production, but also to make the fodder accessible round the year to all types of dairy animals adequately.

Importance of feeding green fodder

(i) Fulfils bulk of the animal easily and quickly

(ii) Good palatability and digestibility

(iii) Good source of water as they contain 75-85% water depending upon the type of fodder hand stage of harvesting

(iv) Main sources of fibrous carbohydrate, which are well utilized by the animals(the non-legume fodders are rich in carbohydrate)

(v) Major sources of vegetable protein (legume fodders are rich in protein content)

(vi) Good sources of important minerals like calcium and iron.

(vii) Rich source of carotene (vitamin A) and vitamin E, required to maintain optimum fertility of the animals.

(viii) The milk and milk products of the dairy animals fed on green fodders are rich conjugated linoleic acid (CLA), which has major health benefits in human beings.

Constraints in availability of green fodder: The major constraints in the availability of green fodder may be broadly categorized as follows.

1. Small land holding size of the dairy farmers
2. Unawareness for fodder cultivation
3. Land utilization pattern and crop production system
4. Seasonal variation

Strategies for round the year good quality fodder availability

Fodder crops are the plant species that are cultivated and harvested for feeding the animals in the form of forage (cut green and fed fresh), silage (preserved under anaerobic condition) and hay (dehydrated green fodder). Efficient utilization of limited land resources and other agricultural inputs for obtaining the best from the harvest in the form of herbage per unit area and time should be the primary objective of forage production system. An ideal system, besides giving higher yields and making the maximum use of available resources, must have favourable effect on soil productivity and provide sustainability to the production system. In fact, intensive cropping is the only alternative to boost forage yield from irrigated lands and overall productivity which covers about 30% of the cultivated area in the country. The multicut nature and flexibility in manipulating the duration for several forage species are desirable traits to increase cropping/harvesting frequency. The supply of protein to animals from legumes is cheaper than from concentrates. The non legume fodder is rich in energy. It is therefore, essential that fodders are grown as mixtures in which such as cowpea, guar, berseem, and non legumes such as sorghum, maize, bajra and oat are grown together.

The strategies for round the year fodder availability for the dairy animal scan be chalked out as follows. Green fodder round the year can be supplied mainly by growing various fodder crops with proper time of sowing by planning strategically in such a way that fresh green fodder is available to the animals round the year. Second option is preservation of fodder by nutritional technologies. The green fodder can be preserved for silage making for lean period or for whole of the year. A good quality green fodder round the year to the dairy animals can be supplied by mainly two ways.

1) Fresh green fodder
2) Silage

Fresh green fodder : Table 1 shows the sowing and harvesting time of various fodder crops most suitable in the area. To ensure regular supply of green fodder round the year, sowing strategy should be made by considering number of animals, quantity of feeding green fodder and types of crop to be sown. Normally a multicut fodder crop is ready to give second cut

after 30-35 days of previous cut. So sowing strategy should be in such a way that a cut harvesting should be completed within 30-35 days for efficient utilisation of resources. Similar strategy should be adopted for single cut also because once the harvesting of single cut crop is started, it should be completed within 35-40 days, after that fodder quality start deteriorating.

Table 1: Showing and harvesting time of various fodder crops

S. No.	Crop Name	Sowing time	Harvesting days after sowing	Harvesting time	Seed Rate Kg/acre
1	Sorghum Multicut	March-July	55-60 and subsequent cut after 35-40 days	May-Nov.	20
2	Sorghum Single Cut	April-Aug.	70-75	July-Nov.	20-25
3	Pearl Millet (Multicut)	March-May	50-55 days and subsequent cut after 35-40 days	May-Oct.	4-5
4	Peral Millet (Single Cut)	Last March-Sept.	50-55 days	Mid May-Nov.	3-4
5	Maize	Mid Feb-Mid April	60-75	Mid April Mid Nov.	40
6	Cow Pea	March-July	60-65	May-Oct.	16-20
7	Guar	March-Aug.	65-70 days	May-Oct.	16
8	Chineese Cabbage	Last week of Sept.-Nov.	65-70	Dec.-Feb	1.5-2.0
9	Berseem	Mid Oct.-Nov.	55-60 and subsequent cut after 30-35 days	Second fortnight of Dec. to end of April	8-10
10	Oat	Last week of Sept. to last week of Dec.	65-70	First week of Dec. to Mid April	40
11	Napier	Feb.-March and Aug.-Sept.	65-75 and subsequent cut after 45 days.	Mid Feb.- Mid Dec.	11000 cuttings with 2-3 eyes

For example make the fodder sowing and harvesting plan for 10 adult buffaloes to feed green fodder 40.0 kg of green fodder per buffalo round the year. Strategic planning for this is as follows.

a) Green fodder requirement for 1 month = 120.0 qtls

b) Green fodder requirement for 12 months= 1440.0 qtls To supply 120.0 qtls of fodder the sowing and harvesting plan is as follows.

a) Sow 1.5 acres of multicut sorghum in first week of March

b) This area will supply 120.0 qtls/month of green fodder in months of May, June, July and Aug.

c) Then sow 0.75 acres of bajra in last of June or first week of July.

d) It will give 120.0 qtls of green in Sept

e) Sow 1.0 acre of single cut sorghum or maize by the end of July or first week of August, it will give 240.0 qtls of green in months of October or Nov.

f) In the mean time multicut sorghum field will be empty, prepare it for Chineese cabbage sowing in last week of Sept. From this land sow chineese cabbage in 0.75 acres and it will give 120.0 qtls of green in Dec and in 0.5 acres of this land sow oat in first week of Oct. It will give 120.0 qtls of oat in months of Dec and Jan.

g) In 1.0 acres (0.25 reaming from Multicut Sorghum + 0.75 acres from bajra) sow berseem between Mid Oct- Mid Nov), it will give 480.0 qtls of green in the months of Jan, Feb, March and April.

h) From Maize field, Chineese cabbage field and Oat field spare 1.5 acres for multicut sorghum for next cycle and use remaining land for emergency purpose.

Fodder Mixture : It is advisable to grow fodders, whenever possible as mixtures rather than as single crop. In crop mixture combine a non legume fodder crop such as maize, sorghum and bajra with a legume such as guar and cow pea provide a balanced diet for animals because legume are important sources of proteins and non legumes are rich in energy. Oat can be sown as a mixture with chineese cabbage. Berseem is also sown with oat. Harvest the fodder mixture when maize is in milk ripe to dough stage. Sorghum when one half to one third heads out and bajra when shows the emergence of ears from the flag leaves. Bajra and sorghum mixture with guar is the famous fodder crop mixture of Haryana.

Silage: Silage is widely used in dairy cow rations and is also used to some extent in buffalo rations at organised farms in India. Good quality silage is very palatable to cattle and buffaloes and little wastage occurs during feeding. Production of good quality silage involves fermentation of high moisture forage by anaerobic lactic acid bacteria, and inhibition of growth of aerobic microorganisms that result in spoilage of silage.

Advantages: It is prepared from the plants, which have thick stems like sorghum, maize oat etc. The weeds can also be utilized along with the main fodder crops for silage making. The silage making destroys majority of the weed seeds. It is highly palatable by the dairy animals. The organic acids produced in the silage are similar to those normally produced in the

digestible tract of the ruminants and therefore are used in the same manner.

Principle: The fresh fodder, when packed in a container and allowed to ferment under anaerobic condition produces some volatile fatty acids, which preserve the forage material for a long time with minimum loss of nutrients. The material produced is known as .silage. and the process of conserving green fodder as silage is called ensiling. The quality of silage formation is achieved by controlled bacterial fermentation in the absence of air (oxygen), where the sugars and sugar like compounds in the fodder crops are converted into lactic acid. The acid is bacteriostatic (inhibits growth of micro-organisms), hence helps in conserving the green fodder as stable silage.

Crops suitable: This process is highly dependent upon the sugar content of the fodder. The fodder crops rich in soluble carbohydrates are most suitable for ensiling. Then on-leguminous fodders like maize, jowar, bajra, and napier bajra hybrid have more sugar content as compared to the leguminous fodders like cowpea and guara and are therefore, more suitable for silage making. The leguminous fodders are not suitable for silage making due to low dry matter, high protein and high buffering capacity. The crops should have solid stems so that small amount of air is trapped. However, hollow stemmed crops can also produce good silage but the trampling should be adequate.

Stage of harvesting : The dry matter or moisture content and sugar content of the fodder crop are the important factors for the production of good quality silage, which are again related to the stage of harvesting of the fodder crops. The dry matter percentage at the time of ensiling should be 30-35% i.e. the moisture content of 65-70%. The thumb rule for determining the optimum dry matter or moisture content is to press a handful of chaffed fodder in hand palm, known as .Grab Test.; if the dry matter or moisture is appropriate, the hand palm will remain almost dry. If the fodder crops are cut when too immature, these crops being high in sugar and water content may cause excessive acid formation. Therefore, the fodder crops should be harvested at right stage to produce good silage. The fodder maize crops should be harvested at grain in milk stage. The fodder jowar (sorghum or chari) and bajra crops should be harvested at flowering to dough stage and flag leaf stage, respectively. The fodder napier bajra hybrid crops should be harvested at one and half meter height. If silage will be prepared from natural grasses, then it should be harvested at flowering stage.

Site for construction of silo pit : It should be near to the cattle shed and on a higher and sloppy ground to avoid seepage of rain water into the silo pit. The water table of the pit area should not be high.

Type and size of silo pit : Bunker type rectangular silo pits can be constructed, which should be cemented. The size of the silo pit depends up on the quantity of green fodder available with the farm. However, depending upon the availability of space, numbers of one cubic meter (length, depth and width one meter each) silo pits can be constructed. Every one cubic meter of pit can hold 500 kg of green fodder.

Method : After harvesting, chop the fodder crops to the length of 8-10 cm and then spread in the pit uniformly. Press the chaffed fodder with adequate trampling by manual labour or tractor. Cover the material with polythene sheet or if possibly by 10-15 cm straw layer followed by 5-7 cm layer of soil and then plasters it with mixture of clay and dung. Care must be taken that if any crackor hole develops, then it should be plugged immediately to avoid entry of air or water into the pit.

Silage fermentation : Silage is produced by controlled fermentation of high moisture forage by anaerobic microorganisms that grow in the absence of oxygen. Producing good quality silage is dependent on: 1) rapid removal of oxygen from the silo, 2) an adequate amount of soluble carbohydrates to produce lactic acid, 3) the correct moisture level, and 4) lactic acid producing bacteria dominating the silage fermentation.

Oxygen is present when forage is blown into the silo. During the early stage of silage fermentation, aerobic bacteria, yeast, and moulds grow rapidly, and ferment water soluble carbohydrates to carbon dioxide and water until oxygen is depleted from the silo. Prolonged exposure to oxygen during the fermentation process is associated with poor palatability, and reduced dry matter and energy content of silage.

Aerobic organisms are replaced by anaerobic bacteria once oxygen is depleted from the silo. Anaerobic bacteria convert soluble carbohydrates to organic acids, such as acetic, butyric, and lactic acid, causing a reduction in pH of the silage. A rapid decrease in pH helps inhibit the growth of anaerobic bacteria, such as clostridia, that are associated with reduced silage quality. When silage pH drops to approximately 5, the anaerobic fermentation is dominated by lactic acid producing bacteria. As the pH of silage is reduced to 4.5 or lower, further microbial activity is inhibited by the low acidity. At this point the silage is stable and can be stored for a considerable period of time.

Lactic acid is the primary acid found in good quality silage. Conversion of soluble carbohydrates in forages to lactic acid results in the lowest losses of dry matter and energy during silage fermentation. Silage high in lactic acid is also highly palatable to cattle. A good quality silage has little or no butyric acid. Silage high in butyric acid results from fermentation by clostridia bacteria, and has a lower energy value and reduced palatability.

10 Micronutrients supplementation during different life stages of buffalo

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Micronutrients (minerals and vitamins) are the nutrients required in very smaller quantity by farm animals. However, they are of paramount importance, as their deficiency can have a marked effect on productivity, particularly on reproductive performance and health. The relevance of micronutrients gains more significant under the tropical farming system where crop residues form the major source of roughage for ruminants. Straws and stovers are low in many minerals and vitamins and also contain certain anti nutritional factors like silica and oxalates which affects the utilization of micro nutrients. Deficiency of minerals also remained associated with the deficiency of soil where the feed was grown. Here we will cover mineral and vitamins separately including their classification, function and effect of supplementation.

Classification

Minerals are classified according to their need by the animal body. Minerals that are needed in relatively larger amounts are referred to as major or macro minerals and those that are needed in very small amounts are referred to as trace or micro minerals. The minerals are widely distributed in the body, with calcium (Ca) and phosphorus (P) being present in large amounts followed by magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl), and sulphur (S). Calcium constitute about 46 % and phosphorus about 29% of the total body mineral, while, micro or trace minerals like Iron (Fe), copper (Cu), cobalt (Co), iodine (I), zinc (Zn), manganese (Mn), Selenium (Se) etc. comprises of about 0.3%. Bones, muscles and other soft tissues are the primary storage sites for these minerals.

Vitamins are classified as per their solubility either in fat or water. Vitamins A, D, E, and K are fat-soluble and the B-vitamins and vitamin C are water-soluble. Vitamins have diverse functions including involvement in many metabolic pathways, immune cell function, and gene regulation. A clinical deficiency of a vitamin results in a specific deficiency disease such as rickets when vitamin D is deficient. Subclinical deficiencies may occur in which clinical signs of the deficiency are not evident but performance or overall animal health is less than optimal.

Functions

Macrominerals are important structural components of bone and other tissues and serve as important constituents of body fluids. They play vital roles in the maintenance of acid-base

balance, osmotic pressure, membrane electric potential and nervous transmission. Trace minerals includes cobalt, copper, iodine, iron, manganese, molybdenum, selenium, zinc, chromium and fluorine. Other elements have been suggested to be essential based on studies in other species but these are generally not considered to ever be of practical importance in dairy animal. The trace minerals are present in body tissues in very low concentrations and often serve as components of metalloenzymes and enzyme cofactors, or as components of hormones of the endocrine system.

Vitamins are organic nutrients needed in small quantities to perform specific functions. They do not provide energy but are necessary in the use of energy. Vitamins aid an animal by helping regulate body functions, keeping the body healthy, and promoting resistance to diseases. The deficiency of a vitamin can lead to disease or death. Vitamins are classified as fat soluble and water soluble.

Fat-soluble vitamins are vitamins stored in the fat and released as they are needed by the body. Fat-soluble vitamins can be stored for extended periods. They include vitamins A, D, E, and K.

Vitamin A helps maintain internal and external linings and is necessary for a healthy reproductive tract. Vitamin is not readily available in most feeds. A lack of Vitamin A affects the eyes. Carotene is a precursor of vitamin A. Carotene is found in plants; the body transforms it into vitamin A. The amount of vitamin A in roughage is typically indicated by the degree of greenness. Vitamin A deficiency is the most common deficiency in cattle. Signs of a vitamin A deficiency include watery eyes, a rough hair coat, and a reduced growth rate.

Vitamin D regulates the absorption of calcium and phosphorus. Animals make their own vitamin D when exposed to sunlight.

Vitamin E promotes good health. A lack of vitamin E causes failure in the reproductive system. Alfalfa is a good source of vitamin E.

Vitamin K is important in blood clotting. Vitamin K is typically not necessary in the diet, as bacteria in the digestive system are capable to produce it.

Water-soluble vitamins are vitamins dissolved in water. As water passes through the body, it carries out water-soluble vitamins. Thus, these vitamins need to be consumed every day by mono-gastric animals. Water-soluble vitamins are made by microorganisms in the rumen of a ruminant animal and by fermentation in the cecum of the horse. Water-soluble vitamins include Vitamin C and the B vitamins.

Vitamin C is synthesized in animal tissues. Therefore, it is not necessary to add it to feed rations.

B vitamins are classified into two groups. Group I B vitamins include thiamin, riboflavin, niacin, and pantothenic acid. Group I B vitamins are involved in the release of energy from feed nutrients. Group II B vitamins include folic acid and vitamin B12. Group II B vitamins control the formation of red blood cells.

Mineral Supplementation

Use of mineral supplementation in the form of mineral mixture or mineral licks, urea molasses mineral blocks (UMMB) are most commonly used method. Supplementation can also be achieved through feeding compound feeds, oral drenching or dosing or by administering slow releasing mineral boluses which are retained in the gut and in the form of injectable preparations. Heavy pellets of the mineral or soluble glass which has the specific mineral impregnated into it are lodged in reticulo-rumen are useful in steady supply of specific minerals continuously for long time. This approach is useful during peak period of milk production to overcome certain metabolic disorders like milk fever and grass tetany.

Providing area-specific mineral mixture based on the deficiency of minerals in different agro-climatic zones is most appropriate and cost effective method of mineral supplementation. More practical method is supplementing only the most deficient minerals through mineral mixture by assessing the mineral content in soil, feeds and fodders and in animals of different agro-climatic zones.

One of the other cost effective method of mineral supplementation is to provide feed and plant resources rich in the specific micronutrient, which are commonly being fed / grown in that particular region. For example cakes, brans & rice polish are rich sources of phosphorus. Similarly top feeds / tree leaves and legumes are good sources of calcium, copper and zinc. In general, legume fodders, cultivated green fodders and tree leaves are good sources of Ca, Fe, Zn, Cu, Co and Mn and oil cakes and bran are good sources of P, Zn, Cu and Mn.

Vitamin Supplementation

Digestive systems of young ruminants, before full development of the rumen and its microflora, resemble those of monogastric animals. A reasonable assumption is that ruminants, at the tissue level, require the same vitamins as monogastric animals. Similarity of requirements has been shown for the young ruminant before development of the rumen (usually 6-8 weeks of age). Additional B-vitamin supplementation to veal calves was found to improve measurements of calf health to week nine, but did not improve health parameters from weeks 10 through 23 (Woodet al., 2007). Deficiencies of thiamin, riboflavin, vitamin B6, pantothenic acid, choline, biotin, niacin and vitamin B12have all been produced experimentally in young ruminants prior to the development of the rumen (Miller, 1979).

Dairy cattle require the same vitamins and trace elements as humans and other mammals. However the rumen microbial fermentation supplies a significant amount of water soluble vitamins to the host (cow). In some cases additional supplementation is beneficial, although some water soluble vitamins are degraded to a significant extent by rumen microbes. Fat soluble vitamins A, D, and E are derived naturally from beta-carotene (vitamin A), sunlight (vitamin D), and naturally occurring vitamin E. Fresh forages are rich in beta carotene and vitamin E activity, however the levels decline with maturity of the

forage and during storage. Due to the small quantities required and potential losses in the rumen vitamin A and D are typically supplied in the form of a stabilized, spray-dried be adlet. Vitamin E is more rumen stable than vitamin A and D and is often provided dispersed on fine silica.

It is important that vitamin and trace mineral product forms flow freely and disperse completely in feed mixes. Trace elements required by dairy cattle are found in feeds, soil, and water, as are several potential antagonists of trace element absorption (iron, sulfur, molybdenum, clays, and fiber). Antagonists may reduce the net absorption of both endogenous and supplemental trace minerals in the diet. For this reason a "safety factor" is often used when formulating dairy rations. Absorption of trace elements can be understood based on their chemistry. Absorption of the positively charged trace elements: zinc, copper, manganese, and iron are generally regulated at the gut level, while the negatively charged elements iodine and selenium are regulated primarily through urinary excretion. Antagonisms can occur among the positively charged trace elements at the site of absorption (small intestine). There can be differences in gut absorption of iodine and selenium due to chemical forms (inorganic vs. organic).

Cobalt is a special case in that it is only required as component of vitamin B12, the largest and most complex of the vitamins. In ruminants vitamin B12 is synthesized by rumen bacteria, so cobalt bio availability is related to how well rumen microbes are able to incorporate a given form of cobalt into vitamin B12. High grain diets and subclinical acidosis may interfere with this synthesis.

Steps in vitamin and trace mineral formulation:

1. ***Assessment*:** Step one is to assess the animals, their requirements and their nutrient status and determine the optimum level of supplementation. The animal type, age, stage, and level of production will determine the NRC requirements. Visual assessment of the cattle and an oral history of animal health and production from the herd manager can be used for a gross assessment of trace nutrient status, i.e. are there ongoing health or reproductive problems? Is production (growth or milk yield) up to expectations? Forage analysis and sometimes water analysis is used to infer the presence of antagonists (high iron, sulfates, chlorides, molybdenum, ash) that may make it wise to add an additional safety factor(s) to the diet formulation.

2. ***Formulation*:** In this the animal description (age, body weight, stage and level of production etc.)is input into a ration formulation system. Dry matter intake will be estimated by the formulation program. Dry matter intake is a crucial input value and the most difficult to assess for a specific group of animals. Vitamin requirements are usually expressed in quantity per day (i.e. International Units, grams or milligrams per cow per day, Table 4). Mineral requirements (Table 1, 2 and 3) however have been expressed largely as diet concentration (percent or parts per million). Experts in the field of trace mineral nutrition are

strongly recommending that trace minerals be expressed as quantity (milligrams) of absorbable trace mineral per cow per day in formulation. This reiterates the importance of dry matter intake (for instance in close-up dry cow and fresh cow diets) as well as net absorption (bioavailability) of the trace minerals in the ration. Safety factors(addition of trace nutrients above base requirements) are used in most dairy rations and are based on the judgment of the nutritionist and responses of the animals.

3. ***Re-Assessment*****:** Vitamins and trace minerals are required by and affect multiple body systems such as the immune system, reproductive system, circulatory system, liver, and tissue metabolism. Many of the effects of dietary vitamins and trace minerals are long term and so an appropriate amount of time must be allowed to correctly assess the effects of a change in vitamin or trace mineral supplementation on dairy cattle or any livestock. Effects on immunity might be observable within 30 to 60 days, for example in terms of clinical mastitis or other infectious disease, especially around the time of calving. Changes in reproduction or hoof health will take considerably longer, 3 to 6 months. Beyond 6 months other seasonal and management factors make it more difficult to assess responses to a change in micronutrient supplementation.

Example of micronutrient formulation

1. ***Assessment*****:** We have been asked to formulate a ration for a mixed group of Holstein cows containing both first-calf heifers and older cows. Body weight is estimated at 1450 pounds on average. Days in milk ranges from one week fresh to ~200 days in milk. Based on calving history most cows are between 30 and 150 days milk (estimated average 90 days in milk). Milk yield average is 85 lbs for this group and 70 lbs for the herd overall with a 3.6% fat test and 3.0%protein. Somatic cell count averages 300,000 but has been up and down in recent months. Fresh cows are generally getting off to a good start although clinical mastitis and metritis have been higher than in previous years, including some heifers. Pregnancy rate has slipped during recent months with lower first service conception rates.

Forages consist of corn silage, alfalfa-grass haylage, and grass silage. The remainder of the ration consists of wet distiller's grains and a grain mix(corn, soybean meal, canola meal, soy hulls, wheat bran, minerals, and vitamins). Current ration formulation is based on 50 lbs dry matter intake. Forage analysis indicates that soil contamination may be an issue in the hay crop silages (ash 10 to 12% DM, iron 400 ppm). Well water supply is ample. Water has not been analyzed for quality.

2. Discussion with herd management**:** Although we have been asked to formulate the lactation ration we need to ask some questions about the dry cow and heifer programs to assess that trace mineral and vitamin supplementation and general nutritional needs are being met. Recent data from diagnostic lab field investigations indicate first-calf heifers may calve with marginal trace mineral status due to low or marginal supplementation during the

late rearing period. I four discussion leads us to question the trace mineral or vitamin status of cows at calving we may need to increase supplementation to dry cows/springing heifers or in the lactation ration. The primary concern appears to be udder health/mastitis/SCC which could be due several non nutritional factors that should be explored (cleanliness, milking procedure, dry cow treatment).Reduced conception rates may well be secondary to mastitis, although it may also indicate marginal trace nutrient status in early lactation.

3. ***Formulation:*** a. First step will be to obtain as sound an estimate of actual dry matter intake as possible and an idea of how much this varies day to day, week to week. This will require learning about feed mixing and ration delivery on the farm, how amounts fed are adjusted and whether dry matters are being measured on wet feeds and adjusted for in the batch mix. We may need to ask that refusals be weighed back and that will require us learning about the feeder daily schedules to determine if this is feasible. This sounds like a lot of work but learning more about feeding practices gives us a much better chance of a successful outcome. Trace minerals and vitamins are required in very small quantities so it is important to ensure that these micronutrients are fed as accurately as possible overtime.

b. Next we should review the trace mineral content of forages and by-product feeds. These are the most variable sources of trace minerals. Ash content of forages should be included to account for soil contamination. The presence of antagonists such as sulphur (>0.4%), iron (>400 ppm), molybdenum (>2.0 ppm) should be assessed. Duplicate (and independent) samples of forages and by-products are recommended for trace mineral analysis. It may be a good idea to take water samples for quality analysis.

c. Based on knowledge of the makeup of the group and estimated dry matter intake we can next formulate a ration. Cow data (parity, body weight, milk yield) will be used by most ration programs to predict dry matter intake and nutrient requirements. A lead factor should be applied either to the level of milk production (upward) or predicted dry matter intake (downward) to compensate for cows less than 50 days in milk in the pen. One standard deviation has been determined to be a good guideline for milk yield, but we rarely know the average and standard deviation for milk yield by pen. Therefore it becomes a judgment call whether to set milk production at 10 to 15 pounds above the pen average. This should be reviewed regularly as the average milk yield and days in milk of the pen changes over time.

d. The last step would be to establish a safety factor for vitamin and trace mineral requirements and to select sources of these micronutrients.

i. Forage trace mineral and ash content
ii. Presence of antagonists in water
iii. Other mitigating circumstances such as health challenges, mycotoxins in feed, large variation of cow age, stage of lactation, production level within pen

Table 1: Macro mineral requirements (g/day) for maintenance of buffalo[a]

B.Wt. (kg)	Ca		P	Na		Cl		K	Mg	S
	Dry	Lactating		Dry	Lactating	Dry	Lactating			
200	8	9	4	3	7	5	20	36	4	1
250	10	11	5	4	9	6	25	44	5	1
300	12	14	6	5	10	8	30	53	6	1
350	14	16	7	6	12	9	35	62	7	1
400	16	18	8	7	14	10	40	71	8	2
450	18	20	9	8	15	11	45	80	8	2
500	20	23	10	8	17	13	50	89	9	2
550	22	25	11	9	19	14	55	98	10	2
600	24	27	12	10	21	15	60	107	11	2
650	26	30	13	11	22	16	65	116	12	3
700	28	32	14	12	24	18	70	124	13	3
750	30	34	15	13	26	19	75	133	14	3
800	32	36	16	13	27	20	80	142	15	3

[a]Taken from Nutrient requirements of cattle and buffalo (ICAR-NIANP), 2013

Table 2: Requirements[a] of minerals during growth, pregnancy and lactation

		Pregnancy		Milk (g/kg production)
Element	Growth (g/per kg weight gain)	Initial 190 days	After 190 days	
Ca	17 (<200k gbody wt)	1	10	4.8
	13(200 -300 kg body wt)			
	8 (>300kg body wt)			
P	9 (young)	1.5	6	1.8
	6 (adult)			
Mg	0.45	Nil	0.33	1.2
Na	1.4	Nil	1.39	0.5
Cl	1.0	Nil	1.0	0.8
K	1.6	Nil	1.027	1.2
Mn	0.7	Nil	0.3	3.0
Cu	1.15	0.5	1.5-2.0	3.75
Zn	24	Nil	12	33.33
Fe		Nil	18	5.0
Co				0.012
I				8.5 to 19.5

[a]Taken from Nutrient requirements of cattle and buffalo (ICAR-NIANP), 2013

Table 3: Trace mineral requirements[a] (mg/kg DMI) for maintenance

Mineral	Requirement
Cobalt	0.11
Copper	10
Iron	50
Manganese	15
Selenium	0.25
Zinc	40 (80)[1]
Iodine	0.25 (0.15)[2]

Table 4: Requirements[a] of vitamins in buffaloes

Vitamins	IU/kg body weight	
Vitamin A	110 (adult)	80 (growing)
Vitamin D	30	
Vitamin E	0.8 (lactating)	3.2 (peri parturient)

[1]During summer and transitional animals, [2] in extreme summer

[a]Taken from Nutrient requirements of cattle and buffalo (ICAR-NIANP), 2013

11 Ultrasonography in the management of buffalo reproduction

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In the recent years, ultrasound has emerged a powerful technique for examination of reproductive organs in large animals. The technique of ultrasonography is non-invasive and non-disruptive similar to per rectal palpation. It is simple and safe to both the subject and the operator. Ultrasound examinations can be performed repeatedly without causing much stress to the animal. Ultrasonographic findings are accurate, documentable, and rapid as they facilitate an immediate interpretation during the examination. In large animal reproduction ultrasound is mainly used for knowing cyclic status of animal, differentiating between silent estrus and anovular buffaloes, ovarian pathological conditions, early pregnancy diagnosis, fetal age determination, fetal sex determination, fetal viability and assessing the uterus and its content.

To achieve the target of a calf per year in buffalo, it is essential that estrus is detected at the earliest post-calving and inseminated at the end of the voluntary waiting period of 45-60 day. Buffaloes not inseminated, either cyclic or acyclic should be treated accordingly. Furthermore, pregnancy diagnosis should also be made at the earliest and re-insemination should be made in non-pregnant buffaloes. Failure to detect the estrus is the most serious and widespread problem that affects breeding efficiency in buffaloes. Detection of estrus is necessary for a planned insemination program and is the key to the successful use of artificial insemination (Al) in this species.

Major applications of ultrasonography in buffalo reproduction can be described under following heads:

Diagnosis of cyclic status of animal

The diagnosis of ovarian function is usually based on observing the behavioral signs of oestrus by the animal owner, rectal palpation of ovaries for the presence of CL by veterinarian, or progesterone estimation twice at 10 days interval. Poor estrus expression in buffaloes decreases the efficiency of visual observation while small ovaries with embedded CL reduce the efficiency of rectal palpation. On the other hand, progesterone estimation is costlier, a cumbersome laboratory procedure and require at least two sampling. Therefore, transrectal ultrasonic scanning is very useful for providing

accurate and immediate information about the cyclic status of the animal. These observations can also be helpful in predicting the day of oestrus and ovulation.

Mature CL in buffaloes ranges from 16-25 mm in diameter. On ultrasound scanning, a developing CL appears as an irregular mildly echogenic structure, while mature CL is more echogenic and appears as a well defined granular oval structure with a line of demarcation from ovarian stroma. The line of demarcation becomes faint in regressing CL, due to minor differences in echogenecity from that of the ovarian stroma.

Buffalo in oestrus have good uterine tone and discharge on rectal palpation and have a well develop large dominant follicle of 12-17 mm on ultrasound scanning in one of the ovary with presence of fluid in the uterine lumen. CL of previous cycle is occasionally visible during this period.

Ovulation can be detected by sudden disappearance of the large pre-ovulatory size follicle on subsequent ultrasound examination. It is also possible to detect the corpus haemorrhagicum on the first day after ovulation. The developing corpus haemorrhagicum is a poorly delineated, irregular, slightly echogenic grayish black structure within the ovary.

Buffaloes those ovulated recently, have a corpus haemorhagicum in which fluid may be visible and size is around 12-14mm in diameter. During this period, all follicles are generally less than 6-7 mm and more often uterine tone as well as discharge can be observed.

Ovarian cysts and pathological conditions

Ovaries are said to be cystic when they have one or more fluid filled structure of more than 25 mm that persist for 10 days or more. On Sonographic scanning cysts appear as large nonechogenic round structures (black) that are either single or multiple in one or both ovaries. Differential diagnosis between a follicular cyst and a luteal cyst can be made on the basis of thickness of the cyst wall. The thickness of the wall of a follicular cyst is <3mm while it is >3mm in case of luteal cyst. A small fluid filled cavity may be present in the corpus luteum. Such condition is called cystic corpora lutea and is non pathological. Parovarian cysts appear similar to the follicular cysts but are located outside the boundary of ovarian stroma. Pus in ovarian abscesses usually turns dry and presents hyperechogenic image.

Follicular growth pattern

Ultrasonography can also be used to monitor the growth and regression of ovarian follicles. It is now well established that follicular growth occurs in a wave-like pattern. A follicular wave involves synchronous growth of a group of follicles in both ovaries, from

which one follicle attains dominance over others to become the dominant follicle (DF). Each DF has a growing, static and regressing phase. There may be one, two or three waves of follicular growth during estrous cycle in buffaloes (Baruselli et al.,1997). The first wave begins on day 1, the second around day 9-11 while the third wave appears on day 17 of the estrous cycle. Only the DF of the final wave ovulates, whereas the DFs of the preceding waves undergo atresia. Sequential ultrasonic monitoring of individually identifiable follicles is a powerful tool for studying follicular dynamics during the estrous cycle, pregnancy, postpartum period, superovulation and in relation to hormonal treatments.

Uterus and cervix

Pathological and other physiological conditions of uterus can be identified using ultrasonography in large animals (Fissore et al., 1986). Shape and echotexture of the uterus varies during the different phases of estrous cycle. It is possible to distinguish endometrial folds and myometrium as well as small accumulations of fluid in uterine lumen, during estrus. However, during diestrus, fluid is absent and uterine wall is less distinctive in shape and size. Endometrium can be distinguished from more echogenic myometrium. Ultrasonic image of cervix on day 0 is characterized by presence of non-echogenic fluid collections within a hyperechogenic image. However, during diestrus phase non echogenic area is seen. Uterine pathological conditions, such as endometritis, pyometra, mucometra and mummified/ macerated fetuses are generally characterized by a thickened uterine wall and a distended lumen, filled to varying degrees with partially echogenic snowy patches. In fetal maceration, the fetal bones are identifiable as echogenic particles in the uterine lumen suspended in the fetal fluids. In mummified fetus, the uterine fluid is absent and fetal mummy appears as a poorly defined echogenic mass.

Early pregnancy diagnosis

The greatest advantage of ultrasonography is diagnosis of early pregnancy. With this technique confirmed diagnosis can be made around day 30 post-insemination. The accuracy widely varies depending on a number of factors, including the stage at which pregnancy is examined, experience of the operator, frequency of transducer selected, age and parity of animal and number of times animal is examined. Early diagnosis of pregnancy is based on the detection of a discrete, somewhat linear non-echogenic structure within the uterine lumen. Using ultrasound it is better to diagnose pregnancy around 30-32 days pregnancy in cattle and buffaloes. This is because by this time fetus and fetal fluid inside uterus is quite appreciable and those found non-pregnant can be treated by single injection of prostaglandin to bring them in estrus.

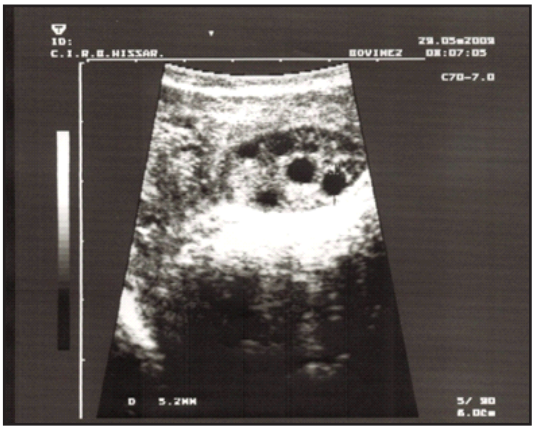


Fig 1. A normal size buffalo ovary with several follicles of medium to small size without corpus luteum (CL)

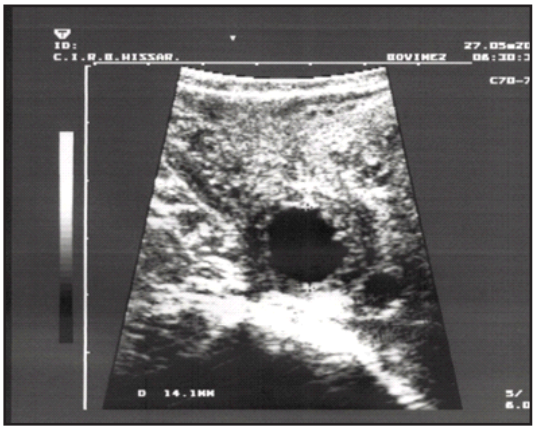


Fig 2. Buffalo ovary with Preovulatory follicle of 14.1 mm

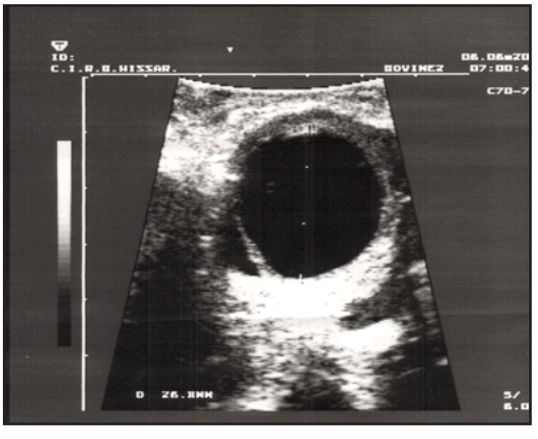


Fig 3. Buffalo ovary with a follicular cyst. Size of cyst (26.8 mm).

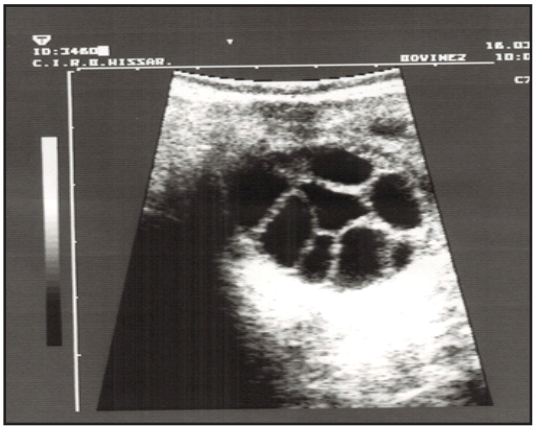


Fig 4. Buffalo ovary with multiple preovulatory follicles on the day of estrus after superovulatory treatment with FSH.

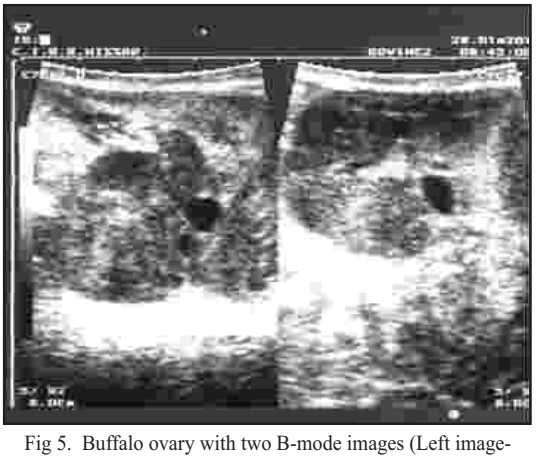


Fig 5. Buffalo ovary with two B-mode images (Left image- Left ovary ; Right image – Right ovary) Day 5 post-estrus having Multiple CL on both right (6 CL) and left ovary (5 CL).

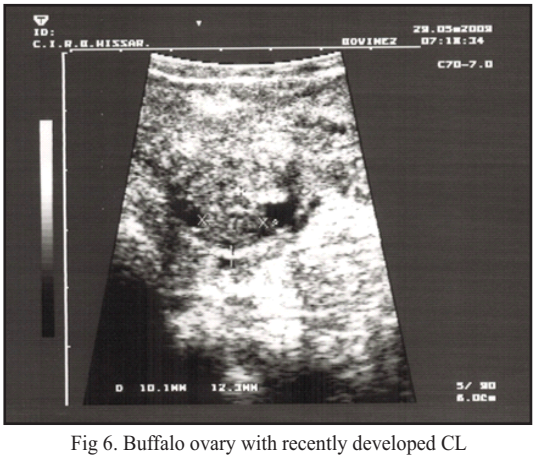


Fig 6. Buffalo ovary with recently developed CL on Day 2 post-estrus. 2 small follicles also present.

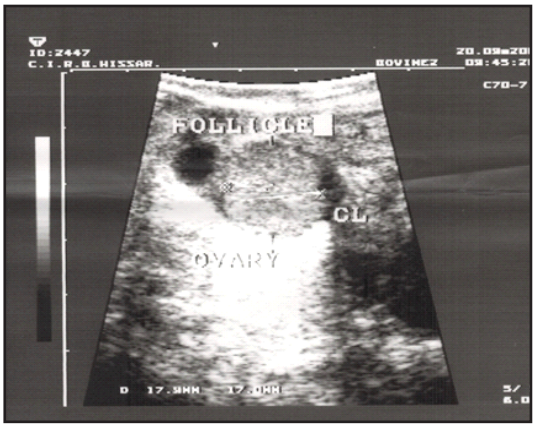


Fig 7. Buffalo ovary with a well developed CL on Day 10 post-estrus.

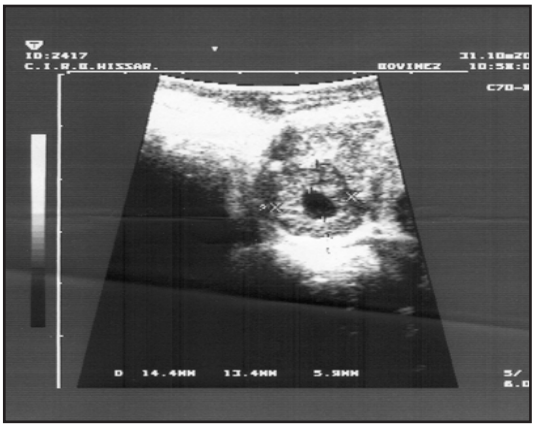


Fig 8. Buffalo ovary with a cystic CL on Day 10 post-estrus. A small cavity is present inside luteal tissue.

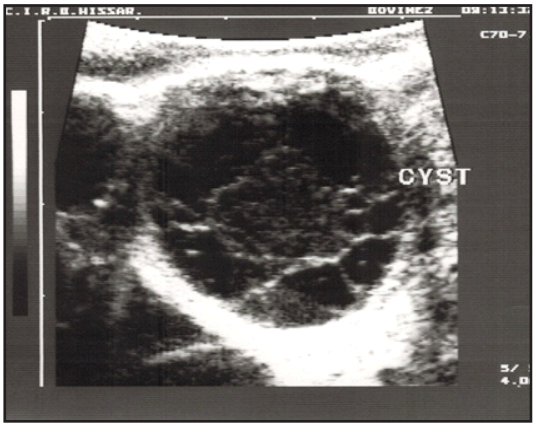


Fig 9. Buffalo ovary with a luteinized follicular cyst. The inner cavity shows a network of echogenic reflections

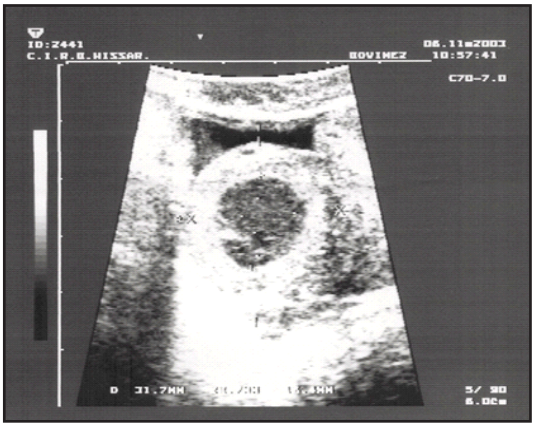


Fig 10. Buffalo ovary with a luteal cyst.

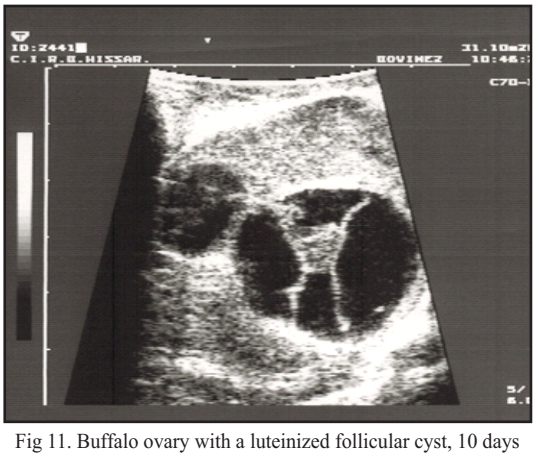


Fig 11. Buffalo ovary with a luteinized follicular cyst, 10 days after GnRH injection. The wall of follicular cyst is luteinized and inner cavity shows a network of echogenic reflections.

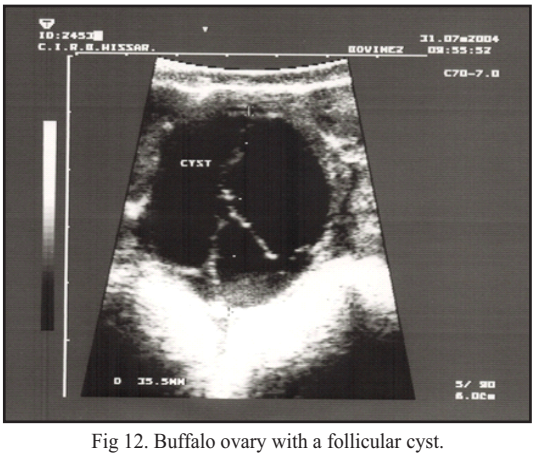


Fig 12. Buffalo ovary with a follicular cyst. The inner cavity shows a network of echogenic reflections.

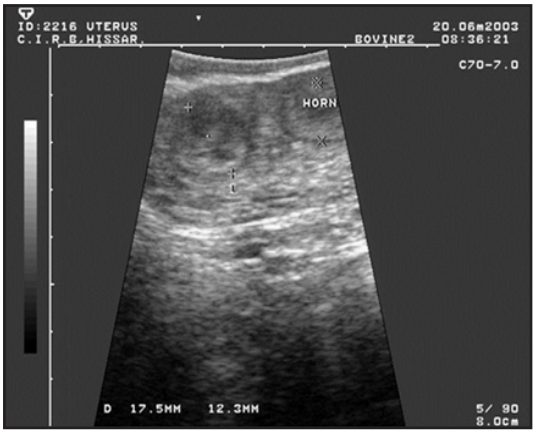


Fig 1. Ultrasonogram of a non-pregnant uterus.

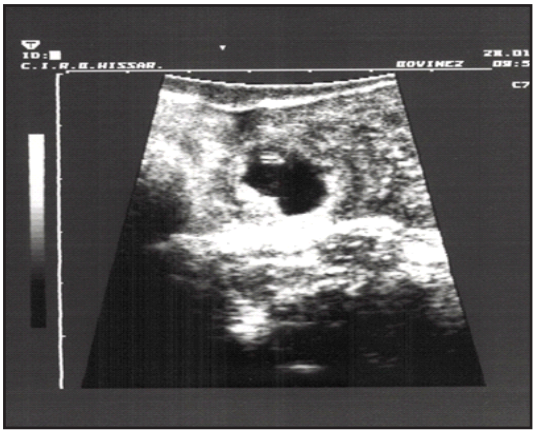


Fig 2. Ultrasonogram at 28 days of pregnancy showing a small fetus.

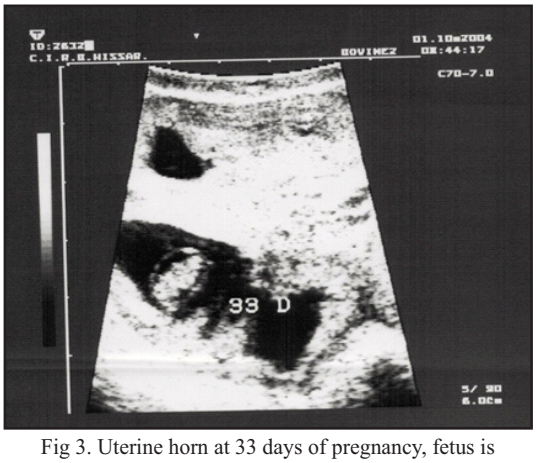


Fig 3. Uterine horn at 33 days of pregnancy, fetus is clearly visible as elongated echogenic structure within an amniotic membrane.

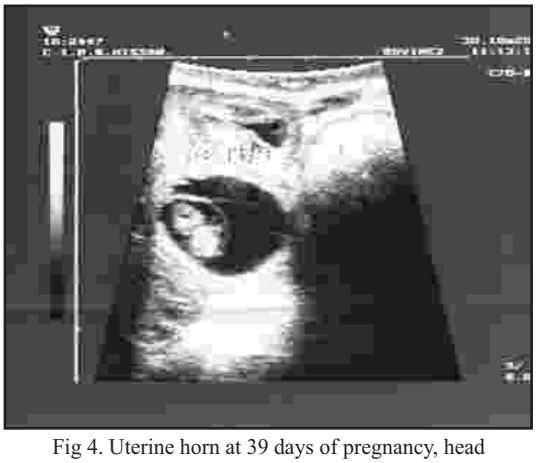


Fig 4. Uterine horn at 39 days of pregnancy, head is clearly visible, developing eye is seen as anechoic spot.

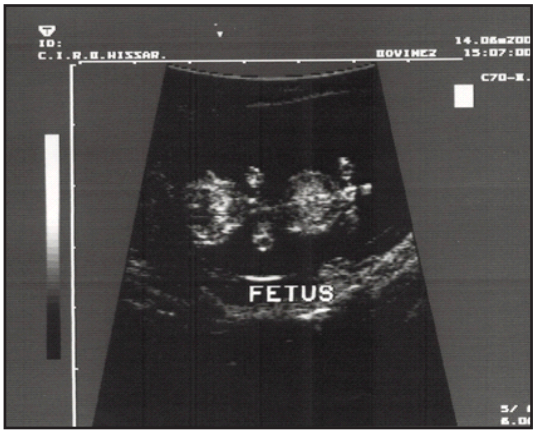


Fig 5. Fetus at 50 days of pregnancy. Head, body, tail and all four limbs are clearly visible.

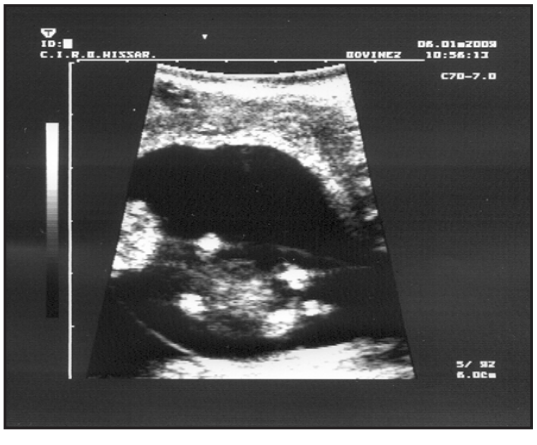
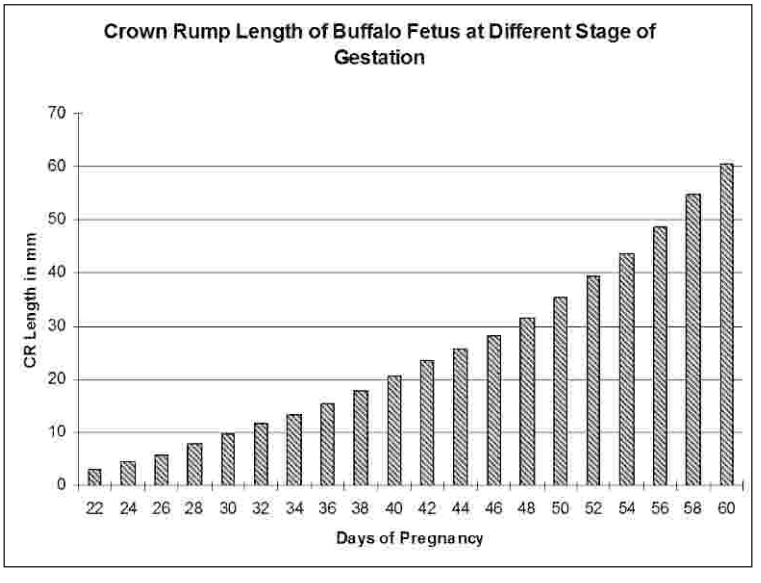


Fig 6. A female fetus showing genital tubercle on day 60 post-insemination near tail

Fetal age determination

Determination of gestational age helps in appropriate management of animal around parturition. Palpation per rectum allows only rough assessment of the fetal age. Crown rump length (CRL, distance a straight line between the fetal crown and the origin of tail) is most excellent for calculating ages of embryos less than 60 days (Sharma et al., 2012) and head or trunk diameters are more easily obtained for fetuses above 60 days old. The mean gestational days at first detection of various fetal structures in buffalo fetus are as follows: heart beat, 27.1±0.3; amnion, 30.4±0.2; optic area, 32.4±0.2; forelimb buds, 34.8±0.2; tail bud, 36.7±0.2; optic lens, 40.8±0.3; umbilical cord, 40.9±0.2; split hooves, 42.7±0.3; fetal movements, 45.0±0.4 and genital tubercle, 49.8±0.4 days (Sharma et al., 2012).



Fetal sex determination

Fetal sex determination is one of the important applications of ultrasonic imaging in buffaloes. Fetal sex determination has the economic advantage for animal owner since a buffalo carrying female fetus is likely to fetch more prices compared to that carrying a male fetus. Similarly, it also helps in deciding whether or not to retain a pregnant buffalo already earmarked for culling, besides being useful in progeny testing programme. In bovines, diagnosis of fetal sex is based on the presence of scrotal swelling and mammary glands or the location of genital tubercle which leads to the formation of penis or clitoris (Curan, 1992). In buffaloes, genital tubercle appears around day 50 in between the hind legs as a prominent bilobular structure. Thereafter, it migrates in the vicinity of the umbilical cord in males and the tail in females. Correct diagnosis can be made between day 55-100 post breeding in buffaloes (Sharma *et al*., 2011).

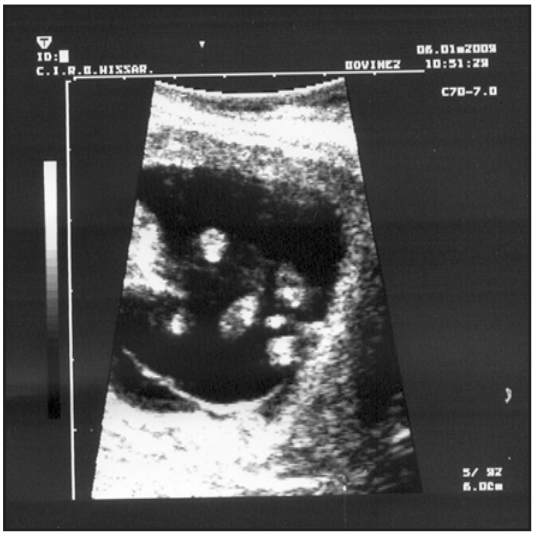


Fig 7. A male fetus showing echogenic structure (genital tubercle) on day 60 post-insemination just behind umbilicus.

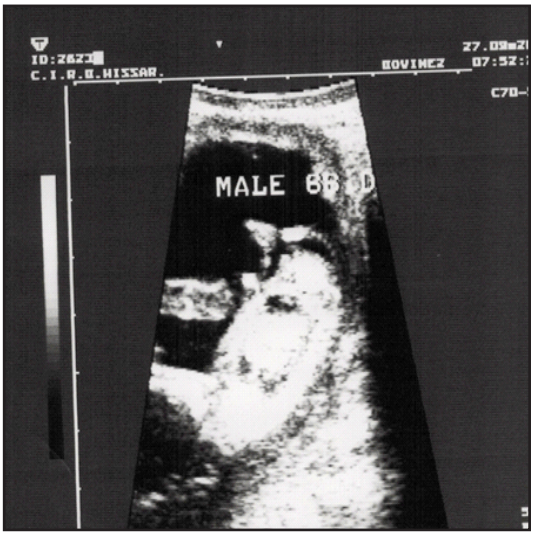


Fig 8. Fetus showing attachment of umbilicus with body having an echogenic structure (genital tubercle) near its attachment on day 66 post-insemination- A male fetus.

Ultrasonography in Embryo Transfer program

In ET programme ultrasound can be used to evaluate superovulatory response and devising suitable strategies which could enhance ovulation rate in donors. The response to a given quantity of gonadotrophin is extremely variable both between and within individuals. Furthermore, embryo must be transferred into the uterine horn, ipsilateral to the side ovary bearing corpus luteum. Accurate identification of early CL by rectal palpation is extremely difficult especially in buffaloes. Ultrasonic examination of recipient buffalo for the presence of CL on the day of embryo transfer (Day 5/6) is very helpful for its correct diagnosis.

Ultrasound guided ovum pick up and IVF technique

Considerable progress has been made in harvesting oocytes from slaughter house ovaries and creating suitable media for in vitro maturation, fertilization and subsequent embryo culture. However, limitation in this technology has been the pedigree status of oocyte collected from abattoir. Curiously, almost 99% population of the follicles present in the ovary undergoes atresia and only less than 1% of it is used in the active reproductive phase of buffaloes. Therefore, use of transvaginal ultrasound guided oocyte aspiration technique in live animals is a better alternative to trap these unutilized oocytes and produce elite embryos of known genetic make up using IVM-IVF technology.

Apart from oocyte aspiration, ultrasound guided puncture technique can also be used for aspiration of fetal fluids for sex determination, biochemical analysis and hormonal estimation; sampling of uterine contents for diagnostic purposes and injection of substances into the ovaries, follicles, corpus luteum and uterus for research purposes.

Oestrus induction and ovulation synchronization

Ultrasonic scanning of ovaries provides useful information for rational treatment of silent estrus and anestrus conditions in buffaloes. Buffaloes detected with a mature CL in the ovary, can be administered with single or double injections of $PGF_2\alpha$, 11 days apart. This is followed by insemination at observed estrus, usually occurring within 2-5 days post-injection. Acyclic buffaloes (no CL - True anoestrus: delayed puberty, post partum anoestrus, summer anoestrus), on the other hand, essentially require progesterone priming to induce fertile ovulatory oestrus. It has been revealed by ultrasonic examinations that wave like pattern of ovarian follicular growth continues even during anoestrus period and diameter of the largest dominant follicle (DF) may attain a size equivalent to that of the preovulatory follicle (Sharma et al., 2004). This implies that anovulatory anoestrus condition is due to failure of the DF to ovulate rather than its absence. In anoestrus buffaloes, ultrasonography revealed that the DF undergoes atresia rather than ovulation, possibly due to failure of appropriate preovulatory LH surge. Exogenous GnRH induces ovulation / luteinization of large DFs, leading to subsequent formation of CL which can be readily identified with ultrasonography (Sharma et al., 2004).

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12 Application of oestrus induction and synchronization protocols in buffalo

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Failures to resume cyclicity after calving and silent oestrus are the two most serious problems that affect reproductive efficiency in buffaloes. To achieve the target of a calf per year in buffalo, it is essential that estrus is detected at the earliest post-calving and insemination is made at the end of the voluntary waiting period of 45-60 day. Detection of estrus is necessary for a planned insemination program for buffaloes and is the key to the successful use of artificial insemination (Al).

As a short term strategies hormonal treatment are preferred to induce cyclicity or bringing them in heat within a narrow window. There are several hormonal preparations commonly used to induce estrus in cyclic as well as acyclic buffaloes. These include administration of prostaglandin, progesterone, gonadotrophins and gonadotrophin releasing hormone or their synthetic analogues, either alone or in various combinations. Some of the commonly available hormonal preparations are as follows.

Gland	Hormones	Ingredients in commercial preparation	Commercial Product	Packing
Hypothalamus	GnRH	Buserelin 4mcg/ml	Receptal, MSD	2.5 ml, 10 ml
		Buserelin 4mcg/ml	Gynarich, Intas	2.5 ml, 5 ml
		Buserelin 4mcg/ml	Pregulate, Virbac	5 ml
Pituitary	hCG	Chorionic gonadotrophin	Chorulon, MSD	1500 IU Vial
Pituitary	FSH	Follicle stimulating hormone	Folltropin V, Bioniche	400 mg Mostly used for superovulation
Uterus	PMSG	Pregnant mare serum gonadotrophin	Folligon, MSD	1000 IU vial
Uterus	Prostaglandin	Dinoprost tromethamine 5 mg/ml	Lutalyse, Pfizer	5 ml, 10 ml
		Cloprostenol 250 mcg /ml	Estrumate, MSD	2 ml
		Cloprostenol 250 mcg /ml	Pregova, Virbac	2 ml
		Cloprostenol 250 mcg /ml	Vetmate, Vetcare	2 ml
		Cloprostenol 250 mcg /ml	Pragma, Intas	2 ml
		Luprostiol 7.5 mg/ml	Prosolvin, MSD	2 ml, 10 ml, 20 ml
		Tiaprost trometamol 0.196 mg/ml	Iliren, MSD	10 ml

Ovary –CL	Progesterone	1.38 g Progesterone implant	CIDR, Pfizer	Pack of 10
		3.3 mg Norgestomet ear implant and 3 mg Norgestomet + 2 mg Oestradiol injection	Crestar, MSD	Pack of 5
		Three 186 mg (each) + one 400 mg medicated progesterone device	Triu-B, Virback	Pack of 10
		Hydroxyprogesterone 250mg/ ml	Prolutek,	3 ml
		Hydroxyprogesterone 250mg/ ml	Duraprogen	3 ml
Ovary- Follicle	Estrogen	Oestradiol valerate 10 mg/ ml	Progynon depot, German Remedies	1 ml Ampoule
		Oestradiol valerate 10 mg/ ml	Pregheat, virbac	5 ml

Diagnosis of cyclic status of animal

Diagnosis of the silent estrus or anovulatory condition is essential for instituting the most suitable treatment for oestrus induction. The diagnosis of ovarian function is usually based on observing the behavioral signs of oestrus by the animal owner, rectal palpation of ovaries for the presence of CL by veterinarian, or progesterone estimation twice at 10 days interval. Poor estrus expression in buffaloes decreases the efficiency of visual observation while small ovaries with embedded CL reduce the efficiency of rectal palpation. On the other hand, progesterone estimation is costlier, a cumbersome laboratory procedure and require at least two sampling. Therefore, transrectal ultrasonic scanning has emerged as a very useful tool for providing accurate and immediate diagnosis about the cyclic status of the animal. These observations can also be helpful in predicting the day of oestrus and ovulation.

Oestrus induction and synchronization

For initiating oestrus induction and synchronization program, it should be the priority of veterinarian that protocol must be simple with minimum use of hormone, minimum handling of animal and minimum visits. For this individual animal must be examined for cyclic status (cyclic animals having CL vs anovular buffaloes). The choice of treatment is based on cyclic status of animal. Following methods are commonly used for this purpose:

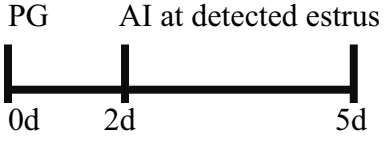
- Estrus and ovulation synchronization using prostaglandin F2α alone in cyclic animals
- Estrus and ovulation synchronization using progestogen
- Estrus and ovulation synchronization using GnRH based protocols
- Estrus and ovulation synchronization using progestogens and GnRH based protocol

For induction of estrus in cyclic animals having a functional CL - either the CL is regressed prematurely (with prostaglandins), or the luteal phase is extended artificially with exogenous hormonal (progestogens) supplementation, so that it can then be terminated abruptly in order to mimic spontaneous regression of the CL. Prostaglandins are effective in inducing estrus in cyclic animals only. However, if given at random stage of estrous cycle oestrus induction rate are nearly 66% as this PG is effective only during two third period of estrous cycle. Acyclic buffaloes essentially require progesterone priming in order to induce overt, ovulatory and fertile estrus. PG administration is ineffective in anovular buffaloes and also not required while administering progesterone implant.

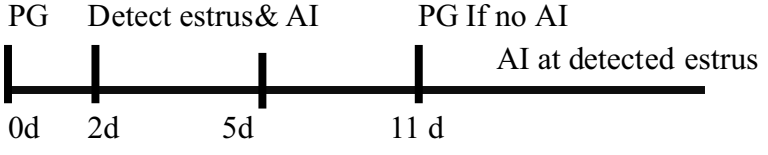
Estrus and ovulation synchronization using prostaglandin F2 alone in cyclic animals

PGF2α causes luteolysis of CL and there is sharp decrease in concentration of progesterone hormone. After PG treatment animals are reported in heat between 2-5 days. PGF is not effective in animals that do not have a CL. Therefore, prepubertal heifers, postpartum acyclic females and even cyclic animals between first five to six days of the estrous cycle do not exhibit any response to PG administration. PGF alone is a very effective management tool in cyclic animals having a sound heat-detection program in the herd. There are three methods for estrus synchronization using PG injection.

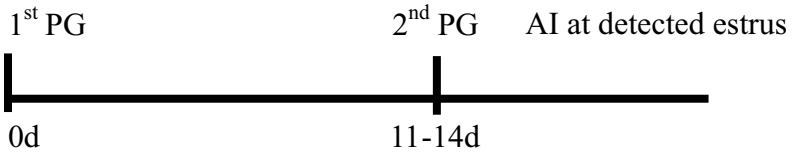
First method : All animals having a detectable CL are given an injection of PG and observed for estrus for five days. Animals found in heat are identified and inseminated. Conception rates are near spontaneous estrus (Sharma et al., 2009).



Second method: In this method PGF2α is injected to all and animals are inseminated at observed estrus between day 2-5. Animals not inseminated during this period are given second injection of PG 11days later and insemination is done again at observed estrus.

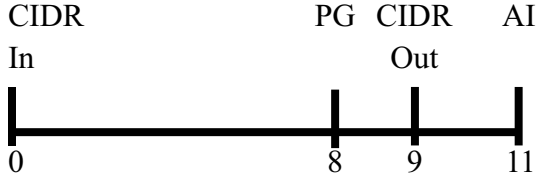


Third method: Inject the PGF2α in all the animals at day 0 (Day of treatment) and again at day 11. Animals found in heat after 2nd PGF2α injections are inseminated at observed estrus.

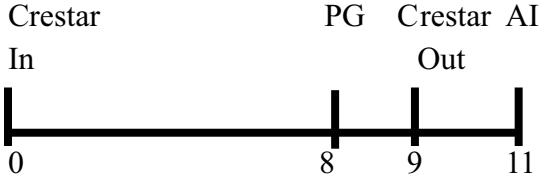


Estrus and ovulation synchronization using using progestogen

CIDR intravaginal Pessary: The CIDR is a "T" shaped device with flexible wings that collapse to form a rod that can be inserted into the vagina with an applicator. On the end opposite to the wings of the insert a tail is attached to facilitate removal with ease. The backbone of the CIDR is a nylon spine covered by progesterone (1.38g) impregnated silicone skin. Progesterone concentrations are maintained at a relatively constant level during the seven days the insert is in the vagina. It is always good to squeeze the vagina after CIDR removal by back racking to clear the vagina for any pus type discharge at heat. An injection of PG is also administered preferably 24 h before implant removal or at the time of implant removal. Animals are found in heat within 48-72h of CIDR removal. [Lamb and Larson, 2004].



Crestar ear implant : The implant has two part-Crestar ear implant (3.3 mg Norgestomet) and Crestar injection (Crestar injection of 2 ml, containing 3 mg Norgestomet and 5 mg Estradiol valerate). The implant is placed for 7-9 days subcutaneously in ear along with Crestar injection. An injection of PG is also administered preferably 24 h before implant removal or at the time of implant removal. Animals are found in heat within 48-72h of CIDR removal (Nayak *et al*. 2009; Kumar and Mandape, 2004) There is no need to inject the Crestar injection and PG if animal is acyclic.



Estrus and ovulation synchronization using GnRH based protocols

For estrus and ovulation synchronization in cyclic cows Pursley and coworkers (1995) developed a protocol combining $PGF_{2\alpha}$ and GnRH injections, which provides greater synchrony of induced estrus and ovulations thus permitting fixed-time

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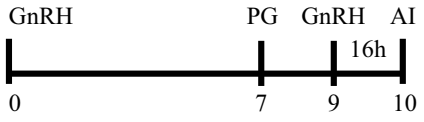

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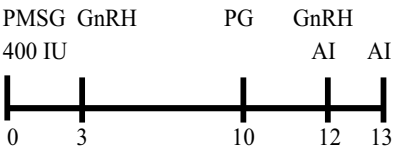

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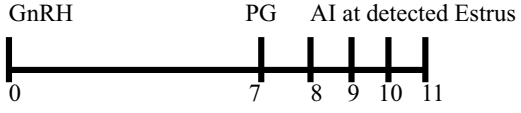

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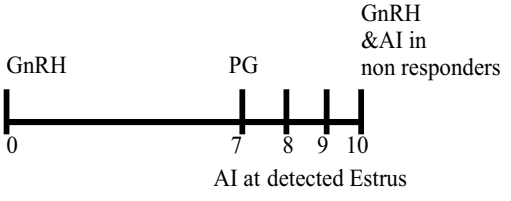

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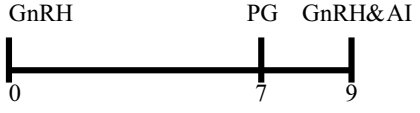

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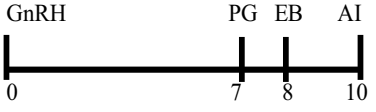

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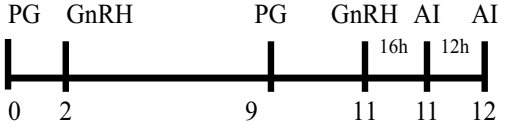

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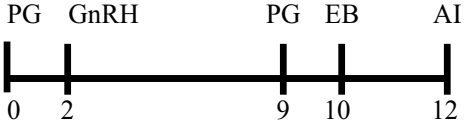

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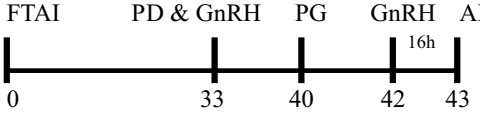

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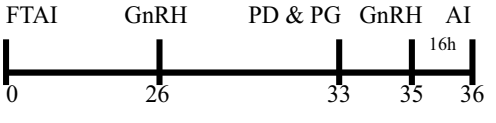

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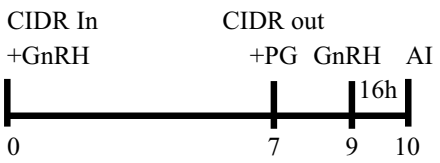

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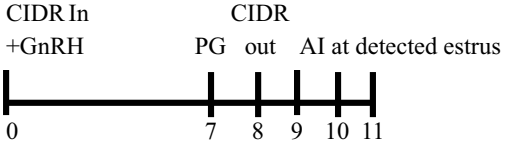
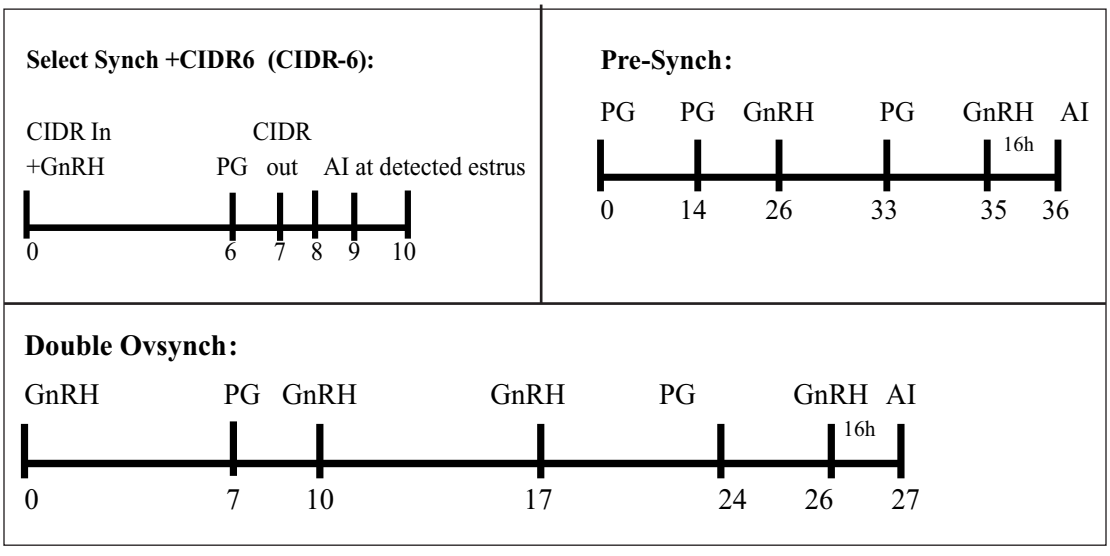




inseminations, without the need for estrus detection. This protocol is based on the concept that first injection of GnRH (day 0 of treatment) given at random stage of the cycle causes ovulation of the largest dominant follicle and starts a new wave of follicular development. Subsequently an injection of PG is given 7 days later to cause the lysis of the CL. Later on, a second GnRH injection is given on day 9 to facilitate ovulation and animal is inseminated around 16 h after the second GnRH. This fixed time AI (FTAI) protocol was named as Ovsynch and several variations have been developed subsequently in the quest for higher conception rate, better ovulation synchronization and scheduling resynchronization program.

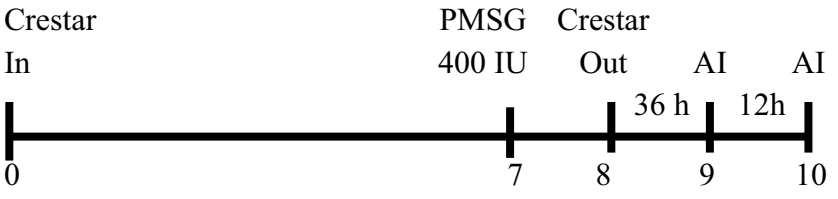
Choice of treatment

It is very difficult to choose the protocol among different available protocol. It should be kept in mind that these protocols are not a replacement of poor heat detection system but help in reducing days open in those animals which are not detected in estrus at proper time. In GnRH based protocols, estrus and ovulation synchronization is good however, conception rate is not so high. It should be the priority of veterinarian that protocol must be simple with minimum use of hormone, minimum handling of animal and minimum visits.

Therefore, if animal is cyclic and having a mature CL, inject PG and inseminate the animal on observed estrus. If animal is not observed for heat within 2-5 days, inject one more injection of PG 11-14 days later after first injection and bred at observed estrus. If animal is acyclic use progesterone implant (CIDR/ Crestar) for 7-9 days and inject 400 IU PMSG one day prior to implant removal. Animals are in heat between 36-48 h after implant removal and should be inseminated. Using progesterone implant, acyclic buffalo should be inseminated

36 and 48 h whereas cyclic buffalo should be inseminated at 48 and 72h after implant removal. There is no need to inject PG in confirmed acyclic animals. However, if animal cyclicity is not confirmed then PG injection should be given 24 hr prior to implant removal. If these implants are not available then Ovsynch Plus protocol is better choice in cyclic as well as acyclic animals.

Progesterone Implant for confirmed acyclic buffaloes



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13 An overview of estrus detection in buffaloes

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The domestic water buffalo (*Bubalus hbubalis*) is very important livestock resource in Asian continent particularly south-east Asia including India, ASEAN countries, the Mediterranean region and Latin America. Out of total world population of buffalo, 96% belong to Asia. The world population of buffalo is estimated to be 199 million (FAOSTAT, 2015) with more than 97% of the population located in Asia. In recent decades, buffalo farming has expanded widely in the Mediterranean and Latin America in addition to, in Central/Northern Europe where several herds were introduced. Milk is the most important livestock commodity. In this perspective, buffalo has emerged as the "black gold" (Borghese, 2005) of India and proved itself as the favourite milch animal of farmers. It has a significant role in the agricultural economy of many developing nations by providing milk, meat and draught power. The domestic water buffalo, being one of the most important livestock animals in India has an important role in the agrarian economy and will continue to have a bigger role in future as well. The successful breeding and thus milk production depend on the fertility performance of female animals, which must be bred at the right time after estrus is observed. The buffaloes can utilize poorer quality roughages, adapt to harsher environments and are more resistant to numerous bovine tropical diseases. Despite these merits, buffalo have relatively poor reproductive efficiency irrespective of their location throughout the world. Buffalo exhibit many of the known reproductive disorders including delayed onset of puberty, poor estrus expression, longer postpartum ovarian quiescence, and most importantly lowered conception rates particularly when bred artificially (Gordon, 1996).

However, it can be understood that the true realization of production potential in buffaloes is not yet achieved because of several constraints including impaired reproduction. In general, the reproductive efficiency in buffaloes has been hampered by late puberty, seasonality of calving, longer postpartum anoestrus and subsequent calving interval. Visual signs of estrus are not prominent in the buffalo. While accurate and timely detection of estrus is pre-requisite for successful artificial insemination program, lesser overt or weak signs of estrus in buffaloes has been one of the key reason for the highest percentage of the conception failures during the first service. In this background, we are summarizing the prevalent estrus detection methods and a way forward for it.

2. Buffalo reproductive cycle and the 'bottlenecks'

In buffaloes, the mean length of the estrous cycle is 21 days, with greater variation than observed in cattle. The signs of estrus in buffalo are less evident than in cattle and homosexual behaviour between females is rare. Estrus behaviour in buffalo has a lower intensity than in cows and is, consequently, much more difficult to detect. Acceptance of the male is considered as the most steadfast estrus indicator in buffalo. Frequent urination, bellowing, vulvar swelling, mucus discharge are also salient estrus signs in river buffalo, but their expression is extremely weak (Barile, 2005). The duration of estrus has been found to be similar in river and swamp buffalo, varying between 5 and 27 h, and ovulation occurs about 24-48 h (mean 34 h) after onset of estrus, or 6-21 h (mean 14 h) after the end of estrus (Kanai *et al*., 1990; Perera, 1999). The hormonal changes occurring in peripheral circulation are similar to those observed in cattle, but the peak concentrations of oestradiol-17β and progesterone are less. The number of follicular waves during an estrous cycle generally varies from one to three and influences the span of the luteal phase as well as the inter-ovulatory interval. Under most favorable conditions, dairy types managed with restricted or no suckling resume estrus cyclicity by 30-60 days after calving, while swamp types with liberated suckling do so at 60-90 days. However, in many farming systems extended postpartum anoestrus is a major problem. Buffaloes are shy, poor and difficult breeders apart from being predisposed for late maturity and anoestrous conditions. Onset of estrus is not very pronounced and silent estrus is another major problem. In other way, they don't exhibit obvious signs of estrus and express the behavioural signs of estrus during a limited period of year, which is pronounced more during September to February months.

Estrus detection has been one of the most important components for any effective breeding program (Pelissier, 1982). Therefore, the lesser overt signs of estrus in buffaloes has been one of the main reason for the highest percentage of the conception failures during the first service. This seriously jeopardizes the buffalo entrepreneur's or farmer's effort to achieve one live birth per 14 months, and consequently more numbers of live births during a buffalo cow's productive life. All these culminates in to ruthless economic losses to farmer and finally to nation's economy. In actual terms, each missed breeding is a missed opportunity which imposes a loss of milk production for 21 days, in addition to bearing the feeding cost for maintaining dry animals. The success rate of artificial insemination is approx. 50% in buffalo or even less at field level. Highest conception rates are achieved when the insemination is done 8 to 12 hours after the start of estrus (known as “standing heat”).

3. Need of estrus identification and ovulation prediction in buffaloes

Estrus detection in buffaloes is an important phenotype for effective breeding. Loss of revenue due to failure to detect estrus or flawed diagnosis of estrus has been estimated to the tune of over $300 million to the USA dairy industry (Senger, 1994) and in India, the approximate loss is about several crores. That missing event leads to a huge economic loss to

India having ~55 million breedable buffaloes. The success of artificial insemination (A.I.) depends on critical examination of animals to sense heat at appropriate time as late insemination leads to failure of conception. Correct timing of insemination with ovulation helps to achieve the objective of successful fertilization.

4. Current methods of estrus detection in buffaloes : There are various methods for detection of estrus in buffaloes. However, it has to be understood that each one has its own flaw in terms of accuracy and efficiency and are not absolute. Different signs are studied carefully for detection of heat. Heat detection aids are very vital tools for efficient reproductive management if used in combination with expert eye. For example, Visual observation with tail paint is 98 % efficient as compared to heat watch alone *i.e.* 91 %. It has already been reviewed extensively by Rao et al. (2013).

1. Visual symptom/ change in parlor behavior based heat detection: Estrus behaviour in buffalo has a lower intensity and it is more difficult to detect. Acceptance of the male is considered as the most reliable estrus indicator in buffalo. Continuously urination, bellowing, vulval swelling, mucus discharge are salient estrus signs in buffalo, but their expression is weak and vary from season to season. Other symptoms include inadequate appetite, nervousness, riding on other buffaloes or allow other buffaloes to mount on her, reduction in milk yield and crutching the back and lumber region etc.

2. Vaginal cytology, pH, conductivity measurement : The differential staining properties of vaginal smears shows cytoplasmic lipids in vaginal smear and urinary sediments. Smear also shows increase in cornified acidophil cells during estrous period. The pH is also a good indicator of animal in estrus. The pH falls from 7.0 to 6.72 one day prior to estrus which further fall to a level of 6.45 immediately before ovulation. Vaginal resistance varies with stage of cycle. The decrease in electrical resistance or rise in conductivity of the vaginal tissues and discharges during estrus were well reported. Vaginal probe approach also includes intra-vaginal or implantable resistance devices with transponder to send the information directly to computer. Measurement of vaginal conductivity entail repeated insertion and repeated measurement can produces inflammation which may affects the reading. Vaginal resistance can differ with site of probe in animal i.e., measurement of resistance in posterior vagina is less reliable than anterior vagina.

3. Fern pattern of saliva and cervical mucus discharge: The saliva and cervical mucus can be collected from buffalo/cow suspected of heat. It is smeared on slide and dried naturally in air. If fern pattern appears in slide in microscope, it indicates animal in estrus. Salivary fern pattern is also an unorthodox indicator of estrus(Ravinder et al., 2016). If fern pattern show more branching, it shows appropriate time for insemination.

4. Manual heat detection by uterine tone: The female reproductive tract of the buffalo is similar to that of the cow in structure and position, although the cervix is less conspicuous and the uterine horns are more coiled. Similar to cattle, the uterine horns are turgid and coiled

and have marked tone during estrus; they are flaccid with lack of tone during diestrum. Docile response of the animal to the placing of palm of hand on the rump and response to light massage of vulval lips were seen in the heifers in estrus. The maximum tone in uterine horn remains on day of estrus. The conception was directly proportional to the degree of tonicity of uterus (Gunasekaran et al., 2007). Yet it is one of the most reliable indications, although it requires expert hand for the purpose.

4. Cervical mucus glucose content & endometrial biopsy : The glucose test is more positive on day of estrus than on the other day. Endometrial biopsy shows rise in phosphate activity around estrus.

5. Milk yield fluctuation : Sudden drop in milk (75% of its usual yield) on estrus followed by recovery at next milking is good indication of estrus. Such drop in milk is due to concentration of estradiol in blood. Hitherto it is good indicator but it requires milk yield recording.

6. Temperature measurement : The temperature of skin, deep body, vagina and milk is measured as means of detecting estrus. Radio telemetry based vaginal temperature measurement was also used with reliable result. The ruminal temperature also raised during time of estrus measured by sensor based intra ruminal electronic radio-telemetric bolus. On the other hand estrus detection rates by temperature monitoring rarely exceed 70-80 percent.

7. Heat expectancy charts : This simple management aids allow heat to be recorded and the time of next heat to be predicted so that buffaloes can be viewed more closely at the time of the next expected heat. Both manual and computer based system are developed which assist in easy detection of heat.

8. Tail painting : Tail painting/chalking is easy method of heat detection. It is commonly used in combination with visual observation. Fluorescent paint may be used in night for heat detection with artificial provision of electric lamp. The result is not good in buffaloes due to wallowing activity and false positive reading can occur if smearing occurs from false contact with low tree branches or from lying in free stalls. A detection rate of 94 percent was shown to be possible.

9. Use of androgenized female : This female is just like male for estrus detection. The chin ball device may be fitted on the female. Such system would be cheap as well as there would be little risk of introducing venereal disease in herd. The efficiency varies from 39 to 74 percent.

10. Chin ball device: The device placed under the chin of the bull, causes paint to be smeared on back of the cow if mounting takes place. It works on the principle of "ball point pen" i.e., if chin is pressed on rump it will mark animal in estrus. A buffalo bull fitted with chin ball detects heat efficiently if used at least twice daily. The efficiency of estrus detection is around 50 percent if used once daily.

11. Use of teaser bulls: Teaser animals are used for heat detection, especially in close housing system. Marking device such as chin ball may be fitted to teaser bull for proficient heat detection. Gomer bulls can also be used where the bulls are altered, so that they cannot make sexual contact with female. If the bull mounts, it shows that the female must be in heat.

12. Bio-stimulation: Presence of male in the vicinity of the females, will improve expression of estrus to be detected. It is used commonly as curative measure for silent heat problems especially in buffalo.

13. Pressure sensitive KaMaR or BeaCon heat detector: It can be fitted on sacrum of female. It shows good result in cattle. Moreover, in buffaloes the method is not satisfactory. Wallowing might interferes with the efficiency of heat detection in buffaloes. Such detectors were significantly more efficient than chinball-harnessed steers. The mistake can be made with these aids if they are not utilized in conjunction with heat detection records and good judgment. Proper fixation is also important to avoid loss of device. The efficiency is 80-90 percent. Heat patch with visible colour change can also be employed. The heat patch is applied on the tail head with fixing device and after mounting, the colour of dye changes.

14. Electronic heat mount detector: Electronic heat mount detector also known as heat watch system, is a radio-telemetric system that sense the mounting activity. The data recorded is transmitted to a receiver then recorded by computer for subsequent retrieval (At-Taras and Spahr, 2001). A cow declared to be in heat if she shows mounting 3 times within 4 hours. A similar instrument the "mount count" is pressure sensitive estrous detector glued at sacral area of cow. The mount count signals through LED lamp which gives exact time of mounting. The efficiency of this system is around 91 percent.

15. Pedometer and activity meters: The buffaloes in heat are more mobile and walk two to four times as compared to non-estrous animals. Activity meters are used at the neck or leg and they may be read by receiver and pass on to computer for retrieval. Some pedometer emits signal in form of light when animals show increased activity. Careful observation is however required to remove high false positive reading. Data of animal activity recoded with the help of pedometer has good correlation with estrus (Lopez-Gatius*et al*., 2005). ALT (activity, lying time and temperature) pedometer is a real time watch used for measuring time interval for activity rise. The lack of acceptance has been due to initial cost and expense of replacing lost device. Efficiency of heat detection is 90 to 96 percent.

16. Use of CCTV and video camera: This system of recording is unique for round the clock observation and data recording of herd. Using time-lapse and fast play back, the estrous activity of the night can be viewed in half an hour. It is applicable in intensive system of housing (close housing), however the range of camera may either miss cows because they are not within view area of the camera. This method may not be applied in loose house and range system.

17. Electronic odour detector/ electronic nose : The principle of the device is based on detection of pheromones related to heat. The pheromones are the natural olfactory signal for bull that cow is in heat. Trained dogs have the ability to detect estrus odour correctly in approximately 80 percent of estrus cow. Dog can detect estrus by urine and milk, after being trained with vaginal fluid samples (Fischer-Tenhagen et al., 2011). The odour is not emitted by vaginal mucus or urine was also reported. The BOVINOSE (pheromone based sensor system) for estrus detection is based on the principle of detection of sex pheromones that are secreted by the cows, exclusively during estrus. Sex-pheromones are associated with estrus (Weigerinck et al., 2011). The pheromones are actually released by the dung of cow in estrus. Pheromones are volatile fatty acids i.e., Acetic acid (AA), Propionic acid (PA) and 1-iodoundecane (Sankar and Archunan, 2008). The synthetic compounds (volatile fatty acids) when rubbed on to dummy cows elicited similar responses in bulls. In future, it could be a better technology.

18. Biochemicals or bioanalytes detection: Greater variability of estrous cycle length in buffalo can be attributed to various factors including adverse environmental circumstances, nutrition and irregularities in secretion of ovarian steroid hormones The concentration of blood progesterone is at its nadir/rock bottom (0.1-0.3 ng/ml) during estrus and remains close to 1 ng/ml for the next 3-4 days The first significant increase in progesterone concentration occurs about 7 days after estrus. Peak progesterone values of 4.0-5.1 ng/ml have been recorded about 15 days after estrus. Circulating estradiol concentrations remain low during the luteal phase with minor fluctuations 10-20 pg/ml around Days 4 and 10 of the estrous cycle in river buffalo, but not in swamp buffalo. Peak concentrations of estradiol (30-35 pg/ml) were detected on the day of estrus or one day before followed by a decline to 5-10 pg/ml within two days. This pattern is indicative of enhanced estradiol production by the pre-ovulatory follicle during proestrus. One of the most common manifested and measurable changes that occur during estrus and prior to ovulation is the presence of luteinizing hormone (LH) in the blood and urine. LH is important in the ovarian activity since its pre-ovulatory peak is responsible for follicular wall rupture and the ovulation. The pre-ovulatory LH surge has been detected in the buffalo by enzyme-immunologic assay. Basal LH levels decrease towards the mid-luteal phase and then progressively increase during the follicular phase. Circulating concentrations of LH reached a peak 20-35 ng/ml at the onset of estrus followed by a quick decline within a day; LH remained low 1-3 ng/ml during the luteal phase. During most of the estrous cycle, baseline values of 0.72-20 ng/ml for luteinizing hormone have been recorded in buffaloes with peak values on Day 0 (day of estrus) of 20.0-40.0 ng/ml. A LH surge occurs prior to ovulation and sets the time for ovulation. These hormones can be analyzed in the biofluids like milk, urine and can provide useful indication about animals in estrus.

19. Use of infrared spectroscopy and magnetic resonance spectroscopy: Infrared spectroscopy and nuclear magnetic resonance spectra are carried out to detect estrus related change (inflammatory reaction) in vaginal mucus, vulva and vestibule.

20. Synchronization of estrus: It is one of the important methods for easy detection of heat and timed A.I. Synchronization of heat is a process by which group of animal are managed in such a way that they will come in heat on same day. Different protocols are used for estrus synchronization like Ovsynch etc. in both cattle and buffaloes. By manipulating the level of endogenous estrogen preovulatory estrus behavior expressed optimally.

21. Use of ultrasonography for monitoring of ovarian status: Monitoring the ovarian function with the help of ultrasound in bovine has improved the knowledge and understanding of follicular dynamics and number of developing follicles. Ultrasonography can also be used to detect ovulation time with respect to different sign of heat. An-ovulation is also diagnosed by ultrasonography. Measurement of endometrial thickness before and during estrus indicates conception and fertility status. Ultrasonography accurately guides the estrus detection and ovulation time in cow but it require expert person to understand the scan image and instrument needs careful handling and maintenance. The efficiency of ultrasonography is around 85 to 95 percent.

(22) Improving estrus detection rate using sensor based fuzzy logic system: Fuzzy function can be used for automatic detection of estrus using fuzzy logic. For input data the system uses previous estrus cases information with data of pedometer for rise in activities. The outputs were organized in three categories: i.e. "in estrus", "may be in estrus" and "not in estrus". The sensitivity was found around ninety percent (Brunassi et al., 2010). Heat can be detected by vaginal fluids using specific sensor (Borecki et al., 2012). This method focuses on solving the problems rather than modeling the system mathematically, however it requires a sufficient expert knowledge for formulation of rule base, fuzzification and defuzzification. The sensitivity is found 84.2 percent, indicating that the system may improve automatic estrus detection.

Future Perspectives and conclusion : Current methods of estrous detection and hence ovulation prediction are not always reliable, labor-intensive, and need skill and experience. Hence, development of a simple sensor or sensing device to ascertain the exact time of A.I. is of much interest to farmers, buffalo owners, A.I. workers and other stakeholders. Such a sensor could result in increased reach and success of A.I. by small farmers.

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14 Methods of early pregnancy diagnosis in dairy animals

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An early and accurate diagnosis of reproductive dysfunctions or aberrations is key to better reproductive management. For example, early pregnancy diagnosis is important in order to shorten the calving interval as it enables the producer to identify open animals and then to treat and/or rebreed them in due time. This becomes more relevant to a species which shows seasonality in reproductive function. Similarly, early detection of significant changes in some key biomarkers related to normal pregnancy and/or cyclicity of the animal can be of immense help in formulating economic management strategies in dairy production systems. Though studies on levels of progesterone, pregnancy associated glycoproteins (PAGs), interferon tau and early pregnancy factor are some of the clinically practised pregnancy detection methods in bovines, yet none of the bio-molecules identified for pregnancy till date qualifies as an ideal biomarker for early pregnancy. In the absence of any such reliable bench top pregnancy diagnosis method in large dairy animals, the quest for suitable early pregnancy biomarkers continues, while the traditional method of rectal palpation continues to be the undisputed method of choice the world over.

Direct method of pregnancy detection

a. Per-rectal palpation

Cowie (1948) first described transrectal palpation of the uterus as a method for pregnancy diagnosis in cattle which makes it the oldest and most widely practised method for early pregnancy diagnosis in large dairy animals even today. Traditionally, to confirm pregnancy at about Day 30 of gestation onwards, the practitioners have relied on the palpation of amniotic vesicle and slipping of the chorioallantoic membranes between thumb and forefinger. In buffaloes too, palpation per rectum is a simple, economic and the most widely practised method for pregnancy diagnosis; however, this method is only accurate from Days 45 of pregnancy. Though per-rectal palpation is the cheapest pregnancy diagnosis method, several studies have suggested that examining pregnant cows early in gestation by transrectal palpation increases the risk of iatrogenic embryonic mortality.

b. Ultrasonography

By per rectal palpation an expert can accurately diagnose an animal pregnant only after Day 35 of gestation, but the application of ultrasonography has made diagnosis possible as early as Day 28 post insemination or even earlier. The first visible changes appearing by Day 21

post breeding, when fetal heartbeat can be visualized, also helped confirm a viable pregnancy. Transrectal ultrasonography has the added advantage of providing additional information on ovarian structures, identification of twin fetuses, and determination of fetal viability, age and sex. Transrectal ultrasonography made it possible for a thorough examination of the reproductive health of the animal and therefore, it has now become an established research tool to study bovine reproductive biology in cattle and buffalo. Ultrasound is less invasive, more accurate and efficient technique than transrectal palpation for early pregnancy diagnosis and may minimize the rare incidence of palpation-induced abortions.

Most studies on utility of transrectal ultrasonography for pregnancy diagnosis have been conducted in cattle, but lately it has found utility in buffalo cows as well. In buffaloes, transrectal ultrasonography is most commonly used to determine pregnancy, fetal age and sex as well as ovarian activity. In early 1990's, various workers started using transrectal ultrasonography in buffaloes with visualization of the embryonic vesicle and embryo proper in pregnant buffalo cows between Days 19 and 22 after AI. In a field study on 260 buffaloes between Days 30 and 45 post AI, sensitivity of detection of pregnancy was observed to be 97.9%. However, unpublished data from researchers at the Central Institute for Research on Buffaloes suggest accuracy for selecting pregnant buffaloes at Day 21 post-AI to be about 50%, which increases to almost 100% by days 30. These findings support other findings in cattle which claim that transrectal ultrasonography for pregnancy diagnosis between Day 21 and 25 post breeding, has sensitivity and specificity of 44.8% and 82.3%, respectively, which further increase to 97.7% and 87.7%, respectively, when conducted between days 26 and 33 post AI. Per-rectal palpation and transrectal ultrasonography are direct and accurate methods for pregnancy diagnosis, both require a great deal of skill and experience. Veterinary-grade ultrasound machines equipped with a rectal transducer are quite expensive and therefore the high initial cost of this technology limits its practical implementation.

Indirect methods of pregnancy detection

a. Progesterone

Measurement of progesterone is an indirect method for pregnancy diagnosis in many livestock species including cattle, buffalo, sheep and goat. Conception extends the life of the corpus luteum (CL) by preventing luteolytic mechanism to trigger, thus prolonging and maintaining its functional characteristics, ensuring continued high progesterone levels. Progesterone maintains the uterine endometrium in a state which supports embryonic development, implantation and foeto-placental development. Progesterone concentrations vary with the stage of the estrous cycle which makes it one of the most commonly studied reproductive hormones in bovine ruminants for pregnancy detection and ovarian activity. Studies in the bovine estrous cycle indicate that the milk or serum progesterone concentrations reach a maximum value 13-14 days after estrus; and if the animal is pregnant,

these continue to remain elevated up to Day 21 post fertilization and beyond. These high levels of progesterone in serum or milk between Day 18 and 24 post insemination form the basis of establishment of pregnancy in cattle.

The level of progesterone in milk parallels the levels in plasma and the two are highly correlated. In buffalo cows, it is quite evident that the progesterone levels in milk are four to five times higher than in blood plasma. Just like cattle, buffaloes too can be accurately diagnosed as nonpregnant by determination of plasma progesterone concentrations 21 days post-insemination. Progesterone, which is traditionally looked for in the body fluids, is also secreted in detectable amounts in fecal matter of animals and its estimation can help in differentiating pregnant and non-pregnant animals, particularly useful in wild-life. A major constraint in using progesterone assay for pregnancy diagnosis is its use only in cases where AI or breeding dates are known/ recorded and not randomly in the herd. Nevertheless, progesterone analysis remains the most common clinical use of any of the reproductive hormones.

b. Estrone sulphate

Estrone sulphate is a conjugated steroid product of estrone, present predominantly in the bovine placentomes and it is the major estrone present in the fetal (allantoic and amniotic) fluids and maternal peripheral plasma of cow with measurable quantities detectable by Day 52 onwards till the end of gestation. Its concentrations increase from Day 60 and plateau around Day 150 post insemination. However, a reliable pregnancy detection is possible only after Day 100 of gestation and therefore this test can only detect late pregnancy. Concentration of estrone sulphate in the maternal body fluids is a useful indicator for the placental functions especially related to embryonic growth.

Estrone sulphate concentrations have also been frequently correlated to fetal numbers as these are higher when the number of developing foetuses is more than one. Yet, estrone sulphate is not an ideal pregnancy biomarker as the plasma and milk profiles are influenced by many other factors such as genetic makeup, weight, parity status and environment.

c. Early Conception Factor (ECF)

Early pregnancy factor (EPF, also known as early conception factor - ECF) is present in the sera of pregnant mammalian females. EPF remains the earliest serum benchmark for positive fertilization and hence successful conception. EPF is present in serum upto two thirds of the gestation. Cavanagh (1996) described EPF as (i) an extraordinary potent molecule with growth regulation and immunomodulatory properties, (ii) secreted in miniscule amounts after fertilization and (iii) one of the earliest indicators of pregnancy related physiological changes and hence provides an opportunity for development of a useful early pregnancy detection method. This novel pregnancy specific protein has high immunosuppressive ability which is demonstrated by rosette inhibition test, a bioassay first demonstrated in pregnant mice. Although EPF is secreted in early pregnancy, it is not strictly

pregnancy specific because of its secretion from non placental sources such as tumors and transformed cell lines, which makes it an erroneous pregnancy detection method.

d. Interferon τ (IFN-τ)

Moor and Rowson (1964), the pioneers of sheep embryo transfer, transferred embryos on Day 12, 13 and 14 to unmated ewes, instead of the usual practice of transferring embryos by Day 8 suggesting interactions between embryo and uterus influencing the luteal function and hence establishment of pregnancy. Later research proved that the secretions from conceptus are, in fact, responsible for the maternal recognition of pregnancy. Interferon-τ, a novel type I interferon is produced by the conceptus between days 12-13 and 14-16 post insemination in sheep and cattle, respectively. Acting within the uterine environment with extremely low levels in extra-uterine tissues, including the peripheral circulation, makes it difficult to use IFN-τ directly as a preferred early pregnancy diagnosis molecule. Rapid advancement of molecular techniques in the last two decades has opened new avenues for exploring this unique molecule as pregnancy marker for ruminants through studies on IFN-τ stimulated genes (ISG) in peripheral blood leukocytes. Low transcription of ISG15 gene in peripheral blood leukocytes during early pregnancy can be used for differentiating pregnant and non-pregnant cows with high accuracy though extensive validation and development of a bench top test from these studies will take longer.

e. Pregnancy Associated Glycoproteins (PAGs)

Relocation of the extra embryonic trophoblastic cell layers to the endometrium between days 20 to 28 and secretions from conceptus lead to successful implantation and continuation of pregnancy in ruminant species. The pregnancy associated glycoproteins (PAGs) are such secretory products from the mono- and bi-nucleate trophoblastic cells in bovine placentome having role in positive gestation. Among these glycoproteins, two pregnancy specific proteins in the sera of pregnant cows, a 65-70 kDa similar to α_1-fetoprotein and a 47-53 kDa protein showed no reactivity with known proteins and it was given the name 'protein B' or the 'pregnancy specific protein B' (PSPB) in bovine. Further purification and characterization of several isoforms from bovine foetal cotyledons found that the protein B is actually a 67 kDa glycoprotein. Very recently, it has been observed that placental defects, commonly seen during somatic nuclear transfers in cattle, are complemented by unusually high plasma levels of PAGs, probably due to diminished clearance of these proteins following changes in the glycosylation patterns. PAGs are one of the promising molecules for development of bench top pregnancy detection method. PSPB is detectable in serum of pregnant cows over a long period of gestation starting at about fourth week of gestation to a few weeks after parturition. High circulating levels of these proteins 80 to 100-d postpartum restrict their use in pregnancy diagnosis tests.

Sasser and co-workers (1986) developed double antibody radioimmunoassay for the serological detection of PSPB for pregnancy detection in cattle, and found serum levels

increasing progressively from 1 ng/ml after Day 30 to 9 ng/ml, 35 ng/ml and 150 ng/ml after three, six and nine months of pregnancy, respectively. The study claimed PSPB detection to be more accurate than the traditional rectal palpation method for pregnancy detection. Different homologous (RIA-497) and heterologous radioimmunoassay systems (RIA-706, RIA-780, RIA-809 and RIA-Pool) developed for measurement of ruminant blood PAG concentrations are highly correlated, and can be used for pregnancy detection of 30-80 days. Radioimmunoassay of pregnant sera of zebu cattle established PAG concentrations to be 6.0 ng/ml, 196 ng/ml, 1095.6 ng/ml and 348.4 ng/ml at 8 weeks, 35 weeks, at term and 2 weeks postpartum, respectively, a pattern similar to other breeds of cattle. Results of PAG-RIA based pregnancy diagnosis in buffaloes have also been encouraging with high degree of accuracy of diagnosis as early as Day 31with 100% sensitivity and 90-100% specificity.

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15 Seasonality of reproduction in buffalo: Strategies for round the year breeding

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Small and marginal farmers having land holdings of 1-2 ha and 5-15 dairy animals account to 3/4th of livestock wealth and 68% of milk production in India. Moreover, only 19.2% buffalo ownership in India is with large farmers. Thus, to have a major impact on total milk production in India, strategies need to focus on improving the reproductive potential of buffalo reared by sm all farmers. Furthermore, animal husbandry sector in India contributes about 4.1% of total GDP, with Punjab contributing 8.36% and ranking 5th in all India milk production. Milk production is severely affected by Temperature-Humidity Index and it decreases in crossbred cows by 35-40% when THI increases by 72. Punjab's native buffalo breeds are comparatively hardy animals as compared to crossbred varieties to climate variability.

Seasonal variation in overt estrus and pregnancy rate in buffalo: This phenomenon in buffalo is well known worldwide. In a recent study on 1,087 buffalo exhibiting overt estrus and subjected to AI over a period of 12 months in a region of Punjab, India (30°23'N, 75°33'E) revealed that on an average 90.6±11.1 inseminations were carried out in each month (Monthly range: 41-151) with an average annual pregnancy rate of 40.0±2.2% (Monthly range: 27-54%). Within day 90 post-AI, 36.5±2.2% (Monthly range: 26-51%) buffalo returned to estrus, and out of the buffalo not returning to estrus within day 90 post-AI, 36.6±3.4% (Monthly range: 17-56%) exhibited anestrus on day 90 post-AI (Ghuman and Dhami, 2015b). This further confirmed that the non-breeding season had impact on buffalo exhibiting overt estrus and subsequently getting conceived.

Fate of dominant follicle in summer anestrous buffalo: The enigma of seasonal variation in ovarian activity in buffalo remains unsolved, probably due to the lack of information about ovarian activity in the buffalo displaying anoestrus during summer. During peak summer, the fate of dominant follicle in summer anestrous and cycling buffalo was monitored using transrectal ovarian ultrasonography on alternate days. It was observed that the anestrous buffalo exhibited successive development of follicles that reached dominant phase (19 follicles, 12.6±0.3 mm diameter) but remained non-ovulatory. The diameter of ovulatory follicles (6 follicles) in cycling buffalo was 13.1±0.8 mm (Ghuman *et al.,* 2010). Thus, it was suggested that the buffalo displaying summer anestrus had ongoing ovarian follicular activity and the dominant follicles were able to attain ovulatory size but failed to ovulate and regressed.

Improving conception rate following application of fixed-time AI (FTAI) in buffalo during non-breeding season: Applying FTAI protocol in buffalo may provide a potential alternative for increasing their life time productive period. However, the major bottlenecks in wide application of FTAI at small farmer's doorstep are, a) poor conception rate during non-breeding season, and b) failure of non-conceived buffalo to return to estrus following FTAI. In addition, if a buffalo fails to conceive, a farmer has to bear a loss of $4.2/d in terms of loss of milk and other managemental expenses. Thus, the small farmers can be convinced to adopt FTAI if a protocol with good conception rate is available. During non-breeding season, we aimed at the comparison of fertility outcome of two FTAI protocols in buffalo reared by small farmers. A group of buffalo (n=24) were subjected to Ovsynch protocol (cost: $11.63; d0 and d9, 20µg GnRH analogue each; d7 500µg $PGF_{2\alpha}$, both hormones by i.m. route; AI 16h after d9 GnRH), while other buffalo (n=96) received an estradiol, E/progesterone, P -based FTAI protocol (Cost: $26.16) in which 2mg estradiol benzoate was administered (i.m.) on d0 and 500µg $PGF_{2\alpha}$ on d9. Sustained progesterone release device (1.38g progesterone) was placed intravaginally from d0 to d9. On d11, 20µg GnRH analogue and 500IU eCG were administered (i.m.). AI was done 16h after GnRH analogue administration. In both groups, pregnancy was confirmed by ultrasound aided diagnosis on d60 post-AI and the buffalo failing to conceive and returning to estrus were re-inseminated at observed spontaneous estrus without any additional hormonal treatment. The conception rate following FTAI in ovsynch or E/P protocol was 20.83% and 53.50%, respectively. Also, the conception rate following re-insemination in respective groups was 25% and 61.65%, respectively. Overall (1st AI and re-insemination) conception rate in ovsynch or E/P protocol was 25% and 69.8%, respectively (Ghuman and Dhami, 2015b). Thus, although estradiol / progesterone-based FTAI protocol is costlier compared to ovsynch, the former is economical due to much better conception rate in buffalo during non-breeding season.

Melatonin - A potential candidate for alleviating seasonal suppression of fertility in buffaloes: Regulation of reproductive axis by melatonin, a pineal hormone, could be central to the seasonal decline in reproductive efficiency of buffalo. For evaluating the impact of melatonin treatment during non-breeding season on induction of estrus, amelioration of oxidative stress and conception rate, 132 anestrous buffalo were subcutaneously inserted 2x4 mm size absorbable melatonin implants (18 mg/50 kg b wt) at the base of left ear and 60 buffalo were kept as control. Ovarian ultrasonography and jugular vein blood sampling was carried out at 7 day interval starting from day 7 pre-treatment to day 35 post-treatment or the onset of estrus whichever was earlier. Estrus detection (morning-evening) was also carried out during the study period. Control and treatment group buffalo showing estrus were inseminated. Buffalo failing to exhibit estrus were subjected to FTAI using Ovsynch plus CIDR protocol. Plasma melatonin remained elevated till day 35 post-treatment. A greater proportion of melatonin-implanted anestrous buffalo exhibiting initiation of ovarian cyclicity (55.3 vs. 28.3%). Conception rate subsequent to AI at overt estrus or after FTAI was

higher in implanted buffalo (57.6 vs. 35.0%). Treatment had no visible impact on ovarian follicular dynamics. Lipid peroxide in RBC lysate reduced in implanted buffalo from day 21 onwards when compared to their pre-treatment and control group values. Antioxidant enzymes (glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase) in RBC lysate increased following treatment (Ghuman *et al.,* 2015). Thus, melatonin implantation was successful for inducing ovarian cyclicity, alleviating oxidative stress and improving conception rate in buffalo during seasonal suppression of fertility. However, the usefulness of melatonin implants for inducing ovarian cyclicity was not always successful, probably due to farm conditions, body condition of animals and environmental factors (Carvalho *et al.,* 2015)

Melatonin receptor 1A gene polymorphism in Murrah buffalo with possible impact on summer anestrous: Melatonin receptors are classified as MTNR1A and MTNR1B subtypes but only the first is involved in the regulation of seasonal reproductive activity in mammals. Murrah buffaloes were analyzed to investigate polymorphic sites for Mnl I, Rsa I and Hpa11 using three different primers of expected size of 268 bp (1), 824 bp (2) and 856 bp (3). PCR of genomic DNA using primers 1, 2 and 3 resulted in successful amplification of bands of 824 bp, 268 bp and 856 bp corresponding to the main part of the exon II of the MTNR1A melatonin receptor gene. Polymorphism was noticeable at 50 bp and 267 / 110 bp restriction sites upon digestion of 824 bp PCR product with Mnl I and Rsa I, respectively. Genotypic frequency for the presence of 50 bp and 267 / 110 bp restriction sites was 0.62 and 0.95 / 0.33, respectively. Digestion of 856 bp PCR product with Mnl I and Rsa I also showed polymorphism at 236 / 50 bp and 320 / 50 bp restriction sites, respectively. Genotypic frequency for the presence of 236 / 50 bp and 320 / 50 bp restriction sites was 0.04 / 0.76 and 0.10 / 0.05, respectively (Cheema *et al.,* 2016). Thus, a difference was revealed in polymorphism of MTNR1A gene amid heifers and cows. Polymorphism observed in MTNR1A gene in Murrah buffaloes may have impact on summer anestrous and needs to be investigated.

Decreasing the impact of summer season on embryonic mortality: Summer season is known to have impact on follicular development and subsequent functionality of corpus luteum. We assessed the effect of crushed flaxseed (a source of omega-3 fatty acids) supplementation (300 g/100 kg bwt/day for 60 days), over and above the routine feed, on luteolytic signal ($PGF_{2\alpha}$), luteal function (progesterone) and conception rate. On day 50 post-calving, non-supplemented buffalo were treated to synchronize time of ovulation using an Ovsynch + Controlled Internal Drug Release (CIDR) protocol followed by intravenous oxytocin treatment (OT; 100 IU) on day 15 post-ovulation. Thereafter, the same buffalo were supplemented with flaxseed, treated to synchronize time of ovulation starting on day 35 post-supplementation using the same protocol and subjected to same OT treatment. The mean hourly concentration of $PGF_{2\alpha}$-metabolites subsequent to flaxseed supplemented was less than in the pre-supplementation period. Moreover, in another group of synchronized buffalo,

post-AI luteal phase plasma progesterone was greater in the flaxseed supplemented group and conception rate on day 63 post-AI was 66.7% in supplemented and 31.2% in non-supplemented buffalo (Nazir *et al.,* 2013). Thus, the beneficial impact of dietary supplementation of crushed flaxseed on conception rate through attenuation of luteolytic signal and improvement in post-breeding luteal profile can be useful for buffalo during heat stress period. Nevertheless, various hormonal treatments around the time of AI or during early or mid-luteal phase in buffalo can also be used to alleviate the detrimental impact of seasonal stress on luteal profile (Pandey et al., 2013a, 2013b, 2016).

In brief, recent research has focused on understanding the reason behind seasonal pattern of reproductive activity in buffalo and has tried to develop strategic protocols that can be effectively used for inducing ovarian activity, controlling ovulation and decreasing embryonic mortality during seasonal anestrus.

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16 Infectious diseases affecting buffalo reproduction : Causes, diagnosis and control

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India is the top dairy producer in the world (yielding about 125 billion litres per annum from cattle and buffaloes). This milk is of huge importance in providing nutrition to a growing population - whilst also providing economic benefits to the rural communities. Sustaining "white revolution" is a vital component of India's agricultural policy and a crucial part of our rapidly growing economy. Therefore to maintain the sustenance of "white revolution" as a vital component of agricultural policy, it is very important to break the cycle of infectious diseases and their transmission, so as to optimise the production status of our vast livestock resource.

Infectious agents, such as bacteria, viruses, protozoa and mycoplasma interfere with reproduction by manifestation of various disease processes. Bacterial infections of the buffalo reproductive tract lead to anestrus, repeat breeding, delayed return to oestrus after mating, early embryonic death and abortion. The important diseases which are responsible for infertility and abortion in buffaloes are brucellosis, infectious bovine rhinotracheitis, leptospirosis, listeriosis, campylobacteriosis, trichomoniasis, neosporosis, etc. These diseases can result in substantial economic losses, indicating the need for control measures to prevent infections or disease. Infectious diseases are responsible for about 25% of cases of infertility.

Brucellosis

Brucellosis caused by *Brucella abortus*is one of the most important worldwide zoonotic diseases affecting livestock and humans. In India, brucellosis was 1st recognized in 1942, and accounts for the economic losses to the tune of Rs.350 million/year. Brucellosis has been eradicated from most of the developed countries viz., Japan, Australia, Canada and New Zealand.

Brucella abortus is a Gram negative, coccobacillus comprising 9 biovars. Cattle & buffaloes harbor predominantly B. abortus biotype-1; followed by biotype-3; rarely biotypes-2, 4, 5,6 and 9. Principal manifestations of brucellosis are reproductive failure and abortion, which generally occur after the fifth month of gestation as the pathogen has an affinity for uterus resulting in abortion. Subsequent pregnancies are usually carried to term but there is profuse excretion of the organisms in placenta, fetal fluids, vaginal discharge and milk even after normal parturition. Retention of fetal membranes and metritis often occur.

Hygroma of knee joint is a common and only manifestation of brucellosis in non-pregnant animals. In males it causes epididymitis and orchitis.

Brucella abortus is transmitted via ingestion of contaminated placenta or uterine discharge. It spreads through haematogenous route and localizes in the trophoblasts. Incubation period is 50 to 250 days (directly proportional to the stage of fetal development at the time of exposure)

After brucella penetrates the mucous membrane of upper digestive tract, eye or skin, and enters macrophages where it multiplies, survives and gets passed on to the lymph nodes causing acute lymphadenitis and bacteremia. Once in the circulation it gets localized in different organs such as spleen, mammary gland, supra mammary lymph nodes and pregnant uterus. In the pregnant uterus it penetrates the epithelium of chorion, proliferates and leads to placentitis and endometritis causing ulceration of the uterine epithelial lining. Further it invades allantochorion and passes on to the fetal blood circulation causing endotoxaemia, placentitis and ultimately fetal death and abortion. After abortion most of the organisms leave the uterus and invade blood vessels and again get reestablished in the lymph nodes.

Diagnosis

- Symptoms include herd history of stormy abortions in pregnant animals (6 to 8 month). The placenta is usually retained, thickening of inter-cotyledonary area (leathery appearance) and necrotic changes in cotyledons.
- Demonstration of organisms in direct smears (Ziehl-Neelson stain) from fetal stomach content, vaginal discharge and placenta. There is presence of large aggregates of intra cellular, weakly acid-fast organisms.
- Isolation and identification of the organisms on selective media (Furrell's Medium) from samples of fetal stomach, liver, lung, spleen or vaginal discharge from dam.
- Serological detection of specific antibodies in serum of aborted buffaloes using Rose Bengal Plate test/ Rapid Plate Agglutination test. A titer 1/40 in non-vaccinated and 1/80 in vaccinated animals is considered positive. Titers of samples positive for Rose Bengal test are confirmed by Standard Tube Agglutination test or by Compliment Fixation test.
- Milk Ring testis used as herd test for screening of brucellosis.

Treatment : Antibiotics are not very effective in curing the disease; however, Oxytetracycline could be tried as a single dose @ 10mg/Kg BW in non-pregnant infected animals, and two doses @ 10 mg/Kg BW with 12 days interval in pregnant infected animals.

Control : All positive samples in Milk Ring test should be cultured on selective media. Similarly all positive animals in serological testing should be segregated and culled from the herd. In India 'test and segregation' in conjunction with vaccination is the only alternative for controlling brucellosis. Two vaccines have been approved for use worldwide.

Brucella abortus S-19 (calf hood vaccine)

- Produces short lived antibody response upto 6-8 months after vaccination, and the herd builds immunity in 3-5 years period.
- Difficult to differentiate between vaccination and infectious titers using routine serological tests.
- Not recommended in pregnancy
- In males, calfhood S19 vaccination usually results in persistent antibody titres, testicular infection, and hence infertility.
- Furthermore, the vaccination of infected animals with S19 does not cure nor alter the normal course of the disease.

Brucella abortus RB51

- Differentiates infectious titers from vaccination titers.
- Infectious to humans
- Not very effective to control abortions.
- Not Validated in buffaloes

National control programme on brucellosis (NCPB)

- Aim is to reduce the impact of disease on human health and to reduce economic losses.
- NCPB is a time bound 5-year intensive location targeted control program.
- It does not recognize individual infected animals rather it recognizes village as a herd and intends to involve village milk cooperatives in diagnosis and control through vaccination.
- Periodical surveillance using milk ring test for pooled milk and ELISA for random or herd screening.
- Targets *B. abortus* S19 vaccination for all the female calves of 4 to 8 months in infected villages.
- The programme assures very high and sustained cost benefit ratio to farmer & dairy industry and helps to establish accredited herds/ villages

Constraints

- Increased trade movement and commercial dairy farming.
- No policy for slaughter of infected animals and compensation there off.
- Lack of public awareness
- Calf hood vaccination is not practiced
- On disease confirmation owner goes for distress sale of infected animals
- *Brucella* has a wide host range, just screening and vaccination of dairy animals cannot be sufficient. Sheep, goat, pigs and other animals are often reared by the farmers in the same village in the same locality.

Trichomoniasis

It is caused by Trichomonas foetus, a motile flagellated protozoan, which is an obligate venereal pathogen found only in the genitalia of bovine where it lives in low oxygen tension environment. It does not invade through the genital epithelium to other organs/tissues. Instead, it remains as a lumen-dwelling, extracellular organism. Infected animals experience early embryonic death, irregular oestrous cycles, repeat breeding, and abortion in the first half of gestation. Some animals develop postcoital pyometra. Mostly these symptoms appear in the herd following the introduction of new animals or bulls to the herd. Generally, the infection is cleared within 90 days. Some animals carry the infection till term and deliver normal live calf (carrier).

In bulls the organism inhabits on mucus membrane of penis and prepuce. In older bulls, the organisms survive in the deeper epithelial crypts and therefore these bulls are more likely to become a permanent carrier but there are no clinical signs and the semen picture is normal.

Diagnosis

Demonstration of organisms **:** In females the organisms can be demonstrated in placental fluid, stomach contents of the aborted foetus, uterine washings, pyometra discharge, or vaginal mucus. In the infected bull the organisms are demonstrated in prepucial washings.

Mucus agglutination test : Mucus samples are collected from the cervical region of the vagina, preferably few days after oestrus. Antibodies appear in cervical mucus about 6 weeks after infection, and persist for several months. Antibodies may also be found in preputial secretions. The mucus agglutination test is most useful as a herd test, being capable of detecting latent or recently cleared infections. It is specific and does not cross-react with Campylobacter foetus or Brucella abortus, but lacks sensitivity.

Virgin Heifer test mating: The suspected bull is mated with virgin heifer, and then cervical vaginal mucus samples are collected 12-19 days after mating for culture and identification.

Intradermal Tricintest : A dose of 0.1 ml of the 'Tricin' antigen is injected in tradermally in the skin of neck and the reaction is measured 30–60 minutes later. The reaction consists of as hallow plaque observed visually and showing an increase of >2mm in skin thickness.

Treatment : In infected females, the breeding should be stopped for 3 months and should be treated for pyometra. Test the animals regularly by vaginal mucus agglutination test.In infected males, the medicines viz; Trypoflavin (1% solution, 0.5% ointment), Acriflavin (1% ointment), H_2O_2 (3% solution) or Chloramine (0.3-0.5% sol) could be applied topically on penis after withdraw in git out of prepuce under epidural anaesthesia or bilateral internal pudendal nerve block. Sodium iodide (10mg/100 kg BW in 500 ml distilled water) could be infused intravenous (repeat 3-4 doses at 48h interval).

Vaccination : Whole cell vaccines offer protection and are available commercially, either as monovalent 'bacterin' or part of a polyvalent vaccine containing Campylobacter and Leptospira spp. (CL-vaccine). These products are effective in females but not in bulls.

Campylobacteriosis (Vibirosis)

It is an enzootic infectious diseases caused by the organism campylobacter fetus previously known as *Vibrio foetus foetus*. It is Gram-ve, motile,"S" shaped with a single polar flagellum, curved to spiral rods. The organisms do not interfere with the fertilization process but as a result of inflammatory changes in uterine mucosa, the fertilized ovum fails to grow, leading to early embryonic death and long irregular oestrus cycles. The incubation period of the disease varies from 7-9 days, and the organism is killed by disinfectants and dryness, but can resist deep freezing of semen.

The disease is transmitted venerally through natural service from infected bull or A.I. (infected semen).Infection can also spread from animal to animal by improperly cleaned instruments. In males, infection occurs on serving an infected animal or at semen collection with contaminated A.V.

Symptoms

1. Drop in conception rate and infertility
2. Mucopurulent vaginal discharge in newly served animals.
3. Anoestrus due to embryonic death and persistent CL.
4. Abortion in 5-20% of infected animals during 2nd trimester.
5. Retention of placenta may follow abortion.
6. No clinical signs in recently infected bull.

Diagnosis

1. *Symptoms:* History of infertility, early embryonic deaths and abortions. Aborted fetus may not show any specific changes but there may be necrotic placentitis with yellowish brown exudate on fetal cotyledons and oedma of intercotyledonary spaces like that of brucella abortion.

2. *Culture:* Isolation of the *C. fetus* from the aborted foetus (fetal stomach contents, liver, lung), placenta or vaginal mucus of genital tract. Mucus for culture should be collected at the time of oestrus when the mucous flow is maximal and antibodies are minimal, and the organism is multiplying.

3. *Demonstration of organisms:* The organism can be demonstrated in direct smears stained with diluted carbol fuchsine and Gram's stain, and also in samples of cervical mucus and prepucial washings using FAT

4. *Vaginal mucus agglutination test (VMAT):* This test could be used to detect locally produced antibodies in the vagina and cervix (which appear in the vaginal mucus between 4 to 10weeks after infection and remain for a period of 2.5-16months).

Control

1. Prevent use of infected bull. AI should take over natural service in case of outbreak.
2. All bulls kept in AI centres should be tested every 3 months using culture and FAT, and positive bulls must be culled.
3. All animals should be examined by both culture and vaginal mucus agglutination test. Infected animals must be isolated and only clean ones should be served by clean bulls.
4. Clean cows should be vaccinated with killed adjuvant vaccine

Leptospirosis

Leptospirosis is a contagious disease of bovines and is caused by any of the pathogenic members of the genus Leptospira, which is a zoonotic spirochete. Bovines are the maintenance hosts for *Leptospira interrogans serovar hardjo* (type hardjoprajitno) and *Leptospira borgpetersenii sero var hardjo* (type hardjo-bovis) and incidental hosts for *sero var pomona*, which is maintained in swines. Transmission among maintenance hosts is through contact with infected urine, milk, placental fluid, transplacentally or venereally. Transmission to an incidental host occurs via contact with an environment contaminated with infected urine. The bacteria gain access through the mucus membranes of the eyes, nose, vagina, or abraded skin. Infection in pregnant animals can lead to abortion, stillbirth, or birth of weak calves. Abortion following infection with *sero var pomona* occurs in the last trimester, whereas abortion caused by *sero var hardjo* occurs from 4 months of gestation to term. Abortion rates within a temporal "outbreak" are generally higher following infection with *sero var pomona* compared with *hardjo*. Infertility is also considered to occur with *sero var hardjo* infections.

Diagnosis

- Demonstration of organisms in body fluids by dark ground microscopy, FAT, immunoperoxidase staining or PCR.
- Culturing of organisms in EMJH medium by incubating for at least 16 weeks.
- Serologically through MAT and ELISA.

Vaccination : Leptospiral vaccine is available as Leptavoid H.

Listeriosis

Listeriosis is primarily a disease of the central nervous system of bovines, but a small proportion of animals abort. *Listeria monocytogens* is a zoonotic, gram-positive coccobacillus, transmitted through ingestion of spoiled feed or feed contaminated with infected fetus, placenta or uterine discharge. The organism spreads through hematogenous route to the placenta and fetus. Abortion generally occurs at 4-7 month of gestation. Infected animals show clinical signs of fever, weight loss, endometritis, and retained fetal membranes.The symptoms include nervous manifestations and abortion of pregnant animals at about 4-7 month of gestation.

Diagnosis

1. Demonstration of organisms in direct smears prepared from placenta and fetal liver.
2. Isolation of organisms from body fluids and fetal tissues by culturing on blood agar.
3. Detection of antibodies in the serum of infected animals using agglutination test and CFT.

Other Diseases

Mycoplasmosis : Although *Ureaplasma diversum* and *Mycoplasma spp.* are part of the normal flora of the reproductive tract, they can also cause reproductive failure. Transmission can occur through direct contact, environmental contamination with infected urine, and venereally. Infection with *U. diversum* can lead to granular vulvitis, infertility, abortion, and birth of weak calves. *Mycoplasma bovigenitalium* infection can cause granular vulvovaginitis, infertility and endometritis.

Chlamydophilosis : *Chlamydophila abortus* and chlamydia-like *Waddlia chondrophila* can infect bovines. Infection with *C. abortus* can result in abortion (6–8 months of gestation) or birth of weak calves. *W. chondrophila* also causes abortion. Transmission occurs through ingestion or inhalation of faeces, urine, or contaminated discharges (faeces, nasal, ocular, vulvar, uterine, placenta). Both the organisms cause endometritis and multiply in cotyledons.

Infectious bovine rhinotracheitis (Bovine herpesvirus 1) : The disease, also known as infectious pustular vulvovaginitis (IPV), is caused by bovine herpesvirus-1, and is an important cause of infertility and abortions in bovines. The virus may also express respiratory, ocular and neurological forms of infections. Abortions are mostly associated with the respiratory form of the disease and not the genital form. Abortion generally occurs during 4 to 8 months of gestation. In IPV form, lesions consist of pustules and fibronecrotic plaques, which are limited to vulva and posterior vagina of females and prepuce of males. Transmission occurs through contact with upper respiratory, conjunctival or genital tract mucus membranes, aborted fetuses, or through venereal route. Diagnosis is via isolation and identification of virus from nasal and ocular swabs using tissue culture.

Bovine viral diarrhea (BVD) : Bovine viral diarrhea virus is a Pestivirus. Animals with acute infection usually present with fever, nasal discharge, enteritis, and leukopenia. Pregnant animals infected up to 90 days of gestation can have decreased fertilization rates and embryonic death. Infection between 45 and 175 day of gestation can result in abortion; however, fetuses that survive infection with a non-cytopathic strain of BVDV between 70 and 150 days of gestation usually become persistently infected (PI). Animals that are PI shed large amounts of BVDV and generally do not produce antibodies against BVDV. These animals can be stunted in growth or appear normal. Fetal infection occurring at 100–150 day of gestation can result in congenital abnormalities. Fetuses infected between 150 and 285 day of gestation are usually able to clear the virus, develop normally, and exhibit precolostral neutralizing antibodies to BVDV. The virus is transmitted transplacentally or through

inhalation or ingestion of material contaminated with infected secretions. Persistently infected animals may die and reveal erosions and ulcers throughout the alimentary tract: zebra stripes in the colon are common. Diagnosis is through isolation of virus from nasal or mucosal surfaces and aborted fetuses.

Neosporosis : A highly fatal parasitic disease of dogs exhibited by neuromuscular signs and caused by *Neospora caninum*. The disease earned its significance due to its association with storm of bovine abortions in the second trimester. Previously it was misdiagnosed as *Toxoplasma gondii* as it shares a close relationship with *T. gondii* structurally, genetically and antigenically. But both organisms induce biologically distinct diseases: *T. gondii* causes disease in sheep and humans mainly whereas neosporosis is a major disease of cattle

Infected animals can abort repeatedly at any time from 3 months to full term. Congenital transmission may occursfor several generations with the help of tachyzoites but no natural horizontal cow-to-cow transmission has been reported. The definitive host for the organism is the dog that ingests tissue cysts. The animal then ingests sporulated oocysts in feed, water or soil contaminated by dog faeces. The disease could be diagnosed by detecting antibodies against *N. caninum* in serum by using IFAT and ELISA. Positive IFAT or ELISA mean that the cows were exposed to infection. Postmortem lesions indicate non-suppurative encephalitis in aborted fetal tissue. There is no effective treatment & vaccination. Prevention is difficult because vertical transmission can maintain the disease on a dairy farm even in the absence of the definitive host. The most common recommendation is prevention of fecal contamination of feed.

17 Veterinary control of herd fertility

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India ranks top in milk production in the world, and produced 146.3 million tonnes milk during the year 2014-15, which is about 6.24% higher as compared to previous year (2013-14). The national average per capita availability of milk is 322 gram per day during the same year (2014-15) which is comparatively higher than ICMR recommendation *i.e.,* 280 gm/ day. The today's' position of India in milk production is primarily contributed by buffaloes, followed by cattle and very less percent by goats. However, the productivity of cattle and buffaloes depends on their regular fertility. Fertility is the capability of animals to produce offspring at a regular preferred interval that is fixed by the management policy of the herd; on the other hand, an animal that does not satisfy the management requirement for the herd is defined sub-fertile and those unable to produce calf are called as sterile (Noakes et al., 2009). It is well known fact that the milk production and fertility of dairy animals are negatively associated; greater milk production is associated with reduced reproductive performance in dairy cattle. Hence, it is a hard task for the veterinarian to achieve the optimum fertility of dairy animals and to maintain fertility at optimum level. Further, poor fertility increases culling risk and thus causes huge economic burden to the dairy enterprises. Identification of fertility problems or sub-fertility is the key and primary approach to be considered before implementing managemental strategies for improvement of herd fertility. Additionally, production system in which animals are reared also plays crucial role to meet the nutrient demand of moderate to high yielder dairy cattle and buffaloes, thus it should be taken care while approaching to control herd fertility.

Milk production and fertility

Fertility is a multi-factorial trait and controlled by interaction of several factors such as genetic, environmental and managerial factors, that why it is difficult to identify the exact reason for poor fertility (Walsh et al., 2011). Even it has also been reported that the decline in fertility is primarily associated with demand of lactation rather than some inherent predisposing genetic factor (Noakes et al., 2009). With continuous increase in milk production in dairy cattle and buffaloes during past decades there is also continuous decline in fertility. The decreased fertility in moderate to high yielder dairy animals is considered to be significantly associated with increased postpartum clinical puerperal problems including uterine infections, poor estrus expression and defective oocytes/embryos (Dobson et al., 2007; Walsh et al., 2011). In moderate to high yielder dairy cattle and buffaloes, negative

energy balance (NEB) is an unavoidable condition which arises around and immediately after calving. The NEB subsequently increases susceptibility to metabolic and infectious diseases that increases risk of sub-fertility (Walsh et al., 2011). Hence, these animals require considerable veterinary attention around calving and in the early period after calving (Dobson et al., 2007). Further, delay onset of cyclicity, sub-estrus or silent estrus and ovulation failure are results of NEB also negatively affect the fertility. Additionally, failure to detect estrus, fertilization failure, embryonic mortality (both early and late) together reduces the fertility of high yielder dairy animals (Walsh et al., 2011). Thus implementation of proper nutritional strategies during the dry period and immediately during early postpartum period could minimize the effects of NEB, reduce body condition loss which subsequently could reduce the metabolic and infectious diseases. This could lead a healthy cow which is capable to become pregnant at proper time (Roche, 2006; Chagas et al., 2007; Thatcher et al., 2010; Walsh et al., 2011).

Diagnosis of infertility or sub-fertility in cows and buffaloes

Correct diagnosis of poor fertility or sub-fertility in dairy animals relies on previous history particularly breeding history of animals. Based on the breeding history the general clinical examination of animal is practiced, followed by confirmation of poor or sub-fertility is under taken based on standardized diagnostic test or previous experience of the examiner. The different types of infertility that occurs in dairy animals are described below in detail.

No observed estrus

Pregnancy is a common physiological condition in which there is no sign of estrus but sometimes gestational estrus (4-8% within 5-6 months of pregnancy) observed in cattle and buffaloes. The most common method under field condition is rectal palpation at 2 months for confirmation of either presence or absence of pregnancy which is not possible in early stage. In such cases, ultrasonography is helpful to diagnose pregnancy at 26 days post insemination with more than 95% confirmation of pregnancy (Abdullah et al., 2014). Once the animal is observed non pregnant, then the ovarian structure should be evaluated. But it is very difficult to an experienced clinician to determine all ovarian structure accurately and the ultrasonography is ideal option for evaluation of ovaries. Absence of ovaries in cattle and buffaloes is un-common; however, small inactive ovaries are common condition observed. The inactive ovaries indicate true anestrus and confirmed by repeated per-rectal examination and progesterone assay twice at least 7 to 10 day interval. In heifers, anestrus delays age at sexual maturity and age at first calving which is associated with either nutritional deficiency or health problems or combination of both conditions. Prolonged postpartum anestrus leads to increased days open, calving interval and services per conception and decreased fertility and pregnancy rate which causes huge economic loss. Further, ovary may be active but small and in such condition examination of ovary either by per-rectal or using ultrasound at 10 days interval could confirm whether cow is cyclic or not. Presence of cyst either luteal or follicular

also causes anestrus which can be confirmed by per-rectal examination or using ultrasound. The cyst should be treated with proper hormone; for luteal cyst the choice is $PGF_{2\alpha}$ and for follicular cyst the choice is GnRH, hCG or PRID or CDRI (Noakes et al., 2009).

Abnormal estrus interval, repeat breeding and abortion

Sometimes the estrus interval of animals extended beyond 24 days, which is called prolonged estrus interval. On the other hand, estrus interval may be less than 18 days and called as short estrus interval. In such condition the ovaries structure should be assessed by transrectal palpation or ultrasonography. If ovaries are normal, then prolonged inter estrus interval may be due to failure of estrus detection and in such cases the interval may be multiple of about 21 days. If the interval is not multiple of 21 days, then there is early embryonic mortality. Follicular cyst also causes short interval of estrus cycle. In case of repeat breeding the genital tract should be examined for severe adhesion or infections. The possible causes of repeat breeding are infertile bull, incorrect time of insemination, nutritional deficiency, anatomical defect, uterine infections, anovulation or delayed ovulation and luteal deficiency. Mitigation of possible causes could reduce the chance of repeat breeding. Further, abortion in dairy animals may be bacterial, viral, mycotic or parasitic, and proper diagnosis of cause followed by treatment could reduce the occurrence of abortion.

Early attainment of puberty and sexual maturity in heifers

The puberty and sexual maturity in dairy animals primarily depends on body weight rather than age. There are several factors such as genetic, nutritional, environmental and other managemental factors which directly influence body weight. Managemental interventions at proper time could help in achieving sexual maturity in dairy bovines at an early age. In heifer calves below 6 months there is higher need of protein which is generally not met from tropical and sub-tropical pastures, but at or above 6 months they are able to meet the required protein from the pasture. However, the energy requirement is not properly satisfied by the pasture in heifers of 6 months or higher age, hence supplementary feeding is needed to increase growth rate above 500 g/d (Moss, 1993). Heifer calves above 6 months age grazed under tropical pasture achieve growth rate 500g under supplementation of grain or molasses, but below 6 months owing to protein deficiency the growth rate is less <500 g (Moss, 1993). Hence, below 6 months supplementation of both energy and protein are essential to achieve optimum growth. Moreover, supplementation of extra concentrate with green fodder particularly in summer season (fodder scarcity) has beneficial effect on attainment of puberty and sexual maturity at early age in heifers (Rafiq and Chaudhry, 2002).

Recent study at NDRI revealed that nutritional manipulation by targeting average daily body weight gain of Sahiwal heifers about 400-500g/ day and Murrah buffalo heifers >500g/ day from about 18 months age significantly reduced age at puberty and sexual maturity. Meena (2011) reported that nutritional manipulation in Sahiwal heifers (about 14-

19 months of age) could reduce sexual maturity by about 3-4 months with ADG 458 g/d. In this study, depending on seasonal availability of green fodder the concentrate (20% CP and 70% TDN) quantity was adjusted (@2.0-2.5 kg concentrate/head/d), but in control groups fixed amount of 1.5 kg concentrate plus seasonal green and dry fodder were fed. Similar to Sahiwal cow, another study was carried out on Murrah buffalo heifers (15-18 months age) by Boopathi (2013) and reported that heifers fed with modified ration (variable concentrate quantity i.e. 1.5-2.5 kg depending on seasonal fodder) achieved sexual maturity about 3 months earlier with higher (> 500 g) daily growth rate than those (485.66 g/d) reared under fixed quantity concentrate (@1.5 k g/d) plus seasonal greens and dry fodder. However, overfeeding should be avoided in heifers before puberty otherwise it delays sexual maturity.

Additionally, GnRH administration at pre-pubertal period reduced age at sexual maturity only with better nutritional condition. Sahiwal heifers reared under better nutrition (concentrate quantity fixed 2.0-2.5 kg/d/head depending on seasonal green and dry fodder) when administered GnRH (4 dose @4 µg/animal was administered intramuscularly at monthly interval from 17 to 20 months of age) achieved sexual maturity about 3 months earlier than those without GnRH administration and better nutrition (Meena, 2011). Similarly, sexual maturity in buffaloes heifers administered with six dose of GnRH at monthly interval (@4 µg/animal) from 15-18 months, reduced by 36 days under normal feeding condition but with better nutrition reduced by 122 days (Boopathi, 2013). Further, pre-conditioning (dewormed by Ivermectin and after 14 days Vit-ADE and Phosphorous injection) of acyclic heifers before hormonal treatment could result better response (Kumaresan et al., 2012).

The housing system and photoperiod also reduce age at puberty and sexual maturity in heifers. The heifer calves should be group housed after 6 months of age depending on nutritional need based on body size as well as age. For better feeding management, heifers should be divided into 3 groups i.e. 6 months to 1 year age, 1 year to 2nd trimester and last trimester to parturition (Layek et al., 2011). Further, amelioration of heatstress using, fan, sprinkler, mister, fogger etc. could improve dry matter intake during summer and subsequently enhance growth rate and early attainment of puberty and sexual maturity. Similarly, proper housing can reduce effect of cold stress on heifer and divert more energy towards growth instead of body maintenance (Cozler et al., 2008). It has been reported that buffalo heifers aged between 15 and 30 months reared under either fan or fan plus sprinkler had higher growth rate 501.36 and 460.71g/day compared to control group i.e. 440.38g/day (Vijayakumar et al., 2004). They also reported that provision of sprinkling and fan provided favourable macro-and micro-environment to the animals and enhanced dry matter intake which ultimately resulted higher growth rate (Vijayakumar et al., 2004). About 16 hours/ day (long day photoperiod) exposure to light regimen (15-20 foot candle at animal's eye level) significantly reduces age at puberty in dairy heifers compared to those reared under short day photoperiod (8-h/day) (Cozler et al., 2008). Thus, nutritional manipulation together with

hormonal treatment and better housing management could improve the daily weight gain and early attainment of puberty and sexual maturity.

Post-partum anoestrus management

In modern dairy herd, the post-partum anestrus is primarily develops due to NEB. The NEB creates hormonal imbalance and subsequently suppresses the ovarian activity. Further, NEB also predisposes the cows or buffaloes immediately after calving to metabolic and infectious diseases which alter the endocrine system and finally lead to anestrus in post-partum dairy cows (Dobson et al., 2007; Walsh et al., 2011). Thus proper nutritional manipulation could improve energy balance and induce onset of cyclicity in post-partum period. In addition, suckling of calf is also another factor that prolong onset of cyclicity in cattle and buffaloes could be solved by early weaning of calves from mother.

Evaluation of herd fertility

There are several parameters used to evaluate the herd fertility which is pre-requisite in a dairy herd to investigate the sub-fertility of poor fertility status. Farm records are helpful to estimate different fertility parameters of dairy herds, but it is too tedious and time consuming. However, now-a-days computer based management system makes easier to review herd fertility parameters at regular intervals accurately. The parameters that are used to evaluate the herd fertility are well explained by several authors (Noakes et al., 2009; Gordon, 2011; Layek et al., 2011; LeBlanc, 2013; Abdullah et al., 2015) which are as follows:

Non return rate (NRR) : The NRR is defined as the percentage of cows/buffaloes or heifers in a herd that are not subsequently re-bred or inseminated within a specified period of time after an insemination. The periods are usually between 30-60 days, often 49 days as it covers two potential heats. The target of NRR should be more than 75% and for first insemination should be 80%. This parameter is used to monitor the fertility of bulls and performance of inseminators.

Calving interval and calving index : The calving interval is related to an individual cow and defined as the interval in days between two calvings; while the calving index (CI) is the mean calving interval of all the cows in a herd at a specific point of time which is calculated retrospectively from their most recent calving data. The target calving Interval for cattle and buffaloes should be 365 and 405 days, respectively but the existed one are 500 and 520 days, respectively (Baithalu et al., 2014). However, these parameters give information of previous year; even they do not take into account of fertility of heifers and over optimistic assessment of fertility when cows are culled. Hence, calving index should be interpreted based on culling rate.

Voluntary Waiting Period (VWP) : Voluntary waiting period (VWP) can be defined as the interval during the postpartum period in which producers decide not to breed cows even if

estrus occurs. It usually lasts about 50 days, but may vary a lot from herd to herd according to the breeding strategy in place.

Calving to conception interval (CCI) : The calving to conception interval is the number of days from calving to effective service i.e., service that resulted pregnancy. This is good measure of fertility but depends on the pregnancy diagnosis positive rate. The mean CCI is combination mean calving to first service interval and the mean first service to conception interval.

Days open (DO) : Days open is defined as the interval in days from calving to the subsequent effective service date of those cows or buffaloes that conceive. However, DO ignores the duration from calving to culling or death of those cows that did not conceive.

Calving to first service interval: Calving to first service interval is defined as the average days from calving to first insemination in a herd. It primarily affects the CCI and if first service interval is late then there is longer CCI. Mean value of 65 days calving to first service interval results 85 days of mean CCI and subsequently one calf per year in cattle. This also depends on the breeding policy of the farm that means the voluntary waiting period fixed in a dairy farm.

Service rate (SR) : This measures the proportion of eligible cows and heifers served during a given 21-day period. Theoretically, each individual cow or heifer within a group of non-pregnant animals should exhibit estrus once during a 21-day period. Thus, SR and estrus detection rate will be the same when breeding animals based on signs of behavioural estrus. With good management practices a herd should attain 90% of open cows in estrus within 45 days.

Conception rate (CR) : It is the proportion of cows which have conceived among inseminated cows during a specified period, usually by examination of pregnancy between 28 and 50 days post insemination. A high CR can only be achieved if the cows are in good body condition both in terms of general and reproductive health at the time of breeding. In addition the timing of estrus in relation to detection of heat is also an important point to be considered in relation to conception rate. In farms with good heat detection rate the AM-PM rule holds good. In well managed herd practising A.I., there should be a 50-60% conception rate.

Number of services per conception: The inverse of the conception rate is number of services per conception but rarely used for pregnancy. In a better fertile herd the average number of services per conception should be 2.0.

Pregnancy rate: It is the best and single available measure of overall reproductive performance at the herd level. It measures the possibility that open cows become pregnant per unit of time. It describes the speed at which cows are becoming pregnant in a herd. It is determined by an interaction between SR and CR, and approximates pregnancy rate in large

herds. Thus, maximizing both SR and CR provides opportunities for management control of reproduction and profitability in a herd. At herd level PR is calculated on a 21 day basis (as reproductive cycle is 21 days, so on average, cows should be in estrus every 21 days). A practical method for determining PR is to observe the number of successful pregnancies that occur during 3 weeks periods when eligible cows are at "risk" to become pregnant. PR can vary between 25 and 50 percent in a herd that practising A.I. Pregnancy rate(PR) in mathematical terms, is the product of the heat detection rate (HDR) and conception rate (CR) : PR = CR x HDR.

Pregnancy diagnosis positive rate (PDPR): This measure is defined as the proportion of cows those diagnosed positive for pregnancy divided by the total number of cows presented for pregnancy diagnosis and it should be >70% if pregnancy diagnosis done after 30 days and >85% if pregnancy diagnosis done after 42 days (Gordon, 2011; Abdullah et al., 2015). The PDPR value is a good indicator of farm reproductive performance as it reflects the efficiency of oestrus detection and conception rate in inseminated animals.

Inter-estrus or inter-service interval: The distribution of inter-estrus or inter-service interval provides valuable information about the reproductive status and managemental practices of the herd. These intervals are divided into different groups such as 2-17 days, 18-24 days, 25-35 days, 36-48 days and more than 48 days. Here, if the interval of 2-17 days and 36-48 days is high and 18-24 days is low then it indicates inaccurate identification of estrus or cystic ovarian condition in the herd. Further, large number of 25 days indicates late embryonic mortality or early fetal death problems.

Heat detection rate (HDR): The HDR is defined as the ratio of twenty one to mean inter service interval and expressed as percentage. The target of HDR in a dairy herd is >70%. Though HDR is simple to calculate but can be over estimated as it is influenced by number of short interval services. An overall heat detection rate is calculated as follows:

$$\left\{\frac{(21 \times \text{Average services per pregnancy})}{(\text{Average days open} - \text{VWP} + 11)}\right\} \times 100 = \text{HDR}$$

For a complete analysis of HD, there are two time intervals that should be evaluated: pre-service heat detection efficiency and post-service heat detection efficiency. Pre-service HD efficiency examines the period between calving and first insemination and tells how quickly cows are bred after the voluntary waiting period (VWP):

$$\left\{\frac{21}{(\text{Days to 1st service} - \text{VWP}) + 11}\right\} \times 100 = \text{Pre - service HD efficiency (\%)}$$

Low rates may indicate poor heat detection methods and/or non-cycling cows. Post -service heat detection efficiency examines the time between first service and when cows become pregnant. It relates to how quickly cows are rebred if they do not become pregnant:

$$\left\{ \frac{\text{Services per pregnancy} - 1}{\text{Average days open} - \text{Days to 1st service}} \right\} \text{X 21X100=Post-service HD efficiency (\%)}$$

Days between inseminations can also be grouped by 21-day intervals to identify problems with accurate heat identification, early embryonic deaths and missed heats.

Heat detection effciency and accuracy : Heat detection efficiency is usually expressed as the percentage of possible estruses that were observed in a given time period. Several formulas have been developed to determine the efficiency of heat detection. However, inter-estrous interval method is most common; defined as the ratio between lengths of normal estrus cycle to the average interval between consecutive services or heats for all eligible cows and expressed as percentage. The estrus detection efficiency should be more than 70% in a well managed herd. On the other hand, accuracy of detection of estrus is the percentages of estruses observed that are true estruses. Accuracy would be 80% if 2 of every 10 cows inseminated were not in estrus.

First service submission rate: This measure indicates that how quickly the cows are served after they have become eligible for service i.e. after the end of voluntary waiting period. This measure is defined as the number of eligible cows or heifers served within a 21 or 24 days period divided by the total number of those eligible to breed at the start of 21 or 24 days period. The target of first service submission rate should be >70%.

Reproductive efficiency: Reproductive efficiency is considered as a single measure that provides an overall measurement of fertility and takes into account of different parameters. It is defined as the product of submission rate and overall pregnancy rate divided by 100.

Fertility factor: Like reproduction efficiency, fertility factor is a composite measure defined as the product of estrus detection rate and overall pregnancy rate divided by 100. This measure estimates the number of cows those become pregnant during a 21 days period after being detected in estrus and inseminated.

Culling rate: The cows that are slow to produce calf are culled from the herd to maintain the target calving interval. Overall culling should not exceed 5%, and remaining 95% cows those calved and are served again and become pregnant. However, it has been reported that though optimal herd-level culling rate varies from 19 to 29%, in most of the US dairy herds the actual culling rate varies between 30 to 40% (Cozler et al., 2008). Similarly, at NDRI herd average annual culling rate of crossbred cows varies from 26-30%, whereas annual replacement rate was 24-26% (Singh and Gurnani, 2004).

Fertility index: The fertility index is another single index that is combination of several measures. It is calculated by considering pregnancy rate to first service, service per conception, calving to conception interval and culling rate.

Recording system :

Previously information in a dairy farm was recorded manually in different register but with advancement of technology computerized system has been used which are easy and time saving. For recording, each cow should have unique identification number for entry of information in the record. In a dairy farm the basic informations to be recorded are date of calving, artificial insemination date and results of pregnancy diagnosis. Additionally, date of estrus within voluntary waiting period, sire identification, puerperal complications.

Management Information System (MIS): MIS has been introduced in the livestock sector to provide farmers with a computerized tool that allows them to manage large complex livestock performance dataset, to summarize current information and prepare customized reports. The first computer management system "AFIKIM", developed in 1984 provides tools for daily routines (fertility, health and milk production follow-up) and decision making on the dairy farm, alongwith in-depth periodic performance (production, health, fertility and herd planning) analysis of the herd and farm. It is reported that "AfiFarm" significantly reduces calving interval by 65 days within 6 years (Diepersloot, 2011). "DairyMAN", a computerised MIS, has been used in New Zealand dairy herds. It is found that the herds using DairyMAN had significantly superior calving rates at 4 and 8 week and also the submission rate of animals at 21 day were significantly greater."Herdman", a window based herd management software developed by Vetindia Infotech Solutions, Mumbai, in collaboration with the Department of Medicine, Bombay Veterinary College, India which enables the farm manager to schedule the daily operation in the farm as per the list generated by the program. This list is based on the data entered and default pre-decided parameters. For example, if you have chosen 21±3 days as the parameter value for heat interval, then the program will generate list of animals that had shown heat 18 days earlier to the current data and will continue to display their ID number until 24 days, unless the action taken is entered. For cooperative dairies an analogous version 'Herdmancoop' is also available, using this data of individual village/county dairy society can be maintained. "DeLaval Herd Navigator", developed by the Danish company during 2000s includes various biometric and biological models. The biological model developed to create warning for time of heat, risk of post partum anoestrus and risk of cystic conditions. It has been observed that after installation of herd Navigator the estrus detection rate increased to 97% and reduction of days open by 22 days (Blom and Ridder, 2010). "MOIRA" (Management of Insemination through Routine Analysis) is a computer program which can be used to determine when to inseminate cows based on results of milk progesterone tests (Williams and Esslemont, 1993).

Conclusion

It is now widely recognized that reduced fertility in dairy herds is one of the most important factors affecting a producer's profitability. Having a healthy cow is the starting point for good fertility. The starting point in fertility management has to be recording of events and use of database system for data recording. Poor fertility is caused by a number of often complex factors. It is easy to highlight the negative correlation of yield per cow and fertility because this can be clearly measured, but this is not the whole picture. The multifactorial complexity of dairy cow fertility makes it difficult to improve with a single magic ingredient. Each farm will have different fertility issues and it is important to isolate these issues. The impact of proactive veterinary advice and veterinary intervention will be the key for improving output through improved fertility. This strategy definitely cost less than that of treatment cost. Improving fertility starts with balanced nutrition and heat detection.

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18 Frozen semen production and bull management

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Artificial insemination (AI) is the single most important reproductive biotechnology which has revolutionized the breeding of animals. This technique is the most widely used for dissemination of superior genetic material of outstanding males, progeny testing of males to improve the rate and efficiency of genetic selection, introduction of new genetic materials by import of frozen semen at negligible cost compared to the import of live animals, the use of frozen semen even after the death of bull and control of venereal diseases is need of hour for breeding industry. To produce the quality frozen semen, following steps are mandatory to obey as per the guideline under the minimum standard protocol for frozen semen production.

A) Semen freezing protocol

1. Preparation of extender

- Prepare the extender on the day of collection or on the previous day in the evening.
- Keep the extender in water bath at 32^0C before start of collection**.**

2. Semen collection

- Semen is collected in the early hours of morning before feeding.
- From each bull two ejaculate should be taken on the day of collection and the interval between the two collections should be 10 -12 minutes (as per MSP).

3. Semen evaluation

- As soon as the neat semen is received, it should be kept in a water bath at 32° C under Laminar air flow unit, after recording the volume of semen.
- Immediately after collection, evaluate semen for volume, colour, consistency, presence of foreign matter, mass motility, individual motility and concentration.
- Place one drop of semen on glass slide and observe under microscope for mass motility. Ejaculates having more than +3 motility are selected for further processing.
- Sperm concentration and volume of extender to be added are determined with the help auto diluter and digital photometer manufactured by a reputed company

4. Initial dilution

- Till the final dilution rate is decided, semen is diluted with an equal quantity of diluents and kept in the same water bath.

5. Final dilution

- The diluter and semen ejaculate should be kept in water bath at same temperature (32^0C).
- As soon as final volume of diluted semen is calculated, the remainder of the diluents is added to pre diluted semen so that each straw should contain 20 million progressive motile spermatozoa (as per MSP).
- The progressive motility of extended semen is assessed with the help of phase contrast microscope and the ejaculate showing more than 70 percent of motility is taken for further processing.

6. Printing of straws

- The name or number of bull, breed, name of semen bank and date of semen collection are printed on the straws by using automated straw printing machine before filling of straws. Thereafter, straws are sterilized in ultraviolet ray by keeping in UV switched on laminar flow for few minutes.

7. Filling and sealing of straws

Manual as well as automatic filling and sealing machine are available for filling and sealing of semen in the straws. Nowadays, manual method of filling is not commonly used because automatic filling and sealing machine are more efficient and easy to handle. The diluted semen is filled either in mini or medium straws. In automatic filling and sealing machine, semen is sucked into the straws and on touching the polyvinyl alcohol powder plug at one end and impervious seal is made. The other end is pinched together. Filling and sealing of straws are done in cold cabinet at 4^0C during the equilibration period.

8. Racking

Once the filling and sealing is done, the straws are carefully spread over freezing rack inside the same cold cabinet at the same temperature. Thus, in the large cold cabinet, semen storing during equilibration period, filling and sealing of straws, and racking of straws are done.

9. Equilibration of semen

The pre-freeze storage of diluted semen is known as 'equilibration' of diluted semen. Equilibration period refer to the time from adding the glycerol fraction of extender until freezing. In order to extend the fertile life of the sperm, temperature is reduced to decrease the metabolic rate. A thumb rule is that metabolic rate doubles for every 10 degrees, so cooling semen from body temperature of about 39^0C to 4^0C reduces the metabolic rate to about 1/10 of that at body temperature. After the initial cooling period from 32^0 C to 4^0 C, the extended semen should be allowed to equilibrate at 4ºC for at least 4 hours. The changes

occurring in the sperm cell membrane during this period increase sperm survival during freeze- thaw process. It was originally considered that this was the period over which glycerol penetrated the sperm, although more recent studies indicate that the penetration of glycerol is very rapid and most of the equilibration period is concerned with membrane stabilization during exposure to low temperatures. During this time, the straws are filled with extended semen and sealed and placed on racks for freezing in cold cabinet.

10. Freezing of straws

The filled and sealed straws can be frozen by two methods, namely: Static vapour freezing and Forced vapour freezing

Static vapour freezing

Vapour freezing is carried out in a wide mouthed container (LR 320, LR 250) especially designed for freezing of straws or in the thermo cool boxes designed for this purpose. The equilibrated straws are placed 4-5 cm above the level of liquid nitrogen thereby exposing to the vapours of liquid nitrogen. Thereafter, lid is placed over the thermo cool box for settling the vapour. The temperature at this height is -180^{0}C. When the temperature of semen reaches -130^{0}C to -150^{0}C by about 10-15 minutes, straws are immediately transferred to pre-cooled goblet and the goblets are plunged into the other thermo cooled box containing adequate liquid nitrogen. These goblets are now carefully transferred into the desired LN_2 container for further storage.

Forced Vapour Freezing

Forced vapour freezing is carried out by computerized programmable bio freezer. The computerized programmable freezer has mainly three components: a freezing chamber, a computer with a screen and a liquid nitrogen container. Before placing the straws in freezing chamber, freezer is run to lower the temperature up to 4^{0}C. After reaching at the temperature 4^{0}C, the lid of the freezer is opened and the racks containing straws are transferred carefully from the cold cabinet to freezer. The lid is then closed and the desired freezing curve is chosen and command is given to the computer. The typical freezing curve for bovine semen is given below. The computer monitor displays the temperature curves for freezing chamber and for the straws being frozen. Temperature inside the freezer is controlled by the computer by allowing the appropriate amount of liquid nitrogen flowing to the freezing chamber from the liquid nitrogen container. When temperature reaches to -140^{0}C, the lid of the freezer is opened and immediately all straws are removed from the freezer and plunged into the liquid nitrogen in minimum possible time.

Typical freezing rate for bovine semen

Temperature fall	Rate of fall	Time taken
4^0 C to -10^0 C	@5^0 C/ min	1.8 min
-10^0 C to -100^0 C	@40^{0}C/ min	2.2 min
-100^0 C to -140^0 C	@20^0 C/ min	2.0 min

Post-freezing examination

Post thaw motility of semen samples is assessed after 24 hours of storage. Samples having less than 50 percent progressive motility are discarded. Good samples are transferred to permanent storage containers.

A) Bull management

The objective of daily care of bulls is to ensure a satisfactory state of cleanliness. For proper management of bulls, the following points shall be considered:

a) The bulls shall be kept under hygienic conditions at all times.

b) The coat of the bulls shall be kept clean and generally short. The hooves shall be regularly trimmed.

c) The length of the tuft of hairs at the preputial orifice, which is invariably soiled, shall be cut to about 2 cm. The hair would not be removed altogether, because of its protective role. If cut too short, it may cause irritation of the preputial mucosa.

d) Bulls shall be brushed and groomed regularly, and where necessary, special attention shall be given to the underside of the abdomen, a day prior to semen collection.

e) Cleaning of the prepuce with sterile normal saline solution may be done every ten days if the microbial load is within the prescribed limits. Cleaning prior to the day of collection can be practiced if the microbial load in frozen semen is beyond the prescribed limit.

f) In the event of obvious soiling, careful cleaning of the preputial orifice and the adjoining areas with soap or a detergent is recommended; followed by thorough rinsing and drying.

g) Scientific feeding schedule shall be followed for the bulls. Semen station shall carry out routine quality analysis of feed and fodder for arriving at a balanced ration.

B) Recent advancement in buffalo semen cryopreservation at CIRB

Semen cryopreservation is important technique for long-term storage of sperm, required for wide application of artificial insemination for genetic improvement of buffalo but its application has been reported on a limited scale in buffalo, because of poor freezability of buffalo spermatozoa when compared to cattle. In addition, conception rate in buffaloes inseminated with frozen-thawed semen under field condition is very low (30%) as compared to cattle frozen–thawed spermatozoa. One of the possible causes of lower freezability of buffalo bull semen compared to cattle is due to the differences in the lipid ratio of the spermatozoa. Therefore, there is a need to identify suitable additives and extenders for minimizing the loss due to the cryodamage and make improvement in freezability of buffalo spermatozoa. In this regard, first of all, we tried to evaluate the effect of cryopreservation on structure and function of buffalo sperm. We investigated the sperm damages occurring in

acrosome, plasma membrane, mitochondrial activity, and DNA in fresh, equilibrated and frozen–thawed buffalo semen by fluorescent probes. The stability of sperm acrosome and plasma membrane stability, mitochondrial activity and DNA status were assessed by fluorescein conjugated lectin Pisum sativum agglutinin, Annexin–V/propidium iodide, JC-1 and TUNEL assay, respectively, under the fluorescent microscope. The damages percentage of acrosome integrity was significantly increased during equilibration and freezing–thawing process. The frozen–thawed sperm showed externalization of phosphatidylserine leading to significant increase in apoptotic, early necrotic and necrotic changes and lowering of mitochondrial membrane potential as compared to the fresh sperm but all these parameters were not affected during equilibration. However, the DNA integrity was not found to be affected during equilibration and freezing–thawing procedure. It was observed that plasma membrane and mitochondria of buffalo sperm were more susceptible to damage during cryopreservation. Furthermore, the use of fluorescent probes to evaluate integrity of plasma and acrosome membranes, as well as mitochondrial membrane potential and DNA status increased the accuracy of semen analyses (Kumar et al., 2014).

Further, we tried to evaluate the characteristics of frozen thawed semen for predicting the fertility of buffalo bulls. For this, we measured the differences in motility characteristics, head biometry, acrosome, plasma membrane and DNA of cryopreserved semen of fertile and sub-fertile buffalo bulls. The fertility of bulls was classified on the basis of conception rates (CR), where bulls having CR 28–35% and >55% were considered as sub-fertile and fertile respectively. Total motility, average path velocity (VAP), straight linear velocity (VSL) and curvilinear velocity (VCL) of sperm for fertile bulls were significantly higher than sub-fertile bulls. Significant differences were found in the length and width of sperm head between the two groups. The percentage of intactness of sperm acrosome of fertile bulls was significantly higher than sub-fertile bulls. The percentage of apoptotic sperm differed significantly between fertile and sub-fertile bulls. The sperm DNA integrity of fertile and sub-fertile bulls was not significantly different. This study showed that the total motility, VAP, VCL, VSL, length and width of sperm head, acrosome integrity and percentage of apoptotic sperm, are useful for evaluating bulls' semen quality to reduce the risk of using semen of poor-fertility bulls in AI programme (Kumar et al., 2014).

Based upon the above findings, we tried to reduce the damaging effects of cryopreservation and improve the semen functionality with the help of supplementation of sericin in the extender used for semen cryopreservation. In this study, we examined the protective role of sericin on buffalo spermatozoa during cryopreservation. The ejaculates of four bulls were pooled, divided into five equal fractions, diluted with the extender supplemented with different concentrations of sericin (0, 0.25, 0.5, 1.5 and 2%) and then cryopreserved. Post-thaw motility was objectively assessed by CASA. Sperm plasma membrane integrity was assessed by hypo-osmotic swelling test (HOST). Malondialdehyde (MDA) concentration, glutathione peroxidase (GPx) and superoxide dismutase (SOD)

activities were determined in frozen–thawed extended seminal plasma by spectrophotometry. It was observed that the extender supplemented with 0.25, 0.5 and 1% sericin resulted in the higher sperm motility and GPx activity. Furthermore, plasma membrane integrity and SOD activity were found to be higher ($P < 0.05$) in group supplemented with 0.25 and 0.5% sericin ($P < 0.05$). The MDA concentration was found to be significantly low ($P < 0.05$) in 0.25 and 0.5% sericin treated groups than control and other treated groups. In conclusion, the supplementation of 0.25–0.5% sericin in semen extender improved the frozen–thawed semen quality through protecting sperm from oxidative stress (Kumar et al., 2015). After that several group has been used sericin as supplementation in the extender and validated our result (Sonseeda et al., 2015; Dorji et al., 2015).

In recent years demand for alternative of egg yolk in freezing extenders have increased due to variability in egg yolk composition, risk of microbial contamination and presence of steroid hormones. Therefore, a study was designed to compare soya lecithin-based extender (SL) and liposome-based extender (LP) with conventional egg yolk-based extender (EY) for evaluation of post-thaw quality of buffalo semen. Total, progressive and rapid sperm motility were significantly higher ($P < 0.05$) in LP among these extenders. In vitro assessment of post-thawsperm longevity has also resulted in better maintenance of sperm kinetics and motility inLP in comparison to other extenders. Furthermore, sperm cryopreserved in LP travelled significantly more ($P < 0.05$) distance in cervical mucus as compared to SL and EY. Therefore, it can be concluded that the LP is more efficient than SL and EY for the cryopreservation of buffalo semen (Kumar et al.,2015)

Apart from these, studies of proteins present in seminal plasma associated with male fertility are also one of the thrust areas for improvement in fertility. Among these proteins, leptin has been reported to influence the male reproduction. For the first time we quantified leptin (about 4.52- 5.64 ng/mL) in seminal plasma of buffalo and found that it is negative marker for spermatogenesis (Kumar et al., 2016). Therefore, leptin can be used as a negative marker for spermatogenesis to predict the capacity of buffalo bulls for semen production. Further, for the first time, we have also identify (~40 kDa) the clusterin in buffalo seminal plasma and found that accumulation of clusterin on sperm membrane impair its integrity, sperm kinetics as well as sperm motility. Therefore, estimation of CPS can be used with other conventional methods for selection of bulls. All these studies contribute towards development and modification of suitable protocols for buffalo semen cryopreservation, which will lead to improvement in current success rate of AI.

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19 Stress management through housing and nutrition in buffalo dairy farm

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In general, stress denotes a real or perceived perturbation caused by external body forces resulting from exposure of an animal to a hostile environment that tend to displace the organism's physiological homeostasis or psychological well-being. Dobson et al.(2000) stated that stress is revealed by the inability of an animal to cope with its environment, a phenomenon that is reflected in a failure to achieve genetic potential (e.g. growth rate, milk yield, disease resistance or fertility).Stress has no defined aetiology or prognosis. There are several stressors like environmental stress, diseases, threat of physical harm, high altitude, restraint, hunger, and thirst etc. Environmental stress is not limited to climatic factors but extends to nutrition, housing and any stimuli that demand a response from the animal to adapt to new circumstances (Lee, 1993). Animal environment is affected by various climatic variables including temperature, humidity, radiation, wind, photoperiod and rainfall. In response to stressors, the body tries a constellation of physiological and behavioural mechanisms to maintain homeostasis.

Stress and its effects on physiological function

Physiological and behavioural responses are stressor-specific and so the processes engaged to restore homeostasis also differ. Stressors elicit number of mechanisms that serve to promote physiological and psychological alterations as well as adaptation. Exposure of the animal to high temperature and humidity leads to stimulation of hypothalamus which tries to maintain the body temperature by controlling various physiological processes like triggering the evaporative and non-evaporative cooling systems, appetite centre as well as the adaptive mechanisms against the stressors. The heat stress measured through temperature/humidity indexes (THI) play an important role in the reproductive functions of buffalo and it is suggested that THI >74 has a negative effect on reproductive performances of buffalo. When temperature is measured in Fahrenheit, the equation applied is as follows (LPHSI, 1990): THI = db°F−{(0.55− 0.55 RH) (db°F−58)}, where db°F = dry bulb temperature in Fahrenheit and RH = relative humidity (RH %) /100. The obtained values indicate the following: <72 = absence of heat stress, 72 to <74 = moderate heat stress, 74 to <78 = severe heat stress and 78 and more = very severe heat stress.

As a compensatory mechanism decrease in feed intake leads to lesser production of metabolic heat on the other hand availability of substrates for enzymatic activities, hormone

synthesis is also reduced. High environmental temperature may affect the rumen micro organisms that synthesize Vitamin B complex, amino acids and fatty acids on which the nutrition of ruminants largely depends. In addition, reduction of blood flow to rumen epithelium and reduction of rumination is noticed during dehydration and heat stress. Milk production and reproductive functions of buffaloes are negatively impacted by temperature rise during summer and also by sharp temperature decline in winter. High heat load in lactating buffaloes reduces their milk production and shorten duration of lactation periods. The extent of decline in milk yield were less at mid lactation stage than either late or early stage. The decline in yield varied from 10- 30% in first lactation and 5-20% in second or third lactation.

Heat stress and its impact on buffalo reproduction

Prolonged heat exposure activates hypothalamo-pituitary-adrenal axis leading the secretion of corticotrophin-releasing factor (CRF) to stimulate the corticotrophs of the anterior pituitary gland (Tilbrook et al., 2000). The corticotrophs produce a variety of peptides derived from pro-opiomelanocortin, including ACTH, endorphin and melanocyte-stimulating hormone in response to heat stress (Engler et al., 1989). Of all the domestic animal species, buffalo is notorious for being extra sensitive to heat stress owing to its ability to absorb a great deal of solar radiation because of their dark skin and sparse coat or hair, and in addition to that they possess a less efficient evaporative cooling system due to their rather poor sweating ability. Buffalo are quite resistant to the cold weather and sensitive to hot environment therefore, protection must also be provided in the tropical areas where shade, water access and good management increase the production and reproductive efficiency. In a recent study in buffalo, the lowest overall pregnancy rate (0.25) was obtained in July, while the highest overall pregnancy rate (0.59) was found in November (Dash et al., 2015).

High level of serum corticoids was reported in buffaloes exposed to thermal stress during summer that leads to an altered gonadotropin secretion, which ultimately triggers the state of anoestrus (Singhal et al., 1984). Buffaloes show hyper-prolactinemia during hot summer months (Singh and Chaudhry, 1992). The hyper-prolactinaemia has been proposed to be a possible cause of summer anoestrus in the species (Singh and Madan, 1989). A higher plasma concentration of PRL makes the ovaries refractory to the influence of FSH and LH resulting in anoestrus and this leads to anovulatory oestrous cycle and consequent poor breeding performance in summer (Paraneswaran et al., 1983).The low reproductive efficiency of buffaloes in summer has also been attributed to low luteal activity (Madan, 1984) with low level of progesterone in low breeding season compared to normal breeding season (Qureshi et al., 2000). High environmental temperature coupled with high humidity have been shown to modulate follicular dynamics and estrus expression leading to increased incidence of silent estrus. Buffaloes show a suspension of sexual activity during summer in almost all parts of the world. This clinical entity commonly referred to as summer anestrus is attributed to changes in rainfall resulting in reduced feed availability, to temperature stress

resulting in disturbance of gonadotropin secretion and to changes in photoperiod and melatonin secretion (Das and Khan, 2010). A decrease in intrafollicular antioxidant system and subsequent increase in free radical generation may be central to illicit damage to granulose cells leading to low estradiol concentration during summer has also been speculated (Jan et al., 2011). Heat stress delays follicular selection and lengthens the follicular wave and thus has potentially adverse effects on the quality of oocytes and follicular (Das and Khan, 2010).

Housing and nutritional strategies for management of heat stress

The optimum climatic conditions for growth and reproduction in buffaloes are air temperatures of 13–18°C with an average relative humidity of 55–65%, a wind velocity of 5–8 km/h and a medium level of sunshine (Payne, 1990). Housing for buffalo should provide sufficient shelter against the at, extreme cold and heavy rains. During summer they have to be protected from extreme heat and direct exposure to sun while in winter they have to be protected from extreme cold as well. It must allow good ventilation and should allow enough space for each buffalo. The outdoor yard should preferably be concrete so as to prevent it from becoming an unhygienic mud hole. Cooling systems or controlled temperature housing are not common in buffalo farming units, as this animal is reared by marginal farmers in Indian subcontinent and elsewhere.

Loose housing system is more economical system with increased yields as compared to tied up housing for keeping. The open paddock is provided with shelter along one side under which the animals can take feed and do rest when it is very hot or cold. In loose house animals get optimum exercise. If possible provide buffalo with a wallow. However, the wallow should be one with clean water and not far from the farm. Spending time walking in the sun to and from the wallow costs more than it saves. Showering the buffalo with cool water for three minutes twice a day has proven to be an efficient way for them to get rid of excess heat. If buffalo are not provided with proper shelters, wallowing, cool drinking water, showers their feed intake and growth rate declines, and there could even be loss of body weight. There would be a centrally located water trough where the animals are brought to drink water. There is a definite increase in yield of about 20 to 25% by providing cooled drinking water and showering the animals in the afternoons during summer in lactating Murrah buffalo. A shift from day to night grazing practice has been approved during hot summer months. Extra heat management are needed for higher yielding buffaloes as they requires high feed intake that leads to higher metabolic heat production. Water intake increases and in the case of lactating buffalo there could be a drop in milk production. There is also a marked reduction in fertility. However, it is important that thermal ameliorative measures such as sprinkling and cooling are known to increase comfort levels and feed intake in buffalo (Sastry and Tripathi, 1988; Thomas et al., 2005). In lactating buffalo the use of curtains during winter results in producing about 500 gm more milk daily than those kept in an open shed (Gill et al., 1975).

Preservation of forages or supplemented concentrates should be made available to reduce severe weight losses in breeding stock. Grazing animals have to be supplemented with minerals particularly with those which are deficient in forages and fodders because of intensive cropping. Maintenance of energy balance is equally important and feeding buffaloes during night can reduce the heat load on the animal. Feeding green fodders, silage, hay, provision of night feeding, grazing only in the morning and late in the afternoon and supplementation of mineral mixture can improve the efficiency of reproduction during summer (Sastry and Tripathi, 1988). Added dietary fat is an excellent way to increase energy content of the diet, especially during summer when feed intake is depressed. Fat is high in energy (about 2.25 times as much as carbohydrate), does not add starch to the diet (minimizing rumen acidosis), and may reduce heat load in summer. Antioxidants supplementation (such as vitamin E) has been suggested in order to reduce the peroxidation levels leading to oxidative stress in response to thermal stress.

Summary

Buffaloes are more sensitive to heat stress due to dark body color, lesser no of sweat glands and thinner epidermal layer of skin as compared to cattle and are not able to maintain their core temperature within the thermo neutral zone. Heat stress affected buffaloes show an aberration in endocrine profiles. High heat loads in buffalo and cattle lead to depressed feed intake, decreased milk yield, milk fats and protein %, elevated somatic cell counts as well as increased risk of mastitis, weight loss and reduced reproduction. Heat stress decreases fertility in buffalo. The decrease in fertility is caused by elevated body temperature that influences ovarian function, estrous expression, oocyte health, and embryonic development. Proper environmental, nutritional and housing management can alleviate the effect of heat stress in buffaloes.

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20 Herd health management and emergency care

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Management of the health is an important aspect in veterinary and medical profession. In veterinary sciences if health is not maintained properly then it will lead to reduction in milk yield which directly affect the economy of the farmers. It is the prime duty of the veterinarian to provide service to the farmer so that he can maintain healthy livestock. The veterinarian should advice to the farmers regarding balanced nutrition, proper housing, and timely vaccination and deworming. It has been observed that if livestock is not dewormed and vaccinated well in time than it will cause deterioration in animal health and animal will suffer from fatal diseases such as haemorrhagic septicaemia, foot and mouth disease and black quater etc. In cases of heavy worm load the animal will suffer from certain deficiency diseases such as milk fever, magnesium tetany, and post parturient haemoglobinuria. Such animals will also start taking inanimate objects and suffer from impaction of rumen and caecum. To maintain good health the animal requires healthy environment, balanced ration and proper vaccination. If healthy environment is not maintained then it will not only cause mastitis but also attract flies and ticks thereby causing trypanosomiasis and babesiosis. If the above requirement are not fulfilled then animal will suffer from different variety of diseases. Different category of diseases due to mismanagement are as follows

1. **Diseases due to poor hygienic condition :** The diseases due to poor hygienic condition are multiple but the most common is mastitis. As per our hospital record the mastitis is at number one problem of our medicine section followed by cases of gastrointestinal disorders such as tympany, impaction and enteritis. If proper clean environment is maintained then incidence of this problem can be minimized. Proper milking pattern should also be followed to prevent entry of infection into the teat. eg. Dipping of teat in iodine solution before and after milking. Proper cleanliness of the udder and teat and milkers hand is also essential to prevent mastitis. If there is a subsequent reduction in milk yield from a particular quater and change in taste of milk then milk should be tested for subclinical mastitis. Any delay in this procedure can cause destruction of the effected quater. Any doubt regarding reduction in milk yield in spite of proper feeding should be treated immediately without any delay. The milk should be collected in a sterilized vial or in disposable syringe and send to milk laboratory for cultural examination and sensitivity testing. Due to poor management and hard flooring, the incidence of teat injury and fibrosis of the teat canal is increasing day by day.

2. Diseases due to bite of insect and ticks: These diseases are increasing in alarming rate in spite of proper injection / vaccination for trypanosomiasis and babaesiosis. In our hospital the problem of trypanosomioasis is reported in high number. Although babaesiosis is a major problem of cross breed cattle but this disease is being reported in buffaloes also. To prevent bite of insect use of mosquito net and to avoid ticks infestation use of insecticide spray is required. Due to bite of tabanus fly, the buffalo will suffer from trypanosomiasis. The main clinical signs of trypanosomiasis in buffaloes are drowsiness; lose of body condition and posterior paresis. Sometimes oedematous swelling over different body part can also be observed in cases of trypanosomiasis. The use of quinpyramine chloride should be done after every three months to prevent the disease. Use of insecticide spray in the surrounding area with cypermethrin/ permethrin/amitraz etc. and over body of buffaloes can prevent tick infestation.

3. Diseases due to grain overload : It has been observed that during harvesting season, the buffaloes are free to access wheat grain and other materials lying in open. The farmers are busy with the harvesting of the crops so there is less time to take care about their animals. The buffaloes are voracious eater and greedy in nature therefore when the buffaloes approach near the heap of wheat they will keep on eating until there rumen became full of grain. After overloading of the rumen with grain they will take water ad lib and they will suffer from impaction of rumen. Due to more production of lactic acid in the rumen, there will be weakness in the muscles of the limbs and occurence of laminitis. After grain overload, it is mandatory to perform rumenotomy immediately within 6-8 hrs to take out impacted grains from the rumen followed by use of intravenous fluids, soda bicarb, vitamin C and B-complex along with antihistaminics and antibiotic drugs. If the rumen is not evacuated well in time by doing rumenotomy then effect of lactic acid over the limbs and laminae will occur and the buffalo will either sit down or become recumbent and in such cases prognosis is considered grave.

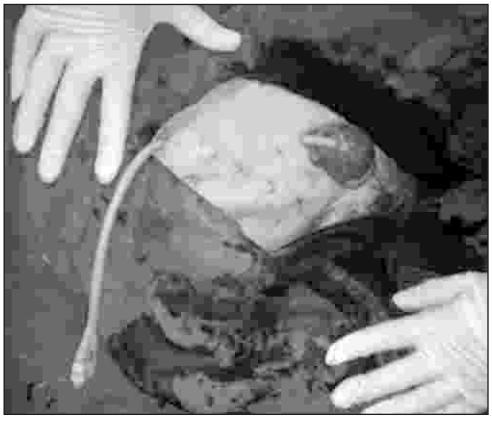
4. Diseases causing respiratory syndrome : There is an increasing trend toward bovine respiratory syndrome. The diseases comes under this syndrome are those diseases which effect the lungs either directly or indirectly. The lungs of the buffaloes are mainly effected by diseases such as haemoraghic septicemia, pneumonia caused by various bacteria

or viruses. The other disease conditions effecting lungs are granulomatous pneumonia, lung abscess, cyst and tumour etc. If animals are not protected against haemorrhagic septicaemia by vaccination then under stressful condition the buffalo will show signs of haemorrhagic septicaemia and there will be heavy mortality if not attended well in time by the local veterinarian. The pneumonia caused by bacteria or virus is a matter of investigation. It has been observed in our clinics that buffaloes are suffering from mixed infection of haemorrhagic septicaemia, foot and mouth disease and typanosomiasis during rainy season.

5. Diseases due to hyperthermia (heat stroke) : Buffaloes has a poor system of thermo-regulation therefore during summer season the buffalo will show sudden rise of body temperature above 108 F. The black colour of the buffalo is also a contributing factor toward sudden rise of body temperature. The buffalo is basically water and mud loving animal and if it is kept in close confinement then during summer season it can show sudden rise of body temperature. If the buffalo is not given regular water bath and ad lib water intake then incidence of this problem will be more pronounced. Whenever there is a case of heat stroke (hyperthermia) then first line of treatment is to provide cool environment ie. (Air conditioned chamber)to the patient. When the temperature is well controlled and become stable up to 104° F then only antipyretic drugs along with fluid and vitamin C and B-complex will work.

6. Diseases due to poisoning/ toxicity : Animals should be maintained on clean fodder. If the fodder is sprayed with insecticide and feed to the animal in ignorance then it will cause toxicity. Therefore, the buffalo should be kept away from insecticide bottles and crop recently sprayed with insecticide. The buffaloes can also suffer from malicious poisoning. In such cases the animals will be dull, depressed with signs of salivation and other nervous symptoms such as ataxia. Therefore, in emergency ward antidote for different toxic substance and poison should be available. The most commonly antidotes available should be atropine sulphate, PAM, barbiturates, different types of fluids, respiratory and cardiac stimulants along with anti shock drugs such as dexamethasone. For critical patients in intensive care unit availability of oxygen and ventilator should also be there.

7. Disease due to eating of inanimate objects : Buffaloes are basically indiscriminate in there feeding habbit and they can easily swallow different types of foreign bodies metallic or non metallic along with their feed. Due to heavy worm load and deficiency of minerals the buffaloes can eat sand, mud and other foreign material. The swallowing of metallic foreign body is a major cause of concern for a veterinarian. The ingested foreign body can lodge in either mouth cavity or in the reticular area. If it get lodged in the mouth cavity then animal will be reluctant to take feed and water and there will be swelling in the throat region. In cases of penetration of reticulum by the foreign body there will be recurrent tympany, rise of body temperature and sharp reduction in feed intake and milk yield. The blood examination and radiography is an important tool to diagnose the cases of traumatic reticulitis. After confirmation immediate removal of the foreign body by performing rumenotomy is required. The common complications of the migration of the foreign body from the reticulum are occurrence of reticular hernia, formation of reticular adhesions and reticular abscess. The rumenotomy is also indicated in the cases of impaction of rumen either due to intake of sand or mud or due to atony of rumen. After rumenotomy the post operative treatment in form of fluids, antibiotics, antihistaminics, probiotics along with daily antiseptic dressing is required till removal of the skin sutures. In cases of reticular hernia, the operation is carried out in two phases; in 1st phase the rumen is evacuated by rumenotomy and in 2nd phase the ruptured diaphragm is repaired by applying lockstich sutures using non absorbable suture material eg. Silk. The herniorraphy of diaphragm is avoided in cases of advanced stage of pregnancy.



10. Disease due to obstruction of urethra : The Urinary tract get obstructed by means of different causes for eg. due to inflammation of urethra (uretheritis), formation of concretions, calculi (obstructive urolithiasis) in urinary passage. The formation of calculus can be attributed due to presence of salts in the water and in the soil. Due to presence of salt in water and in crops harvested over the saline soil, calculus formation will start in the kidney and in due course of time it will get descend down into the urinary bladder and urethra. If the urethras get obstructed then it will cause accumulation of urine in the urinary bladder and kidneys. At this stage the

animal will show colicy signs and there will be history of not passing of urine from the urethral opening. The buffalo will grind the teeth along with straining to pass the urine. There will be switching of the tail along with rectal prolapse in some cases. If the buffalo bull/calf is not operated for removal of calculus then urinary bladder will get ruptured and the colicy signs will disappear. Due to accumulation of urine in the abdomen there will be bilateral distension of the abdomen. At this stage the clinical signs will be salivation and sunken eye balls due to dehydration. The diagnosis of such cases is made on the basis of history, clinical signs, ultrasonography and biochemical examination. The kidney function test will reveal increased value of blood urea nitrogen (BUN) and serum creatinine. In such cases the line of treatment will be urethrotomy in buffalo bulls and tube cystotomy in buffalo calves.

9. Diseases due to trauma and accidents : In surgical section of our hospital the cases of orthopaedics in buffaloes is considered most challenging job because of the low moral and tendency of the buffalo to become recumbent after fracture. The accident can cause injury to tendons, ligaments, joints, bones and hoof area but the most common cases observed are of fracture. In cases of fracture the use of tranquilizer, proper transport and use of splints and casts for temporary treatment play an important role in outcome of the ailment. The advancement in the field of, alternate imaging and anaesthesiology has made the diagnosis and treatment somewhat easier but the availability of strong orthopaedic technique to bear the weight of the buffalo is also essential. The cases of orthopaedics in buffalo should be handled carefully so that a simple fracture may not get converted into compound one during course of transportation and treatment. The main clinical signs in cases of fractures will be swelling and pain over the effected limb. There will be crepitating sound and inability to bear weight over the effected limb when the animal is made to walk. The cases of fracture are diagnosed on the basis of history of accident, clinical signs and radiography. After proper diagnosis it was decided what suitable orthopaedic technique should be used. In cases of fracture of upper long bones, intramedullary pinning, k-nailing is required. In cases of fracture of middle long bones eg. Radius and ulna, tibia and fibula the techniques used are hanging pin cast in forelimb and coaptation splint in cases of tibia and fibula.When lower long bones are fractured then simple plaster cast is advised.The light plaster cast (fibre glass cast) is better than heavy plaster cast (plaster of paris cast). After application of these techniques proper orthopaedic flooring is required so that animal may not get slip again and again along with proper care of the orthopaedic patient. When the fractured bone get healed evidenced by radiography then orthopaedic technique is removed. After removal of the orthopaedic technique, for rehabilitation of the fractured limb different physiotherapeutic methods are used eg. Massage, exercise, fomentation and muscle stimulators.

10. Critical care unit : The critical unit in a veterinary hospital is considered a emergency unit. In this emergrncy unit, the drugs related to emergency and the equipment related to emergency patient should be present. The emergency drugs available in Intensive Care Unit should be respiratory stimulant eg. Nikithamide, precthamide; cardiac stimulants eg. adrenaline; anti shock drugs eg. betamethasone, dexamethasone; antihistaminics eg. pheneramine maleate; tranquilizers eg. xylazine; antidote for poisoning eg. atropine sulphate and barbiturate, haemostat local and systemic both eg. ticture bezoin co A, and etamsylate; oxyzen containing cylinder with proper mask, endotracheal tubes, ventilators for inhalant anaesthetics. Other available drugs in critical care unit should be fluids, plasma expanders, different dressing materials, tourniquets etc.

21 Importance of vaccination and deworming in large animals

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Veterinary vaccines are important for animal health, animal welfare, food production, and public health. They are a cost-effective method to prevent animal disease, enhance the efficiency of food production, and reduce or prevent transmission of zoonotic and food borne infections to people. Safe and effective animal vaccines are essential to modern society. Veterinary vaccines will continue to be an important tool to protect human health, animal health, food safety, and food security and must be accessible and economical.

Vaccines and how they work

Infectious diseases in animals can cost producers a significant loss of production and profit. One way to reduce these losses is to increase the animal's ability to fight disease through good vaccination practices. Vaccines stimulate the body's immune system to build immunity or resistance against disease-causing organisms. Most vaccines are manufactured by growing a particular organism that is later weakened or killed. When a vaccine is introduced to the body, the immune system must first recognize it as a foreign antigen or protein, such as a virus, bacterium, toxin, or parasite. An immune response is then produced in which the body develops specific antibodies and immune cells to remove or kill the infectious agent. Memory cells are then developed for each antigen. When the body is later re-exposed to the same antigen, the memory cells will recognize the infectious agent and remember the most effective way to protect the body. It generally takes 7 to 14 days after primary exposure for the body to develop immunity to an antigen and as little as 48 hours to mount an immune response to that same antigen in a vaccinated animal. It is important to understand that some animals' immune systems fail to develop an immune response sufficient to create immunity to a disease. However, remember that vaccinating your animals is not a 100% guarantee that all the vaccinated animals will gain immunity to that particular pathogen. Two common types of vaccine are killed and modified-Live.

1. **Killed vaccines**

Killed vaccines are made by growing an organism in a growth medium. The organism is then inactivated or killed by utilizing chemicals or heat. A killed vaccine can be produced for viruses, bacteria, or toxins. Adjuvants, which are specific chemical materials that help

stimulate immunity and hold the organism at the injection site to strengthen the immune response, are regularly added to killed or inactivated vaccines. Killed vaccines often require two separate inoculations over 2–4 weeks to obtain a full immune response. Therefore, it is very important to administer both inoculations. Killed vaccines are already constituted and ready to use when purchased. Example: FMD, HS, BQ vaccines.

2. **Modifed-live vaccines**

Modified-live vaccines (MLV) are made with a virus or bacterium that is attenuated, or weakened, so the organism will not cause disease in most healthy animals but will still stimulate immunity. These types of vaccine are not stable in solution so the isolates are freeze-dried to a cake in a vaccine bottle. To use a MLV, a special sterile liquid or a specified killed liquid vaccine is added to the "cake" to make a vaccine solution ready for injection. Modified-live vaccines are very unstable and have a short efficacy life once they are reconstituted, often as short as 1–2 hours. Therefore, reconstitute MLVs only as needed. These vaccines should be mixed gently when reconstituted as some constituents in the vaccine may be damaged or destroyed when mixed too vigorously. Example : Brucella vaccines.

Vaccination schedule for cattle and buffaloes

S. No.	Name of Disease	Age at first dose	Booster dose	Subsequent dose
1	Foot and Mouth Disease (FMD)	4 months and above	1 month after first dose	Six monthly
2	Hemorrhagic Septicemia (HS)	6 months and above	-----	Annually in endemic areas
3	Black quarter (BQ)	6 months and above	-----	Annually in endemic areas
4	Brucellosis	4-8 months of age (Only female calves)	-----	Once in a lifetime
5	Theileriosis	3 months of age and above	-----	Once in a lifetime. Only required for crossbred and exotic cattle.
6	Anthrax	4 months and above	-----	Annually in endemic areas.
7	IBR	3 months and above	1 month after first dose	Six monthly (vaccine presently not produced in India)
8	Rabies (Post bite therapy only)	Immediately suspected bite.	4th day	7, 14, 28 and 90 (optional) days after first dose

Note :- Before any vaccination deworming should be compulsory to get better results.

Points to be remembered while handling/administering the vaccines

1. The manufacturers' instruction on the route and dosage should be strictly followed.
2. Keep vaccine cool in a dark environment and out of sun light when storing and using it.

3. Do not mix products or combine different vaccine products in the same bottle or syringe.
4. The cold chain of the vaccines wherever prescribed should be maintained till the time of administration to the animal.
5. Do not mix modified live vaccines if you won't be able to use them within 1–2 hours of reconstituting them.
6. Animals should be in good health at the time of vaccination
7. A minimum vaccination coverage of 80% of population is required for proper control of the disease.
8. It is beneficial to deworm the animals 2-3 weeks before vaccination for better immune response.
9. Vaccination should be carried out at least a month prior to the likely occurrence of the disease.
10. Vaccination of animals in advanced pregnancy may be avoided even though in most cases nothing untoward may happen.
11. Give injections in front of the shoulder in the middle neck region.
12. Avoid giving injections in the nuchal ligament of the neck region.
13. Do not use the same vaccine gun for different vaccines.
14. Use transfer needles for mixing modified live vaccine to prevent contamination and accidents.
15. Remove air from syringes and/or guns prior to injecting vaccine.
16. Use the correct gauge and length of needle as specified by the label.
17. Change needles every time you fill a syringe or vaccine gun.
18. Change needles that become blurred, bent, or broken.
19. Never straighten and reuse a broken needle.
20. Space multiple injections 4 inches apart on the animal to avoid mixing different products.
21. Discard bottles of killed vaccine that have been opened for more than 2 days because vaccines can be contaminated by repeated introduction of air and needles.

Vaccination failure

1. Lack of maintenance of cold chain from the time of manufacture till vaccination.
2. Poor immune response in weak and improperly fed animals.
3. Lack of herd immunity due to only a few animals being vaccinated.
4. Poor quality of vaccine - Quality will deteriorate if repeatedly thawed and cooled.
5. Low efficiency or ineffective vaccine – May occur in case of strain variation (eg. FMD).

Challenges

Much progress has been made in vaccine development in recent years; however, significant challenges remain. Animal and human infectious disease experts need to work together to prepare for new and emerging diseases. Veterinary vaccines must be pure, safe, potent and effective and they must be economical. Proper standards and production controls in the manufacture of veterinary vaccines are essential for ensuring quality products for animal disease control. Keeping the costs of animal vaccines low will encourage more use of vaccines and less use of antibiotics. It will also enable the use of food safety vaccines that do not have an economic advantage for the producer or health advantage to the animal, but have important public health benefits.

Parasitic disease of buffaloes and their control

Buffaloes suffer from a variety of parasitic diseases, which cause substantial loss to the dairy industry. It is generally observed that buffaloes and cattle share almost identical parasitic infestations. Certain conditions like "Paracooperosis", "Biliary amphistomiasis" and "Ear sore" are exclusively encountered in buffaloes. Parasitic diseases of major importance in Indian buffaloes have been discussed .

Mange

Parasitic dermatitis by the infestation of mites is frequently observed and is characterized by severe irritation, itching and rubbing of the infected parts of body against hard objects.

Sr. No.	Type of Mange	Causative agent	Symptoms
1	Sarcoptic mange	*Sarcoptic scabie* var *bubalis*	Much more severe than other forms. No seasonal specificity. Initial lesion appearing on thin skin parts followed by alopecia and thickening of skin and wrinkled crusts housing the mites and their stages formed. Lack of cleanliness, dirty environment and hot humid environment favor the spread of mange.
2	Psoroptic mange	*Psoroptes natalensis* or *Psoroptes communis* var *bubalis*	Highly contagious form. The mites are usually found around the base of horns, but extensive body infestation may also occur. The condition is often encountered during rainy season in the country

Application of Ivermectin @0.2mg/kg or 1ml/50kg body wt by s/c route gives satisfactory results in infected animals. It can be controlled by repeated dipping of animals and spraying of infected premises with common acaricides like Carbaryl, Malathion, Cypermethrin etc.

Fascioliasis

This is the fluke infestation of liver and bile duct caused by *Fasciola hepatica* and *F. gigantica* in buffaloes. The incidence is quiet high in hot humid regions where grazing is near water resources. Snails (*Lymea spp*.), the intermediate host is in abundance at such places. The disease is mainly manifested in chronic form but heavy infestation in young animal may prove fatal.

Control by reducing the snail population using mollscicides is not feasible, nor is it possible to keep away the buffaloes from infested areas. Regular treatment is, therefore, advisable. Refoxanide and Closantel @7.5mg/kg body wt & Triclabendazole @12 mg/ kg body wt are very effective against fluke infestation. Two or three treatments may be necessary each year in the endemic areas.

Toxocariasis/Neoascariasis

This is the most important intestinal nematode (*Toxocara/Neoascaris vitulorum*) parasitic worm causing the heavy mortality among buffalo calves below 3 months of age in India. In severe infection the calves become anorectic, dull and pass foul smelling clay coloured watery faeces. The characteristic butyric odour may be detectable from the breath. Transmammary transmission via colustrum also occurs.

There should be immediate removal and sterilization of diarrheic faeces of infected calves from the sheds to avoid chances of dispersal of a large number of resistant eggs. Use of Morantel citrate @ 10-12.5 mg/kg and Albendazole @ 5mg/kg are satisfactory to control this infection. The drugs should be administered after fasting the animals.

Strongylodosis

This condition is caused by S*tongyloides papillosus*, the common threadworm of domestic ruminants. This is usually ranked second in frequency and pathogenicity to *Toxocare avitulorum* in young buffalo calves. Infection usually takes place within the sheds as the infective larvae infect the susceptible hosts through active penetration particularly via skin of foot. Intermittent diarrhea with blood and mucus is a common feature. There is loss of condition, retarded growth and occasional death in severe cases. In heavy parasitization, there is catarrhal inflammation with petechial and ecchymotic haemorrhages, erosion and necrosis of mucosa with extensive mucus production in duodenum. The infective larvae while penetrating through skin may cause dermatitis resulting in gross secondary infection.

Thiabendazole, Levamisole and most of available broad-spectrum anthelmintics are effective in eliminating this parasite. Control depends upon elimination of warm, moist areas which are suitable for multiplication of parasites.

Tapeworms

Tapeworms are long, and flat and look like white ribbons. They consist of many segments and live in the intestine. Example *Moniezia expansa, M. benedini* and

Avitellinacentripuctata. Tapeworms produce eggs in the segments which break off and pass out in the dung and contaminating the pastures and the water sources. Animals become infected when they graze the pasture. Parasites feed on the food in the gut and on the blood of the host. The animal becomes weak and loses weight or does not gain weight.

Diagnosis is done by analysis stool sample in which eggs can be detected, or often observation of the gravid proglottids in feces and anus. Niclosamide is most often used. Praziquantel (while not approved for use in ruminants in the US) is also 99–100% effective while albendazole is 19-75% effective and praziquantel + levamisole combination is very effective in reducing worm burden and improvement of weight.

Trypanosomiasis

The disease caused by *Trypanosome evansi* a blood protozoan parasite usually occurs in subclinical and chronic forms in buffaloes. The disease is transmitted by biting flies viz. *Tabanus spp, Stomoxys spp, Hippobosca spp, Chrysops spp*. etc. Outbreaks occur mainly during rainy season and early part of winter due to heavy build up of vetors. Buffaloes mainly act as reservoirs of infection. Stress due to inclement weather or some concomitant infection may flare up the inapparent infection resulting in clinical condition. Per acute cases are characterized by high fever, signs of abdominal pain, sternous breathing and groaning, death taking place in 6-12 hrs.

Antrycide prosalt, 3.5gm in 15ml distilled water @7.4mg /kg b wt by s/c route or diminazine aceurate in a soln of 10mg /kg b wt by i/m route as single doses are recommended for treatment. The control of infection relies upon an infective chemoprophylaxis, control of vectors through spray of insecticides, proper disposal of manure and regular examination of animals during wet season in endemic areas.

Coccidiosis

This disease caused by protozoan parasites, *Eimeri azuemii* or *E. bovis* constitutes major causes for mortality in buffalo calves of 3-6 months age. In most of the clinical cases temperature is either normal or subnormal. Onset is sudden with severe foul smelling diarrhea, fluid faeces containing mucous and blood are often seen sticking on perineum and tail. There is characteristic severe straining and rectum prolapsed may also occur.

It s self limiting disease and subside spontaneously when the multiplication stage of parasite is over. Amprolium and sulphamethazine @10mg /Kg and 140mg /Kg respectively orally for 3-5 days has been found satisfactory for treatment.

Amphistomiasis

A very common amphiostome encountered in the bile duct of buffaloes is *Gigantocotyle explanatum*. The bile duct may be almost packed and wall of the gets thickened. Neoplastic growths may develop at the site of fluke attachment. Pathological changes may also be produced in liver and latter becomes enlarged, pale, hard and studded with haemorrhagic

spots. The bile ducts are congested with papillary projections of the mucosa. The immature parasites develop in the small intestine and in heavy infection there is enteritis, emaciation and death. The treatment used for fascioliasis can be safely adopted for the control of amphistomiasis in buffaloes.

Schistosomiasis

The disease is produced by the infection of blood flukes, *Schistoma nasalis, S. spindale or S. indicum*. The eggs escape through haemorrhages or ulcers into the nasal cavity or into the intestine. Then they hatch into free swimming miracidia which infect water snails and later emerge as cercariae which penetrate skin when the animals enters into water or mucous membrane of the mouth when animal drinks. They migrate in host animals until they reach the appropriate veins, where they develop into the adult form. In Nasal schistosomiasis blood flukes are seated in veins of nasal mucosa causing irritation , mucoprulent discharge, proliferation of epithelial lining and formation of numerous abcesses containing eggs of the worm. In chronic form cauliflower like growth appears in the nasal cavity. In the Intestinal schistosomiasis infection is important in animals under 2 years of age. Haemorrhages and ulcers are formed in small and large intestine.

Positive diagnosis may be made by presence of spindle shaped eggs in the nasal discharge or in the blood and mucus from faeces in the period following patency. Two doses of Praziquantel @25mg /kg body weight at an interval of 3-5 weeks have been found to be effective in animals.

Characteristics of a good dewormer

Deworming is the giving of an anthelmintic drug (a wormer, dewormer, or drench) to a human or animal to rid them of helminths parasites, such as roundworm, flukes and tapeworm. Selecte ddewormer should not be poisonous, easy to administer, effective for all stages of parasites for a longer period of time by acting specifically at the target site and should be economical. Drug resistance in parasites is extremely common The effectiveness of an anthelmintics should always be tested before being used. Animals should not be dewormed unnecessarily. Type of wormer used should be changed/rotated to prevent resistance.

Control and management of parasitic infestation

If Animals get infected our target should be killing the worms within the body and reducing the chances of the animal becoming infected on pasture again and again .For this we should move the stock to new pasture every one to two weeks. Young animals should be separated from old animals and allowed to graze fresh pasture first. If cattle, sheep and goats are kept in the same area, let the cattle graze the pasture before the sheep, as some worms which would infect the sheep will not infect the cattle. If animals are kept in an enclosure, removing the dung and disposing it off properly and timely will prevent the animals picking up more worms or others becoming infected. Do not allow animals to graze on marshy ground or on pasture where the grass is very short.

22 Feed quality assurance program and evaluation of feed ingredients in India

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The quality of animal feeds depends on the quality of raw materials which are mainly cereals, cereal byproducts, oilseed cakes, bran, agro-industrial byproducts. Feed quality can be defined as "any of the features that make something what it is" and "the degree of excellence which the thing possesses". A quality feed should provide all the nutrients in adequate quantity which are easily digestible and ingestible. In India both compounded feed as well as homemade feed are usually fed to the animals. Amongst oilseed cakes category most common are groundnut cake, cotton seed cake, mustard cake, sunflower cake. However, most of the compounded feeds contain limited amount of cakes and grains. In India the quality control is regulated by a statutory body, Bureau of Indian Standards (BIS). It was established under BIS Act 1986. Bureau has setup expert subcommittees for the standardization of different types of commodities. Animal feeds sectional committee is the one responsible to check the quality of animal feeds and feed ingredients.

Quality Control

The purpose of quality control of feedstuffs is to ensure that an unadulterated, true to their nature feeds is made available to the consumers while minimizing the cost of processing. An overview of the complete operations is the primary consideration to organise an in-plant quality control program. The development of quality control manual is first useful guide. It can be used as an employee training tools and as a reference for all others involved in the feed processing unit. A typical quality control manual usually contains an index or outline of contents followed by the company's quality policy. The manual contains procedures for sampling of ingredients and finished products. The manual also document methods of evaluation of feed ingredients and compounded feeds for effective quality control.

General requirements for sampling

The sampling is the first and foremost important steps for the quality assurance program. Following are the precautions needs to be taken:

- Care should be taken so that the properties of feeds are not affected while drawing, preparing, storing and handling samples.
- Samples must be taken at a protected place not exposed to damp area, dust or soot.
- The instruments used for sampling must be clean, dry and sterile when used.
- The samples, the sampling instruments and contains for samples must be protected from adventitious contamination.

- The samples must be preserved in clean, dry and sterile containers. The sample containers shall be of such size that they are almost completely filled with the samples.
- The containers must be sealed airtight.

Sampling procedures

In all cases, at least 10% of the packages should be sampled. A minimum amount of about 1kg should be collected from each load. In India, BIS has laid down the following procedure of sampling of feeds (Table 1).

All the containers in a single consignment of the material drawn from a single batch of manufacture shall constitute a lot. If a consignment is declared to consist of different batches of manufacture, the batches should be grouped separately and the containers in each grouped separately and the containers in each group shall constitute separate lot. The containers must be chosen at random from the lot and for this purpose random number table must be used. Sample should be tested for each lot. Number of containers to be selected from the lot shall depend upon the size of the lot and should be in accordance to the Table 2.

Table 1. Sampling of feeds

Package/bags	No. to be sampled
1-10	1-3
11-25	2-4
26-50	3-6
51-75	6-8
76-100	8-10

Table 2. Sampling from Containers

Lot size	No. of containers to be selected (n)
2-15	2
16-50	3
51-100	4
101-150	5
151-300	7
301 and above	10

Quality control specifications

Ingredients' specifications are essential in a feed quality assurance program. The specifications serve as the basis of laying terms and conditions for the procurement purpose. They also allows set the limits for rejection/acceptability of the feed stuffs. Specifications must be as comprehensive as possible and realistic. Specifications are the foundation of a good quality assurance program as they serve the understanding between nutritionists, purchase and production departments. Quality control specifications of various feed ingredients and compounded feed has been laid down by BIS to ensure suitability for the ingredients to be included in compounded feeds indicating minimum and maximum proportions of inclusions of feed stuffs. In India, the minimum permissible limits for various toxic factors such as mycotoxins, pesticide residues, heavy metals etc. are also prescribed by the BIS which are based on and harmonious with the CODEX standards.

Parameters of quality control

There are two types of quality parameters viz. qualitative and quantitative.

Qualitative ingredient quality : The qualitative ingredient quality constitutes physical characteristics such as colour, texture, odour, taste, particle size and shape. It also includes adulterations, damage and deterioration. The presence of physical contaminants such as metal pieces/nails, hairs, etc. also needs to be checked.

Quantitative ingredient quality : The quantitative ingredient quality is to be checked in terms of moisture, crude protein, crude fibre, ether extract, nitrogen free extract, ash, acid insoluble ash, salts, non-protein nitrogen, micronutrients etc. The chemical analysis for the presence of antinutritional factors such as tannins, saponins, gossypol, cyanogenic glycosides, glucosinolates, mycotoxins, pesticide residues, heavy metals etc. is also must for ensuring quality and safety of the compounded feeds.

Evaluation of feed ingredients and compounded feeds

The feeds and feed ingredients are generally subjected to three types of tests:

1. Physical
2. Chemical
3. Biological

Physical evaluation

Physical evaluation is easy but rough in nature. One must be highly trained to identify the changes in the nature of the feed ingredients. Following are the physical characteristics discussed in details.

Colour : The appearance of the feedstuff reveals its quality. Any change in the colour of the ingredient gives an indication of maturity of the grains, storage conditions, presence of toxins, contaminations due to sand, use of pesticides etc. Orange to red colour of sorghum indicates higher tannin contents. Browning or blackening due to heat shows improper storage conditions. Black coloured fish meal indicates rancidity of fish oils.

Size : Size of grain indicates its energy value due to proportional decrease/increase in seed and its coat. Smaller the grain size will be the metabolizable energy. To evaluate one can take fixed number of grains and weigh. This technique is called test weight.

Homogeneity : The presence of contaminants like other grains, broken grains, husks weed seeds infested seeds reveals can be physically seen. In case of oilcakes closer observation reveals the presence of fibrous material. Rice polish sometimes contaminated with husk, which can be judge well by seeing the homogeneity of feed material.

Taste : As every ingredient has its own characteristic taste, any change in the taste like bitterness in grains, sunflower cake, groundnut cake is indicative of presence of mycotoxins. The level of salt can easily be detected by tasting the ingredients.

Touch : The dryness/rough measure of moisture content can easily be felt by touching e.g. chilliness indicates high moisture content. Clumps can be found out by inserting hand inside the bag. The clumps may be due to excess moisture, improper storage, packing of fresh warm solvent extracted meal. Clumps formed due to excess of moisture will be very hard. To evaluate rice polish, place about 25g on the palm and close the fingers tightly and then open the fingers it will become like a solid mass if the crude fibre is below 12%. In case value exceeds 12 % the mass disintegrates once the fingers are opened. Also pressure will be felt in closed hand in case of high fibre rice polish.

Other physical methods : Dry grains on pouring down or biting produces sound of spilling coins. Winnowing is the best method to detect husk in the feedstuff. Sieving is useful in differentiating contaminants based on particle size. To detect presence of sand a weighed quantity of grains can be soaked in water then by sieving with the hand the grains can be separated. Decant the remaining water and weigh the settled sand to assess the level of contamination.

Chemical evaluation

An analytical laboratory for the precise estimation of nutritive value and contaminants/anti-nutritional factors is of utmost importance. The feed samples collected as per the procedures at the time of supply must be analysed for the proximate principles. This indicates possible constraints on the usage due to the presence of excessive content of crude fibre, fat and total ash. Low crude protein and high fibre in oilseed cakes is indicative of adulteration with urea and or some inferior quality cakes. The amount of acid insoluble ash is a measure of amount of sand or other dirt. It is also desirable to determine fatty acid composition of oil rich ingredients. Chemical evaluation is thus important to ascertain if the ingredients procured conform to the desired quality. The chemical composition/ specification for various ingredients has been laid down by BIS which acts as guidelines for the suppliers, buyers and the users at farm level. Protein rich meals must also be analysed for amino acid composition.

Further, chemical evaluation of anti-nutritional factors must also be carried out so as to achieve the desired feed safety levels. For this advance laboratory with sophisticated instruments such as HPLC with photo diode array detector for the estimations of incriminating factors such as tannins, saponins, gossypol, cyanogenic glycosides, glucosinolates, PCBs etc. and with fluorescence detector (or ELISA reader) for estimating mycotoxins (especially aflatoxins). Inductively coupled plasma spectrometer is a power instrument for the determination of minerals and heavy metals quantitatively. GLC is another instrument highly desired for the analysis of pesticide residues in addition to fatty acid composition amongst the quality parameters.

Biological evaluation

Biological evaluation of feeds involves the use of animals. It needs specialized persons to conduct the digestion and metabolic trials on various species. These methods are time

consuming and are needed for setting the desired specifications and are not for routine quality assurance purpose. However microscopic evaluation of animal feeds can be carried out if so desired.

Microscopic evaluation

Feed microscopy is usually carried out for confirming adulteration and identifying the adulterants. Feed ingredients, adulterants and contaminants must be studied under low and high magnifications. Physical characteristics such as shape, colour, particle size, softness/hardness and textures can be examined at low magnification of 8x to 50x. It is useful in identifying impurities or contaminants and evaluating the quality of feed ingredients. It also serves as a tool to identify missing ingredients in finished feed. The plant cells and structural features of the feeds can be observed at high magnification of 100x to 500x since these characters are retained after grinding and even after powdering the feed ingredients.

A feed scientist must be familiar to feed ingredients and adulterants and must have collection of pure feed ingredients, adulterants and contaminants for the accurate fast quality assurance results. The meshed and sieved food should be used for better observation of plant histology and microscopic appearance.

Conclusions

The effective quality assurance programme needs an integrated approach. The well documented quality control manual is the first thing desired. Another important parameter for the effective quality assurance programme is well defined, exhaustive specifications for the feed ingredients as well as the compounded feeds. The facilities for quality control include specialized experienced manpower and well-equipped laboratory. Sampling is equally important for ensuring the quality control as a lot depends on how well samples have been drawn for reaching accurate and precise inference. The effective feed safety standards can only be maintained if proper quality assurance program is in place.

23 Udder hygiene, health monitoring, prevention of mastitis and therapeutics

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Mastitis is inflammation of the mammary gland usually caused by pathogens, mainly bacteria which have entered the teat canal. Intramammary infection (IMMI) usually occurs as an immune response of animal to bacterial invasion to eliminate invading pathogen. Bacterial presence within the udder results in the movement of white blood cells into the gland to help fight the disease. An uninfected mammary gland will maintain a low somatic cell count (<200,000 cells/ml of milk). Once gland tissue becomes infected, numerous neutrophils will be drawn to the mammary gland, resulting in increased somatic cell counts.

Mastitis can be divided into two major categories on basis of source of infection: contagious mastitis and environmental mastitis. Contagious mastitis can spread from infected gland to healthy gland during milking process by contact with infected milk, splashes or aerosols of milk during milking, milkers' hands or milking equipments.The most common bacteria causing contagious mastitis are Staphylococcus aureus, coagulase negative staphylococci (CNS) and Streptococcus agalactiae. The primary habitat of bacteria causing contagious mastitis is inside udders or on teat skin.Contagious mastitis is usually a chronic or subclinical mastitis.

The primary habitat of bacteria causing environmental mastitis is manure, bedding and soil. It can occur during environmental contact of the teats at milking time or between milkings. The major organisms causing environmental mastitis include the coliforms (Escherichia coli, Klebsiella species. and Enterobacter aerogenes), the environmental Streptococcal species (Streptococcus uberis, Streptococcus dysgalactiae, Enterococcus and other Streptococcus species) and Pseudomonas species. Most environmental mastitis cases are seen in the period immediately before, to a few weeks after, calving when animals are very susceptible to infection because their natural defence mechanisms are low. The environmental mastitis is usually an acute clinical mastitis but subclinical cases do occur too. On the basis of severity, mastitis is commonly categorised as clinical and subclinical, depending on the bacteria causing the infection and the immune response of the animal towards bacteria. Clinical mastitis is characterized by abnormalities in the milk or the udder that can be seen, eg. flakes, clots, and a watery appearance in milk, hardness and swelling of udder. Clinical mastitis can be acute clinical mastitis characterized by a sudden onset of symptoms and shows severe signs. Chronic clinical mastitis persists for a long time but is not

severe. Subclinical mastitis is characterized by an udder infection that shows no external changes in udder and milk but significant changes in milk composition. Subclinical mastitis can only be detected with somatic cell count (SCC), California mastitis test or microbiological culture of milk. Infected animal with subclinical mastitis serves as reservoirs and can infect other animals.

Type	Contagious mastitis	Environmental Mastitis
Source of Infection	Teat and udder	Contaminated environment
Transfer of infection in to udder	During Milking	Between milking and during dry period
Clinical mastitis	Most cases are subclinical	Higher proportion are clinical
Control by	Post milking teat dipping, dry cow therapy, milking hygiene and culling of chronic cases	Environmental hygiene, predipping, dry period teat sealant

Major differences between contagious and environmental mastitis (Blowey & Edmondson, 2010)

Principles of controlling mastitis:

1. Elimination of existing infections in udder: Antimicrobial therapy during dry period is a one of the method of choice. Dry period antibiotic treatment is a formulation of antibiotics prepared for administration into the udder immediately after the last milking of lactation to reduce new infections and elimination of existing infections. In India, ceftiofur hydrochloride 500mg/10ml preparation is available for the treatment of sublinical mastitis at the time of dry off mainly associated with Staphylococcus aureus, Streptococcus dysgalactiae and Streptococcus uberis. There are three phases of dry period namely early, mid and late dry period. In the first two weeks (early dry period); keratin plug forms a teat seal and active involution of udder tissue occurs. In the mid dry period, immunoglobulins and natural inhibitory substances like neutrophils and lactoferrins are formed. In the last two weeks before calving (late dry period), keratin plug slowly dissolves and colostrogenesis occurs. Animals are most succeptible to infection during first and last two weeks of dry period because teat plug is forming and dissolving respectively. Further depressed immune system of dam with approaching parturition contributes to new infections. The persisted subclinical infections during dry period may flare into clinical cases after calving. Most new cases of mastitis occur first four weeks of lactation and 60% of clinical cases by environmental pathogens originate from the infections established during early and late dry period (Blowey & Edmondson, 2010).

2. Prevention of new infection: Premilking and postmilking teat disinfection are the most effective mastitis control practice in lactating animals. Premilking teat disinfection with chlorhexidine in association with post milking teat disinfection reduces new intramammary infection. Post milking teat disinfection is regarded as the single most

effective control practice in lactating animals. Iodophores teat dips with 0.1 to 1% available iodine can be used as post dip. Post milking teat disinfection removes bacteria deposited during the milking process and therefore it is an extremely important control measure against contagious mastitis. Postdip should be applied as soon as milking is over. Teat must not be wiped dry after post dip. Premilking teat disinfection is aimed at reducing the incidence of environmental mastitis.

3. Monitoring udder health status: Implementing and effective system of monitoring udder health involves monitoring at herd and individual level. Use of animal side diagnostic test like California mastitis test - somatic cell count and milk bacteriological culturing are important for udder health and milk quality.

Five points mastitis control programme promoted by The National Mastitis Council (NMC) is as follows:

1. Udder hygiene and milking management
2. Milking equipments maintenance
3. Dry animal therapy
4. Appropriate therapy of mastitis during lactation
5. Culling chronically infected animals

Udder hygiene includes premilking udder preparation by washing teat in water and drying of teat with paper towel followed by use of 0.25% iodine premilking teat disinfectant (Dipping is better than spraying).

The foremilk stripping is checked for clinical mastitis (clot, watery or stringy milk) using a strip cup test. The early recognition and treatment of clinical cases is important part of mastitis control programme (Radostits, 2000).

Therapeutic management:

Mastitis is the most frequent cause of antibacterial use on dairy farms and contributes to a substantial portion of total drug and veterinary costs incurred by the dairy industry (Erskine *et al*.2003). Knowledge on the microbiological profile of mastitis by clinical and laboratory diagnosis (isolation, typification and antibiogram) is one of the basic pillars for the rational use of antimicrobial agents (ATM).The aim in selecting the best antimicrobial treatment regimen for mastitis is administering the drug at a dose and site that will allow accumulation in the mammary gland to maintain an effective drug concentration. There are three pharmacological compartments. The most common target compartment milk and epithelial lining of duct and alveoli of mammary gland. The S. agalactiae, S. dysgalactia, Coagulase negative staphylococci pathogen tend to locate in duct areas of the udder where antibiotics are effective S. agalactiae are very sensitive to penicillin, so treatment has a relatively high cure rate by administering intramammary administration (Erskine *et al*. 2003). Second compartment is deep tissue of the mammary gland. S. aureus can penetrate into udder tissue

and form micro abscesses that are protected from antimicrobials by scar tissue. They are difficult to cure, especially during lactation, so prevention is essential. Coliform mastitis involve animal itself, third compartment. Bacteraemia can occur and respond to systemic therapy. Mild cases of clinical coliforms mastitis generally are self-limiting and the animals own defense mechanisms can successfully clear the infection from the udder, and though antibiotics are not required at all; serious cases supports the use of systemic administration of antibiotics. Pseudomonas infection is impossible to treat so animal must be culled. In mastitis caused by penicillin-susceptible S. aureus strains, best results were achieved using a combination of systemic and intramammary treatment with penicillin G. In infections of the milk compartment such as streptococcal mastitis, there is probably no advantage of systemic administration indeed the concentration of penicillin G in milk remains 100-1000 fold lower than when given intramammarily.

Reasons for treatment failure :

Reasons for treatment failure include lack of contact between bacteria and antibiotics due to scar tissue formation, protection within leukocytes, poor drug diffusion, and inactivation by milk and tissue proteins; microbial resistance to antibiotics; improper treatment procedures like stopping the therapy too soon.

Fig 1 Acute clinical colliform mastitis in buffalo

Fig 2 Chronic Staphylococcus mastitis

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24 Clean milk production, milking techniques and induced lactation in dairy animals

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The international dairy and food safety standards describe milk drawn from the udder of healthy animals, collected in clean dry milking pails and free from extraneous matter like dust, dirt, flies, hay, manure etc., has normal composition, possesses natural milk flavour with low bacterial count and is safe for human consumption as *Clean Milk*. Since it is free from extraneous material, it has high keeping quality and commercial value and therefore can be transported for a long distance without its quality change. Dairy products prepared from such milk will be of high quality and long shelf life.

Milk, naturally, is sterile when secreted into an uninfected udder, but catches contamination during and after milking and subsequent processing like cooling and storage. In case the animal is from mastitis affected, the naturally secreted milk will be with high microbial load indicated by high somatic cell count. Milk is a highly nutritious medium which provides conducive environment for growth of bacteria, yeasts and moulds that are the common contaminants. High temperatures favour the growth of bacteria and spoiled the milk. Better management and good techniques of milking are essential for maximizing milk production and mastitis prevention and hence minimizing economic loss to the farmers.

1.1 Good practices for clean milk production

- Milking area should be ventilated, located separately, free from bad smells, odour and other disturbances.
- Mud, urine, faeces, and feed residues should be removed from the milking shed.
- Stress reduces the milk letdown process hence handle the animal calmly for maximum production.
- Wash teats and udder with clean water and wipe with cloth dipped and squished in disinfectant (Fig.1).
- Animals should be periodically examined for udder swelling, pain and use foremilk from each quarter for infection detection in strip- cup.
- After milking, milk should be filtered through fine sieve, covered and keep in cold place.
- While using machine milking, attach the milking unit within one minute after the start of stimulation.

- Milk from animals being treated for mastitis should not be allowed mixing with normal milk.
- Post milking teat dipping is essential for mastitis control in lactating animals.
- There should be a well-defined 'dry cow therapy' for taking care of mastitis effected animals during resting stage of udder in dried animals

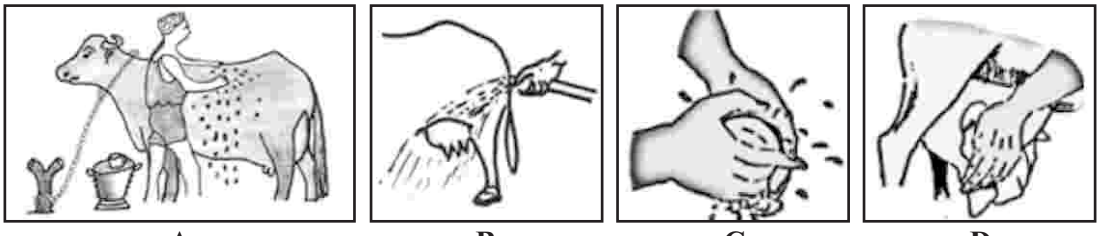

Fig. 1 Prepararion of animal for clean milk production (A) Bathing, (B) Udder cleaning, (C) Cloth dipped and squeezed in antispectic solution and (D) Cleaning of teats and udder

In farms where weaning is not practiced, allow the calf for suckling or led down before start of milking but teat should be wiped with a dipped and squeezed in some antiseptic solution and clean with dry cloth. Before start of milking some feed or concentrate may also provided to the animal so that milking can be performed without any disturbance.

Apart from cleanliness of animal, the milkers as well as the milking pails/utensils should also be clean. The milker should wear clean dress and cover their heads with suitable cap/cloth to protect fallen of loose hairs into milk. Nails of milkers should be well trimmed and hands washed with disinfectant and wiped with towel before and after milking (Fig. 2). Milkers with ill and having filthy habits like sneezing, spilling, blowing nose, infection on the hands etc. should not be allowed, only healthy milker are to be allowed for milking.

Fig. 2 Correct way of washing hands

Lactating buffaloes become habituated to certain routines and sudden change in the routine will disturb the buffalo resulting decline in the milk yield, it should be brought gradually. First calver buffalo are very sensitive and start kicking due to tickle hence only experienced milker should milk these animals. Milkers should be rotate among the buffaloes so that the animal will get accustomed to all instead of single.

After each milking, pails and cans should first be washed with warm water, scrubbed well with detergent or sanitizer and then rinsed well with clean water. Keep the milking

Fig. 3 Milking pails/cans and their drying after cleaning

pails/cans in the revert position on stand for dry. Clean milking pails should be joint less dome-shaped top/ wide mouth and broad base of stainless steel used (Fig. 3).

2 Milking methods

Teat is the first line of defence against mastitis pathogens. The milking process may affect teat's condition, increasing the risk of mastitis. It is well-proven that teat-ends with severe erosions or broken skin will have an increased risk of mastitis. Majority of buffalo owner's follow the hand milking while only a few commercial dairies use machine milking.

2.1 Hand milking

Hand milking is a manual and labour intensive with low costs. Milking is an art which require experience and skill. Milking should be done gently, quickly, neatly and completely. Hand milking is done using clean and dry hands. It is performed by massaging and pulling down the teats, squirting the milk into milking pail. Hand milking exerts pressure in the teat ejecting milk out. For hygienic milk production milking clothes, buckets, udders and hands should be clean. Teats of buffaloes are various in size and shapes therefore, majority of farmers hand milking is practiced from left side in buffaloes. The order of milking the various teats also differs. Milkers may milked the teats cross wise. Hand milking is also done by three methods: (i) stripping, (ii) knuckling and (iii) full hand milking. Out of these three methods the Knuckling and full-hand milking are the two commonly used for milking in buffaloes.

(i) Full hand method: In this method hold the whole teat in between thumb and the first finger encircling the teat. The base of the teat is closed in the ring formed by the thumb and forefinger so that milk trapped in the teat sinus may not go back into the gland eastern. Simultaneously, increase the pressure on teat by squeezed between the middle, ring and little fingers and the hollow of palm, thus, forcing the milk out (Fig. 4). This process should be repeated in quick succession. By maintaining a quick succession of alternate compressions and relaxations the alternate streams of milk from the two teats sound like one continuous stream. Milking by this method animals feel good, no stress on animals during milking and animals became cool and calm.

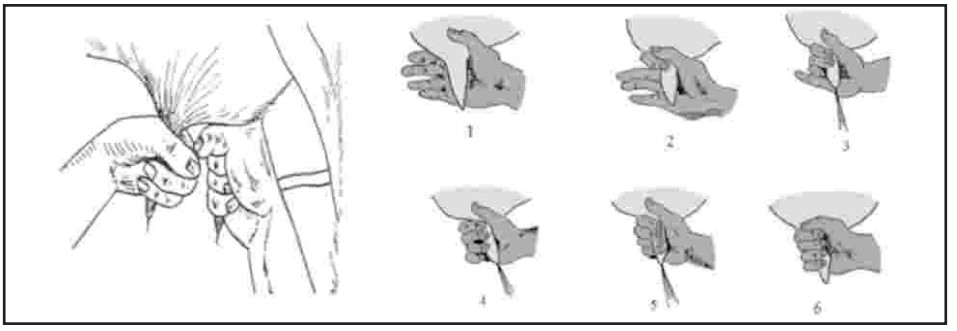

Fig. 4 Full hand milking

(ii) Knuckling Method: It is also similar to full hand milking method. In this method the milkers tend to bend their thumb in, against the teat and the first finger encircling the teat (Fig. 5). Simultaneously, increase the pressure on teat by squeezing between the middle, ring and little fingers and the folded thumb, thus, forcing the milk out. It creates excessive pressure on teat and continuous milking with this method can damage the teats affecting milk production as well as beauty of animals. Animal feels discomfort during milking hence not advised to be practiced.

Fig. 5 Knuckling method of milking

(iii) Stripping method: Stripping consists of firmly seizing the teat at its base between the thumb and forefinger and drawing down the entire length of the teat pressing it simultaneously to cause the milk to flow down in a stream (Fig. 6). The process is repeated in quick succession. Both hands may be used, each holding different teat, stripping alternately. Milkers generally use this method for first calvers as the teats are of small size or at the last stage of the milking. Stripping causes more irritation to teats due to repeated sliding of fingers on teats, sometimes teats of animal become injured which causes mastitis. This method is discomfort to buffaloes so it should be avoided.

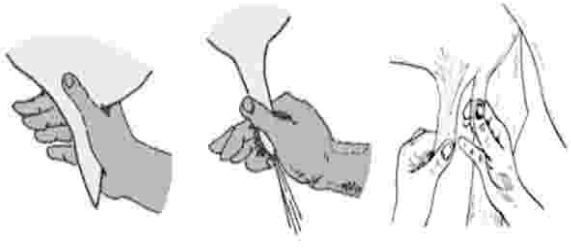

Fig. 6 Stripping method of milking

Full hand milking removes milk quicker than stripping and knuckling because of no loss of time in changing the position of the hand. Buffaloes with large teats are milked by full-hand and knuckling method. Full-hand method is safe and superior to stripping and knuckling

method as it simulates the natural suckling process by calf. In spite of these drawbacks when all milk that is available is drawn out by full-hand method, stripping should be resorted to with a view to milk the animal completely; the last drawn milk is called stripping and is richer in fat.

Post-milking teat disinfectants

The appropriate use of teat disinfecting products reduces mastitis rates and the need for antibiotic use. Just after milking Clean the milking pail by an initial rinse in clean water, followed by scrubbing in a hot detergent/disinfectant solution and finally rinse with fresh water. All equipment must be drained and dry between milking's interval.

2.2 Machine milking :

Machine milking works on the principle of vacuum just like the calf sucking its mother. The Vacuum applied to the teat opens the liner (milking phase) and milk flows down, then the atmospheric air is allowed to enter thus causing liner to close (massage phase). Automatic milking refers to the extraction of milk from buffaloes without human labour. Farmers can select automation as per their needs and budget. In machine milking systems, dairy farmers can reduce costs of labour for milking process. Farmers give more time to focus on farm management issues such as feeding, breeding, health and hygiene. In machine milking important consideration have to be considered as any negligence can cause adverse effect on the animals' health and milk production. The operator has to check the milking vacuum, vacuum level, pulsation rate and pulsation ratio. Also monitor the milking attachment so for avoiding over or under-milking. Over-milking can damage teat ends and leads to mastitis.

Bucket machine milking:

Bucket milking machines were developed to mechanise onsite milking. Each portable unit, consisting of a 15 litre capacity lidded bucket, pulsator and teat-cup assembly or cluster, requires manual attachment to a vacuum supply when it is moved from animal to animal during milking (Fig. 7). Milk is tipped from the buckets into milk cans for transportation and storage in cold place. The system is mechanically simple with relatively requires low investment, running and maintenance costs in comparison to line milking machines in parlours.

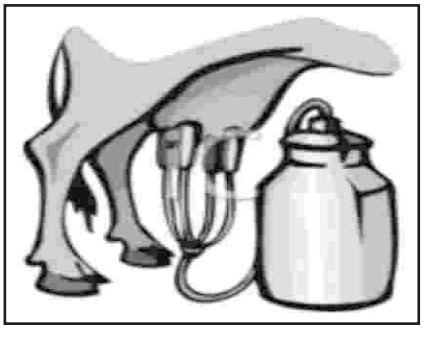

Fig. 7 Bucket milking in buffaloes

Direct-to-Can milking

Direct-to-can milking is a very simple, low cost system of milking, cooling and cleaning specially devised for abreast parlour milking. Milk is drawn directly from udder to milk can, eliminating milk lifting, carrying and tipping and thus enabling each operator to manage 4 or 5 milking units effectively (Fig. 8).

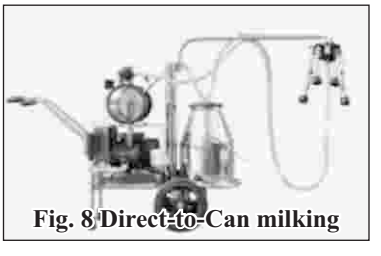

Fig. 8 Direct-to-Can milking

Pipeline milking: Pipeline milking is a high investment, low labour cost system, suited to large sized herds Milk is transported direct from udder to refrigerated bulk milk tank for cooling and storage and plant cleaning is done in-situ. In addition, devices can be inserted into the milking pipeline to reveal clinical signs of mastitis, indicate the milk yield from each buffalo, allow samples to be taken and automatically remove the cluster when milk flow ceases. Pedometer is also fixed by which animal can be sorted for estrus.

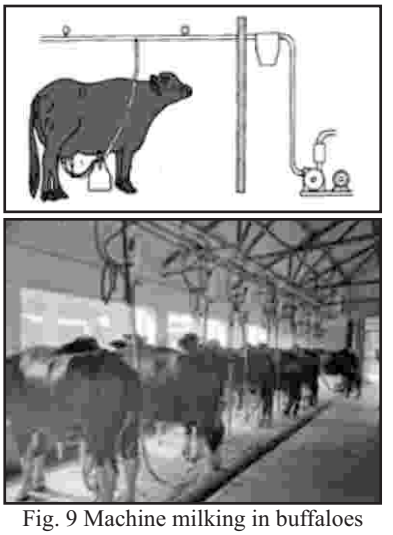

Fig. 9 Machine milking in buffaloes

Prevention of post-milking infection: Post milking disinfection is the most effective way to prevent the spread of mastitis. After milking teat canal remained open and mastitis causative pathogen can easily enter through teat orifice. After completion of milking, animal feels tired and seek relaxation by lying down immediately. The floor is usually spread with waste feed, dung and urine, which are source of infection through teats. So keep animals in standing position at least 20-30 minutes after milking – by sending them to the feeding area/grazing. Always use teat dip just after milking. Milking system should be sterile hence run a complete cleaning cycle by using appropriate detergents and sanitizer after every milking.

3.0 Induced lactation in buffaloes

Sterility and infertility in buffalo account for major economic losses to dairy farmers. Buffalo is the main stay of dairy industry which contributes more than 53% of total milk production. After few lactations, some elite buffaloes become infertile due to one or other reproductive problem i.e. anestrus, failure of conception, cystic ovaries, non-specific abortions etc. Though, these conditions lower the animals reproduction rate, they do not affect production ability of the animal which can be manipulated by artificial means to simulate the changes of reproductive phase - in order to induce changes in mammary gland for initiation of milk production. This process is called artificial induction of lactation and through this process; animals can be brought into optimum production with no adverse effect.

Normally, animals produce milk-after parturition, as a natural mechanism to feed their young-one. The reproductive success in turn initiates the cycle of production. In herds experiencing high levels of infertility, the overall milk production becomes low because of a large number of unproductive animals. This increases the burden on farmer, in spite of his best efforts to maintain high production potential livestock, best management system, housing, feeding and other facilities with favorable climate for milk production. As all dairy farms / farmers are not in a position to replace all infertile animals, these can be made productive through this treatment. The only production that can be harvested from these

animals is therefore milk. Artificial induction of lactation can be done in the high yielder buffalo when they fail to continue the reproductive cycles or become infertile or even sterile.

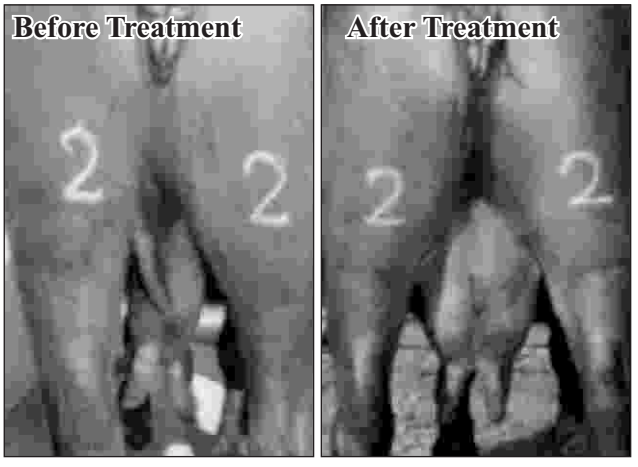

Fig. 10 Udder development in buffaloes undergoing hormone treatment.

More than 50 year ago, scientists succeeded in inducing lactation in dairy heifers by using sex steroids. Later long term treatment with estrogen or estrogen and progesterone combination ranging from 60-180 days in different studies were tried but response of treated animals for milk production was limited. A breakthrough was achieved only after scientists published their results on induction of lactation in cows having reproductive disorders. The procedure described by scientists to initiate lactation in cows involved administration of high dose of estradiol-17 B (0.1mg / kg body weight / day) and progesterone (0.25mg / kg body weight / day) i.e. in the 1:2.5 ratio, for a period of 7 days. Other scientists used different dose of estradiol -17β and progesterone in the ratio of 1:1 @ 0.1mg / kg body weight / day for a period of 7 days in buffaloes and found peak milk yield up to14.5 kg/day which is highest reported so far (Fig.10).

Protocol for milk induction

Before start of the treatment weigh the animals to be induced and should preferably be treated for endo-parasites. A dose of estradiol-17 B and progesterone are to be calculated @ 0.1-mg/ kg body weight/day for buffaloes and 0.1mg and 0.25mg/ kg body weight/day for cattle. The requisite amount of each hormone is separately dissolved in 7.0 ml absolute ethanol for the 7 day treatment. From this solution, 1.0 ml of each hormone is administered daily in equally divided doses given at 12 hour intervals for seven days. After a lapse of seven days, udder massage should be done daily in the morning and evening to simulate milk let-down. This practice has to be followed till the udder becomes turgid (7-8 days) and thereafter, i.e. approximately 21-22 days after the start of treatment, regular milking can be done. In induced buffaloes, milk becomes normal within 2-3 weeks after the start of milking in almost all respect i.e. hormones, fat, protein and lactose content. Generally the treated animals give milk around 200-250 days. It has been observed that ~25% animals which were infertile due to hormone imbalance started showing estrus and conceived after the treatment. Around 75-85% animals respond to this treatment.

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