

NDRI News

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From the Director's Pen



Misti Dahi containing whey protein hydrolysates

One of the major reasons for shortened “life-time milk production” of dairy animal is the transient loss of fertility (infertility). In general, the etiology of infertility in dairy animals are complex and can be classified into congenital, morphological, functional, infectious and of unknown causes, among which post-partum uterine infection alone accounts for more than 25%. Infectious diseases affecting reproduction can cause loss throughout the reproductive cycle by decreasing the ovulation rate, fertilization rate, embryonic survival rate and fetal survival rate. Uterine bacterial infection in cows and buffaloes is a dynamic situation, where regular contamination, clearance of bacteria and spontaneous re-contamination during the first few weeks after parturition takes place, rather than just contamination at the time of calving. A wide range of bacterial species, both Gram-positive and Gram-negative, aerobes and anaerobes have been reported to be isolated from the early postpartum uterus. Most of these are environmental contaminants that are gradually eliminated during the first 6 weeks of postpartum; as such only presence of bacteria in the uterus of post-partum cows does not always result in inflammatory uterine diseases. But, adherence of pathogenic organisms to the mucosa, colonization or penetration to the epithelium and/or release of bacterial products (toxins, enzymes, etc.) may lead to establishment of uterine infections.

Postpartum metritis leads to very high economic losses due to prolonged open days and prolonged inter-calving intervals, resulting in reduced lifetime productivity and also culling of animal. In an estimate from the USA, it is reported that each case of metritis leads to loss of \$304 to \$354 to the producer due to losses in production and performance. Although, the magnitude of this estimate may surprise many, but in



reality, the metritis is an expensive disease in cattle and buffaloes. Uterine infection is also associated with lower milk yield and if this is associated with retained placenta then the magnitude of milk loss is further high. It has been calculated that high yielding cows with mild and severe metritis produced 5.7 and 8.3 kg/day less milk, respectively than healthy cows during the initial 3 weeks of postpartum period. In buffaloes, it has been reported that the milk yield decreased by 239 kg in retained fetal membrane, 181 kg in stillbirth, 173 kg in dystocia, and 98 kg in metritis in a single lactation. Further, in the animals with uterine infection, the release of GnRH from the hypothalamus and LH from the pituitary is also suppressed by LPS (lipopolysaccharide), reducing the ability to ovulate a dominant follicle. Recent investigations revealed that animals with uterine infection had high concentration of LPS that reduced the expression of aromatase, an enzyme required for conversion of testosterone into estrogen. Thus, these animals had lower peripheral plasma estradiol concentrations and are less likely to ovulate, due to poor estrogen positive feedback to the LH. And this ultimately results in increased numbers of inseminations per conception.

In the treatment of uterine infection, generally intra-uterine antibiotics are used, but on several occasions, these antibiotics

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are used injudiciously. The antibiotic should be selected carefully and these should be active against the major uterine pathogens and should maintain its activity in the changed environment of the uterus i.e. altered pH and in the presence of pus, inflammatory exudates and barrier. Antibiotics that are ineffective under anaerobic conditions, such as the aminoglycosides are not recommended in the treatment of postpartum uterine infection. The pharmacokinetic properties of antibiotic preparation should allow the rapid distribution of the antibiotic throughout the uterine cavity, and its good penetration into the endometrium. Further, parenteral administration (i.m, i.v etc.) of antibiotics has been found to be more effective than the intra-uterine infusion in the treatment of post-partum metritis.

In India, although there is no authentic estimate on the loss associated with uterine infection, we can emphasize that

significant financial loss in dairy sector could be curbed by aggressively working on prevention, diagnosis, and proper treatment of uterine infection in cows and buffaloes. Better understanding of etiology, pathogenesis and improvement in diagnosis and therapy would be of great practical and economic importance. Although recently the research on identifying the pre-partum “bio-markers” for early detection of the cows at risk of developing post-partum metritis has been initiated, we are still at infancy stage. If we could develop “on-spot” kits to identify the animals at risk of developing metritis at an early stage, effective management strategies can be planned to prevent the post-partum uterine infection and related consequences.

A.K. Srivastava

(A.K. Srivastava)

RESEARCH

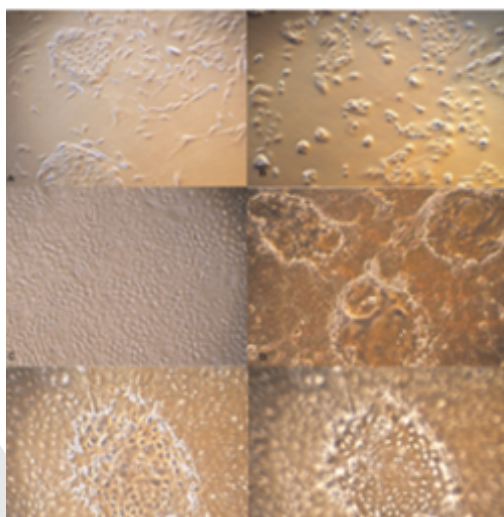
Development of a spontaneously immortalized Buffalo Mammary Epithelial cell line (BuMEC)

(A. K. Mohanty, J. K. Kaushik, Sudarshan Kumar, D. Malakar and A. K. Dang)

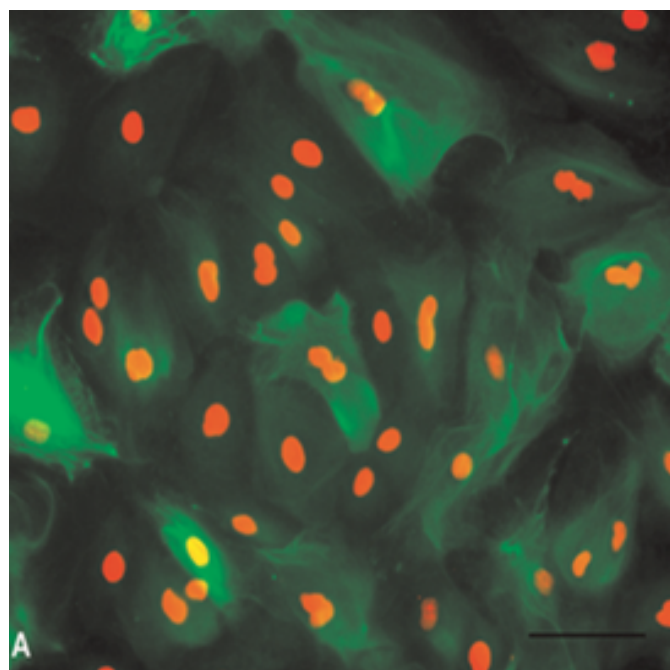
Animal Biotechnology Centre

A spontaneously immortalized Buffalo Mammary Epithelial cell line (BuMEC) has been established for the first time at NDRI. This has been maintained long-term in culture with growth and functional properties unique to Mammary Epithelial Cells. This cell line can be used as a model system for functional study involving mammary cell function,

genomics, transcriptomics and proteomics for understanding mammary gland biology, in general and mammary biology of buffalo, in particular. Buffalo mammary tissue collected from the slaughter house was processed enzymatically to obtain a heterogenous population of cells containing both epithelial and fibroblasts cells. Epithelial cells were purified by selective trypsinization and were grown in a plastic substratum. The purified mammary epithelial cells (MECs) after several passages were characterized for mammary specific functions by immunocytochemistry, RT-PCR and



Photomicrographs of isolation and culture of Buffalo Mammary Epithelial cells (BuMECs). A: Mixed population of epithelial and fibroblast cells (x100); B: Purified BuMEC seeded at low density forming islands (x100); C: Confluent mono layer of BuMECs showing cobble stone morphology (x100); D: Post confluent stage BuMECs forming dome structure (6100); E: Phase contrast image of dome structure with focus on the monolayer (x100); F: Phase contrast image of dome structure with focus set at the top of dome (6100). The dome structure represents a raised layer of cells above the plastic substratum.



Immunostaining for cytoskeletal markers in BuMECs. Fluorescent image of BuMECs stained for Cytokeratin 18 showing intermediate filaments.

western blot.

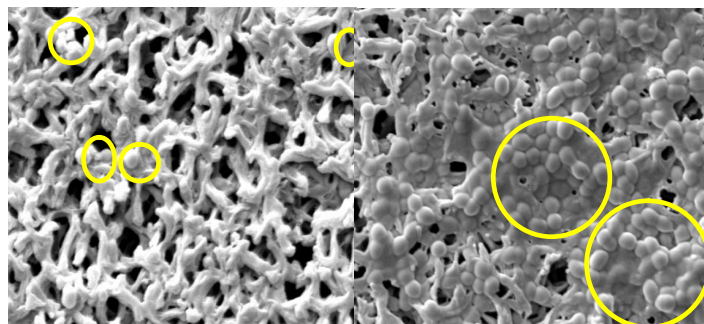
Principal Findings: The established buffalo mammary epithelial cell line (BuMEC) exhibited epithelial cell characteristics by immunostaining positively with cytokeratin 18 and negatively with vimentin. The BuMEC maintained the characteristics of its functional differentiation by expression of b-casein, k-casein, butyrophilin and lactoferrin. BuMEC had normal growth properties and maintained diploid chromosome number ($2n = 50$) before and after cryopreservation. A spontaneously immortalized buffalo mammary epithelial cell line was established after 20 passages and was continuously subcultured for more than 60 passages without senescence.

Characterization of pediocin-resistant *Enterococcus faecalis*

(Rashmi Kumariya, S. K. Sood and Y. S. Rajput)

Animal Biochemistry Division

Enterococcus faecalis is a gram-positive bacterium and is ranked as third most opportunistic pathogens isolated from bloodstream infections. The increasing prevalence of *E.*



Scanning electron micrograph of wild-type and pediocin-resistant of *E. faecalis*

faecalis with resistance to almost all available antibiotics, created a need for development of new ways to combat this bacteria. Antimicrobial peptides (AMPs) such as pediocin also have the capability to kill *E. faecalis*. These bacteria also acquire resistance against AMPs. A resistant variant acquires the mechanism to overcome the bactericidal effect of AMPs. A number of changes occur in cell-wall and cell-membrane in pediocin-resistant *E. faecalis*. A number of resistant variants of *E. faecalis* differing in their degree of resistance were selected and characterized. Morphologically, wild-type strain exists in pairs or in short chains, while pediocin-resistant variants exist in aggregated form. The degree of aggregation increases with increase in degree of resistance. Usually, cell-wall of resistant variant is thick, hard and opaque as opposed to thin, soft and transparent in wild-type *E. faecalis*. The degree of aggregation in resistant variants is linked to hydrophobicity and ability of the variants to form biofilm. The cell-wall of resistant variants acquires reduced negative charge and therefore, cationic AMPs are unable to electrostatically interact with cell-wall and fail to diffuse through it to reach periplasmic space.

Development of Functional Whey Protein Hydrolysate through Enzymatic Hydrolysis

(Alok Chatterjee and S. K. Kanawjia)

Division of Dairy Technology

Whey-the greenish liquid discharge, chiefly of cheese and paneer; once considered as a bane has resurrected into boon for the industry as well as human consumption, with array of techno-functional and bio-functional benefits. Hydrolysed whey proteins contain high levels of bioactive peptides including antihypertensive, anticancer, immunomodulatory, opioid, mineral binding etc. The study herein was envisaged to develop a functional whey protein hydrolysate through enzymatic hydrolysis. Enzymes trypsin and FlavorPro Whey 750P were selected for hydrolyzing, individually and in combination, WPC-70 in order to exploit the antihypertensive and antioxidant biologically active peptides to the maximum. An array of combinations of the selected parameters, individually for either enzyme, was obtained for this purpose employing the Central Composite Rotatable Design model through the Response Surface Methodology approach of Design-Expert®. Based upon several conditions, an optimized set of parameters, with maximum desirability value was obtained for trypsin hydrolysate, with maximum being-sought properties. The further perusal of hydrolysis by FlavorPro Whey 750P was not entertained owing to its lower values for the biofunctional properties and % ACE in particular. The hydrolysis routed by combination of enzymes did not yield significantly promising result. The validated hydrolysate was subjected to peptide isolation, employing ultrafiltration and freeze-drying, followed by characterization of the amino acids and identification thereof by LC-MS/MS. Several peptide sequences were obtained conferring and conforming the sought bio-functional properties (however, mostly antihypertensive) whilst a couple of them reflected some other, though unsought (such as bactericidal and immunomodulatory) reported beneficial properties and a few unreported peptides. Further corroboration for the correctness of the obtained results was established by comparing results to in silico analysis. The hydrolysate was thereafter utilized as a functional ingredient for the preparation of a popular traditional fermented-Indian-dairy-product misti dahi, at three different levels (1, 2 and 3%, v/v), as an attempt to study the effect and acceptance. Initial screening for selection of level was rested upon high values of biofunctional properties, subjected to sensory acceptance. Thereafter, for biofunctional properties in particular, the experimental misti dahi reflected statistically significant difference within treatments and time intervals and recorded 65.92% of ACE inhibition and 26.86% of antioxidant property, by the end of storage period, in relation to the conventionally prepared product (control). During the storage period, the factors indirectly capable of quantifying the shelf life and acceptability of the product, mostly, revealed statistically significant implication, both

within days (time intervals) and within groups (treatments), individually or in conjugation. The study culminated with the calculation for the cost of production of misti dahi that was computed to not only be closely comparable (₹ 8.76 per 100 g) in contrast to ₹ 8.45 for the control but also lower (₹ 12

per 100 g) to that of the misti dahi available in the market, but conventional.

EXTENSION

DAIRY EXTENSION DIVISION

Dairy Education at Farmers' Door

Dairy Extension Division organized the Extension Education Programme "Dairy Education at Farmers' Door" to strengthen the dissemination of dairy production and processing technologies among farming community. Under this programme, a team of NDRI scientists including subject matter specialists from production, processing and management group visited villages viz. Khrijpur and Wazidpur Karnal district on 2nd Saturday of the month and obtained the feedback from the participating farmers.

The main issues in cluster of villages which emerged during

Activities Conducted	No.
• Infertility and Veterinary Aid Camp	14
• Anoestrus and Late Maturity	85
• Repeat Breeding (Conception Failure)	55
• Metritis & Endometritis	25
• Pyrexia	05
• Wound	10
• Eye Infection	05
• Indigestion	10
• Diarrhea	30
• Mastitis	05
• Retention Placenta	25
• Prolapse of Uterus	10
• Cystic Ovarian Condition	25
• Blood Protozoa Diseases	05
• Tick Control Campaign	600
• Deworming (Endo- parasite)	300
Total Cases	1195

the discussion were:

- Tick Control Problem: Tick infestation was a serious problem in village Wazidpur and Khirajpur. Tick control treatment was administered on the animals.
- Anoestrus: The scientists educated the farmers on recommended breeding and feeding practices to address this problem.
- Low milk production during summer: The scientists informed/educated the farmers to offer balanced nutrition to their animals, so that milk yield could be maintained as per animal potential.

Field/Farm Activities

In a total of 14 Veterinary Camps, 1195 cases were treated for reproductive disorders and various veterinary ailments.

Ecto and endo-parasite control programmes were conducted. Special attention was given to improve the productive and reproductive performance of the dairy animals

Kisan Sangosthies

Nine Kisan sangosthies were organized at village level. A total of 75 male and 20 female farmers were benefitted from the sangosthies. The following topics were taken up during the sangosthies.

- Awareness on ecto-parasite infestation
- Awareness of vaccination programme and FMD in

Name of the Programme	Village/ on-Campus	Total Courses Conducted	No. of Participants
Capacity building of farm-women in scientific dairy farming	Subri Shahpur Bazidpur	4	59
Capacity building of farm women in fruits and vegetables preservation	Kulwehri Subri	2	28
Capacity building of farm women in value added milk products	Kulwaheri, Bazidpur Shahpur	4	44
Total		10	131

cross-bred animals and its control measures in all categories of the animals

- Importance of animal deworming
- Role of mineral mixture in animal diet
- Care and management of prolepses of genital organs
- Correct time of breeding of females
- Prevention and control of mastitis
- Clean milk production practices in rural areas
- Role of reducing inter-calving period in lactating animals

Empowerment of Women and Mainstreaming of Gender Issues

Nine women empowerment training campaigns were organized to create awareness in the field of dairying and home science and also to impart skills in these areas so that farm women could generate more income. By these programmes 131 farm women were trained

Educational Visits and Tours: A total of 1907 students (UG &

PG) comprising 1228 male and 628 female students, and 51 faculty members from 35 colleges/universities visited the Institutes. The groups were sensitized about the different research, teaching and extension achievements and facilities available in the Institute.

Training Programmes Organized: In all 30 training programmes (On-campus and Off-campus & training-cum-visits) on different aspects of dairy production and processing, crop and vegetable production, vermi-culture, bee-keeping and home science were organized. In all, 870 farmers, women, rural youth and extension functionaries were imparted training.

Sponsored Training Programmes: Out of the total courses, KVK organized 18 sponsored training programmes on Scientific Dairy Farming, Clean Milk Production, Commercial Dairy Farming and Scientific cultivation of Fruits and Vegetables for 490 farmers, rural youth and extension functionaries. In these training programmes 343 practicing farmers from Bihar, 95 trainees from Rajasthan, 30 trainees from Himachal Pradesh and 22 veterinary officers from Punjab state were imparted training.

Exposure Study Visits Organized: KVK also organized 18 exposure cum study visits for 470 farmers and farm women from different districts of Uttar Pradesh, Madhya Pradesh, Uttarakhand, Bihar, Rajasthan, Punjab, Haryana and Himachal Pradesh.

Animal Health Management Activities: Various Animal Health Management activities were organized through Stockman centers in adopted villages of KVK. At these centers 641 cattle and 400 buffaloes were artificially inseminated and 382 calves were born. Besides these, 39 animals were treated, 43 calves were dehorned and 32 animals were given infertility treatment.

Front Line Demonstrations (FLDs)

Oilseed Crops: During the Rabi season 2012-13, a total 24 FLDs on mustard using varieties Pusa Vijay, CS-54 and CS-56 were laid in 9.0 ha. area in different villages of Karnal district under irrigated conditions. In the demonstration plots, an

average production of 17.36, 16.42 and 16.47 q/ha respectively with aforesaid varieties was observed. The overall performance of these varieties was found to satisfactory.

Pulses: During the Rabi season 2012-13, a total 16 FLDs on gram using BG-1103 variety were laid in 3.23 ha. area in different villages of Karnal district under irrigated conditions. In the demonstration plots, an average production of the demonstrated variety was observed to be 19.83 q/ha. The overall performance of the variety was found to satisfactory.

FLD on Summer Moong: During the summer season 2013, a total 16 FLDs on summer Moong using variety MH-421 procured from CCS Haryana Agricultural University Hisar in 6 ha were laid in different villages of Karnal district under irrigated condition.

FLD on Jowar and Maize (Fodder): A total of 12 FLDs on Jowar Fodder (multi-cut) using variety Sudax Chari-1 in 3.23 ha area and 8 FLDs on maize fodder using variety African Tall in 2 ha area were laid in different villages of Karnal district under irrigated condition.

On Farm Trial on Paddy: Two on-farm trials on paddy in 2.83 ha area at 7 locations using varieties Pusa-1121 and PB-6 (1401) were organized in different villages of Karnal district. KVK also laid trials on different varieties of paddy using direct seeding of rice technology.

Revenue Generation in KVK

Source	Amount(₹)
Training Fee from KVK Courses	23,500
Training Fee from sponsored courses	3,98,021
Tuition fee from visits conducted	9,000
Room Rent from Farmers' Hostel	85,320
Dairy Vikas Kendras	28,840
Fisheries Unit	4,620
Crop Production Unit	10,800
Bee-keeping Unit	17,770
Sale of KVK Publications	81,900
Total	6,59,771

CONSULTANCY SERVICES

1. Contract Research

- Contract Research Project entitled "Evaluating corn hybrid on the quality of silage and performance in cross bred calves" Sponsored by Monsanto India Limited, Mumbai under (Dr. A. K. Tyagi, Principal Scientist, DCN Division) (total project cost ₹ 8,50,43/- including S. Tax); Received 2nd final installment of ₹ 3,50,412/- including S. Tax (i.e. S. Tax ₹ 38574/-) = ₹ 3,11,865/- ₹ 3,11,865/-
- Contract Research Project entitled "Identification and safety evaluation of NCDC 428 culture" Sponsored by Danone Food & Beverage India Pvt. Ltd. Gurgaon under (Dr. R. K. Malik Head, DM Division) (Total project

cost Rs. 3,74,635/- including S. Tax); Received Rs. 3,74,635/- including S. Tax (i.e. S. Tax Rs. 41,211) = Rs. 3,33,425/- Rs.3,33,425/-

Sub Total (1) ₹ 6,45,290/-

2. Contract Services

- Analyses ₹ 25,000/-
 - Sale of Kit ₹ 5,000/-
- Sub Total (2) ₹ 30,000/-

3. Student Training Charges ₹ 2,36,500/-

4. Consultancy (Individual/General) ₹ 63,890/-

Grand Total ₹ 9,75,680/-

EVENTS

Awareness Programme on Multiple Micro-nutrients Fortified Milk

A consumer awareness programme was organized at milk parlour, NDRI, Karnal on 27th April, 2013. Two variants of fortified milk, one containing iron in combination with vitamin A and the second variant containing calcium and vitamin D were introduced for consumers. Sh. Shashank Anand, Supdt. of Police, Karnal was the Chief Guest on this occasion. Some of the leading doctors of



Sh. Shashank Anand, Supdt. of Police, Karnal launching iron and vitamin fortified milk at the milk parlour

Karnal also attended the awareness programme. A 250 ml serving of the iron and vitamin A fortified milk provides about 30-35% of iron and vitamin A requirement on daily basis. Similarly, A 250 ml serving of the calcium and vitamin D fortified milk provides about 40-45% of calcium and vitamin D requirement on daily basis. The product has high heat stability and good sensory acceptability upto 7 days storage.

World Veterinary Day

The World Veterinary Day 2013 was celebrated at NDRI, Karnal on 27th April 2013. The theme of the celebration function this year was "Vaccination to Prevent and Protect". Dr. K. S. Dangi, Director General, Department of Animal Husbandry and Dairying, Government of Haryana inaugurated the celebrations. While addressing the large gathering of scientists, students and farmers, Dr. Dangi called upon the farmers to pay special attention to regular vaccination of animals for the double benefit of profitable animal production as well as safe human health. Dr. Rameshwar Singh, Project Director, DKMA, ICAR, New Delhi said that several livestock improvement technologies are available at NDRI and farmers must take full benefit of it. Dr. AK Srivastava, Director and Vice Chancellor, NDRI, Karnal presided over the function and delivered a lecture on Bird Flu, its symptoms, diagnosis and the ways to protect poultry birds from this disease. He emphasized that contrary to the general public perception; bird flu is very much manageable with proper vaccination plan and it is absolutely safe to consume poultry eggs and meat handled hygienically. On this occasion, the retired veterinarians from NDRI and Govt. of Haryana were felicitated for their outstanding

contribution to the veterinary profession.

Brainstorming Session on "Buffalo Estrus Biology"

The biggest bottleneck in achieving the desired conception rates with artificial insemination in buffaloes is estrus detection. In view the seriousness of the issue, a Brainstorming Session on "Buffalo Estrus Biology" was organized on 28th – 29th April, 2013 at NDRI, Karnal. The objectives of this brainstorming session are to strengthen the research on understanding the estrus biology and to define a strategy for developing effective tools/methods for improving estrus detection efficiency in buffaloes. Dr. A. K. Srivastava, Director NDRI presided over the inaugural function and addressed the audience about the importance of estrus detection in buffaloes. Dr. R. K. Sethi, Ex-Director, CIRB; Dr. B. K. Joshi, Director, NBAGR and Dr. A. Asokan, Dean, MVC, TANUVAS also addressed the audience on the theme of the brainstorming session. The technical session was held on 29.04.2013. During flagging of the issue, Dr. A. K. Srivastava stressed that each missed heat is a missed opportunity and in animals that show a seasonal trend in reproduction, like in case of buffaloes, loss of 21 days is equal to loss of two cycles adding further to financial consequence. Experts presented their views on estrus behavior in buffaloes, estrus synchronization protocols, use of pheromones and sensors to improve estrus detection and differences in estrus biology between cattle and buffaloes. The presentations were followed by thorough discussion. Dr. Inderjeet Singh, Director, CIRB informed the house that understanding the follicular dynamics and dominance in relation to estrus behavior and fertility is very important to device effective estrus detection tools/protocols. Although it is generally believed that the biology of estrus in buffalo is similar to that of cattle, there are several unique features in buffalo. Thus, all the participants were of the opinion that understanding the estrus biology (which is very basic but yet not understood fully) is of paramount importance to develop tools/methods for improving estrus detection efficiency and accuracy. Around 50 participants including scientists, researchers, academicians, field veterinarians and students across the country participated in the session. The inputs from all the participants are being crystallized in the form of status paper, which would help us to address the problems associated with estrus detection in buffaloes in more judicious way. Dr. A. Kumaresan was the Organizing Secretary of the brainstorming session.

Training Programme on "Chemical and Microbiological Quality Assurance for Dairy Supply Chain"

The Division of Dairy Chemistry and Dairy Microbiology jointly organized a short training on "Chemical and Microbiological Quality Assurance for Dairy Supply Chain" from 1st - 7th May, 2013. The training programme was inaugurated by Dr. S. K. Bhalla, Chief Operations Officer, Mother Dairy Fruits and Vegetables Pvt. Ltd. Delhi. A lecture compendium was also released in the inaugural session.

The training programme covered various quality assurance techniques in the area of chemical and microbiological testing of milk. The training programme was attended by 18 participants from various dairy federations working in the quality control and milk procurement sections. Dr. A. K. Srivastava, Director, NDRI, gave away the training certificates to the participants in the valedictory session.

Hands on Training on Composite Dairy Foods

A five days' training entitled "Hands on training on Technological Aspects of Composite Dairy Foods" was organized by Dairy Technology Division of NDRI, Karnal from 23rd - 27th April, 2013. The major objective of the training was to provide opportunity to researchers, entrepreneurs and industry personnel to learn the concept of composite dairy foods. Integration of non-dairy solids with milk although looks simple but from technological angle it is quite complicated mainly because of the interaction among milk and non-dairy ingredients. Initially such experimentations were done to enhance the sensory acceptability but later on it was observed that such composites have more to offer in terms of nutrition, functionality and sensory quality. With these objectives, a training programme was conceptualized with major focus on composite dairy foods and also included topics related to quality assurance, packaging, equipments and food safety standards.

A total 23 trainees drawn from milk producer's union, businessmen, technical persons, trainers, entrepreneurs, students and SHG members were selected for the training. During the training, 9 theory and 12 practical sessions were organized in batches to demonstrate possible options for processing of composite dairy foods. Participants evaluated the developed products and interactive sessions were organized for getting the feedback and possible options for improvement, if any.

Consultative Meeting on Pricing of Milk

Consultative Meeting on Pricing of Milk was organized at NDRI, Karnal on May 31, 2013. The meeting was organized jointly by Haryana Kisan Ayog and NDRI, Karnal. The

meeting was chaired by Dr. M. L. Madan, Former Vice-



Consultative Meeting on Pricing of Milk in progress

Chancellor, Akola and Mathura University and Former DDG, ICAR. The meeting was also attended by Mr. P. K. Das, Principal Secretary, Animal Husbandry and Dairying, Haryana, Mr. R. S. Dalal, Member Secretary, Haryana Kisan Ayog, Dr. B. S. Beniwal, General Manager, DMS. On this occasion, Director, NDRI gave presentation on issues related to pricing of milk.

World Milk Day

World Milk Day was celebrated at NDRI Karnal on 1st June 2013. In order to create awareness about the role of milk in the child nutrition and combating malnutrition, National Academy of Dairy Science (India) and Indian Dairy Association (NZ), Haryana Chapter, jointly organized a



Prof. V. N. Sharda, member ASRB addressing the faculty of NDRI at the World Milk Day

National Symposium on "Child Nutrition Security in India: Way Forward with Milk". Prof. Dr. V. N. Sharda, a renowned Agricultural Scientist and Member, Agricultural Scientists Recruitment Board (ASRB), New Delhi was the Chief Guest at this function that was presided over by Prof Dr. A. K. Srivastava, President NADS (I) and Director & Vice Chancellor, NDRI, Karnal. Prof Sharda, in his inaugural address talked about the role of milk in the nutritional security of the country and called upon the scientists and innovators to develop novel technologies so that milk could be made available to masses at a very reasonable price. Prof Srivastava in his key note address spoke on "Standards and Priorities for Infant Formula with Reference to Indian Context". He also raised certain pertinent issues related to child malnutrition. Dr. G. R. Patil, Joint Director, Academics, NDRI, Karnal, Dr. Alka Mohan Chutani Chief Dietician & Head Department of Dietetics, AIIMS, New Delhi, Dr. Bharti Kalra, a renowned gynecologist & neonatologist, Dr. M. B. Bera, Prof. and Head Department of Food Technology, SLIET, Longowal, Punjab and Dr. Sumit Arora, Principal Scientist, Dairy Chemistry, NDRI, Karnal were the other prominent speakers at this function who highlighted the importance and role of milk in the balanced growth of infants and children. Another highlight of the World Milk Day celebrations at NDRI Karnal, this year, was the presentation of novel and innovative ideas to combat malnutrition in infants through oral and poster presentations by the young

and budding students of NDRI.

Quinquennial Review Team Report Finalized

Quinquennial Review Team (QRT) chaired by Dr. V. K. Taneja VC, GADVASU, Ludhiana and supported by Members Dr. Satish Kumar (CCMB), Dr. B.N. Mathur Ex-Director NDRI and NAARM, Dr. S. N. Maurya, Ex-VC Mathura Veterinary University, Dr. K. T. Sampath Ex-Director NIANP, Mr. G. R. Ralhan from M/S G. R. Associates, Ludhiana and Member-Secretary Dr. Y. S. Rajput, NDRI finalized report pertaining to period April 2007-March 2013 after wide ranging



QRT team interacting with administrative staff

discussions at NDRI Head Quarter and Regional Stations at Bangalore and Kalyani. At NDRI Head Quarter, discussions were held at various Research Divisions, Sections and with select groups from students, administrative staff, industries and farmers. The Chairman QRT presented broad recommendations in an interactive session with BOM.

Visiting Faculty

Dr. Mohamed Bahgat Moustafa, Head, Dairy Technology Research Department, Animal Production Research Institute, Cairen, Egypt visited NDRI, Karnal under Indo-Egypt Work Plan 2012-13 from 8th – 20th April, 2013 for study in the field of “Quality Control and Assurance of Livestock Products”. During his stay, Dr. Moustafa visited various laboratories in Dairy Chemistry, Dairy Microbiology and



Dr. Moustafa interacting with Dr. A. K. Mohanty at Animal Biotechnology Centre

other divisions/sections of the Institute

Training Programme for Govt. Officers from Bangladesh

A training programme on “Dairy Technology and Quality Control of Milk and Milk Products” was conducted from 17th 21st June, 2013 at NDRI, Karnal for the six. Officers deputed by Govt. of the People Republic of Bangladesh. Participants were exposed to modern scientific practices of Milk Production and Processing both at the small scale and industrial level. Considering that the dairy production



Officers from Bangladesh at livestock farm of NDRI

system in Bangladesh is still at evolving stage and the strength of the India in the field, this training was of special significance. The trainees expressed their satisfaction for attending this training and appreciated the overall development in this field in India, particularly in regions around Haryana. They were keen to learn about the advantage of Buffaloes in dairying, the evolvement of women dairy cooperatives and world class milk processing facilities available both in govt. and private sectors. The leader of the delegation Mr. Md. Shafiquel Islam, Deputy Secretary, Ministry of Fishery and Livestock, Govt. of Bangladesh remarked that this interaction opportunity for him and his team will go a long way in strengthening dairy development work in Bangladesh. Dr. G. R. Patil, Joint Director, NDRI assured the trainees that NDRI is committed for any help for Govt. of Bangladesh in this regard and taking on the Indo-Bangla friendship.

HONOURS/AWARDS

Dr. Gurpreet Kaur was awarded “Jawaharlal Nehru Award for PG Outstanding Doctoral Thesis Research in Agriculture and Allied Science 2012”. She worked on “Bacteriocin Resistance among Dairy Pathogens and Spoilage Bacteria and Strategies for its Mitigation” under the guidance of Dr. R. K. Malik, Head, Dairy Microbiology.

Dr. S. Jeyakumar, was awarded “Best Poster Prize” by the Indian Association for the Advancement of Veterinary Research during 13th Indian Veterinary Congress and XX Annual convention of IAAVR and International Conference on “Thrust Areas in Veterinary Research, Education, Regularity

Reforms and Governance for Quality Service to Farmers" organized at Institute of Animal Health and Veterinary Biologicals and Veterinary College, KVAFSU, Hebbal, Bangalore on 16th-17th April, 2013.

PERSONALIA

Joining

- Dr. Ashok Santra, Sr. Scientist joined at ERS of NDRI, Kalyani w.e.f. 8.4.2013.
- Dr. (Ms.) Rubina Kumari Baithalu, Scientist (DT) joined at NDRI, Karnal w.e.f. 10.4.2013.
- Dr. Shaik Abdul Hussain, Scientist (LPM) joined at NDRI, Karnal w.e.f. 11.4.2013.
- Dr. Mohan Mondal, Sr. Scientist (AP) joined at ERS of NDRI, Kalyani w.e.f. 16.4.2013.
- Dr. Rakesh Kumar, Sr. Scientist (ABT) joined at NDRI, Karnal w.e.f. 20.4.2013 on his transfer from CIFT, Kochi.
- Dr. K. Ponnusamy, Head, Dairy Extension Division joined at NDRI, Karnal w.e.f. 31.5.2013.
- Dr. Chitra Nayak, Sr. Scientist (Electronics & Instrumentation) from CIRCOT, Mumbai joined at SRS w.e.f. 11.6.2013.
- Dr. Rakesh Kumar, Sr. Scientist (Agronomy) joined at NDRI, Karnal w.e.f. 25.6.2013.
- Dr. Mohan Mandal, Scientist promoted as Scientist (SS) w.e.f. 8.1.2011.
- Dr. P. Heartwin Amala Dhass, Scientist (SS) promoted to the Senior Scientist w.e.f. 2.9.2009.
- Dr. Menon Rekha Ravindra, Scientist (SS) promoted to Senior Scientist w.e.f. 22.2.2011.
- Smt. F. Magdaline Eljeeva Emerald, Scientist (SS) promoted to next higher grade w.e.f. 4.7.2011.
- Mrs. Deepa, T-4(F/FT) promoted as T-5 (F/FT) w.e.f. 09.07.2009.
- Sh. Sanjiv Kumar, T-5(F/FT) promoted as T-6 (F/FT) w.e.f. 10.01.2011.

- Sh. Prem Singh, T-5(F/FT) promoted as T-6 (F/FT) w.e.f. 01.01.2010.
- Sh. Deshwart Sharma, T-5(F/FT) promoted as T-6 (F/FT) w.e.f. 01.01.2010.
- Smt. Bhpinder Kaur, Ex. T-7/8 (Information) promoted as T-6 (Information) & T-7/8 (Information) w.e.f. 03.02.2000 & 03.02.2005, respectively.

RETIREMENTS/TRANSFERS

- Dr. G. K. Sachdeva, Principal Scientist, Sh. T.L. Jaggi, T-5 (F/FT), Sh. Balbir Singh, T-5 (L/T) and Mr. S. C. Biswas, T-5 (L/T) retired on superannuation from Council's service on 30.04.2013.
- Dr. R. A. Dey, Senior Scientist (Agri. Extension) retired on superannuation from Council's service on 29.06.2013.
- Dr. A. S. Harika, Principal Scientist/Incharge Forage Section retired on superannuation from Council's service on 26.06.2013.

VISITSABROAD

Dr. A. K. Srivastava, Director NDRI, Karnal attended CGIAR Livestock and Fish Annual Review Meeting at Ethiopia during 20th - 22nd May, 2013.

Dr. A. K. Puniya, Principal Scientist, Dairy Microbiology Division was deputed to attend the training on development of molecular markers for identification of rumen fungi at U.K. from 11th May to 2nd November, 2013.

Mr. Pravin Sawale, Ph.D. (Dairy Technology) has successfully attended the 6th International Granulation Conference at The University of Sheffield, United Kingdom w.e.f. 26th - 28th June, 2013.

DISTINGUISHED VISITORS

21-22.05.2013 Mr. Sipke Joost Hiemstra, Head, Animal Genetic Resources Group Centre for Genetic Resources the Netherlands Wageningen University and Research Centre.

urces the Netherlands Wageningen University and Research Centre.

राजभाषा एकक

नगर राजभाषा कार्यान्वयन समिति करनाल की 57वीं छमाही समीक्षा बैठक विगत दिवस डा0 दस्तूर सभाभवन, राष्ट्रीय डेरी अनुसंधान संस्थान, करनाल में डा. ए. के. श्रीवास्तव, निदेशक, एन.डी.आर.आई की अध्यक्षता में आयोजित की गई। बैठक का शुभारंभ दीप प्रज्ज्वलित कर किया गया। बैठक में सभी कार्यालयों के प्रकासनिक अध्यक्षों तथा निदेशक/अध्यक्ष नराकास की सहमति से निम्न निर्णय लिए गए।

केन्द्र सरकार के सभी कार्यालयों में फाइलों पर टिप्पणीयां अधिक से अधिक राजभाषा हिन्दी में लिखने के साथ-साथ मूल पत्राचार का प्रतिकृत बढ़ाया जाए।

सभी कार्यालयों द्वारा कार्यालय की कार्य प)ति में राजभाषा हिन्दी के प्रयोग के लिए संदर्भ साहित्य तैयार किया जाए।

आगामी सितम्बर, 2013 में सभी कार्यालय अपने-अपने कार्यालयों में

राजभाषा सप्ताह/पखवाड़ा तथा मास आयोजित करके सभी कर्मचारियों के लिए राजभाषा प्रतियोगिताओं का आयोजन किया जाए, जिससे कार्यालयों में राजभाषा में काम करने के प्रति जागरूकता बढ़े।

सभी कार्यालय अध्यक्ष यह सुनिश्चित करें कि उनके कार्यालय में सभी रबड़ की मोहरें, नामपट्ट, होर्डिंग्स आदि तथा कार्यालयों में आयोजित किए जाने वाले सभी कार्यक्रमों में अनिवार्य रूप से द्विभाषी बैनर ही लगाए जाने चाहिए। यदि बैनर में स्थान की कमी हो तो केवल राजभाषा हिन्दी में ही बैनर तैयार करवाए जाएं।

वर्ष 2012-13 के लिए राजभाषा हिन्दी में उल्लेखनीय कार्य करने के लिए इस संस्थान को द्वितीय पुरस्कार प्राप्त हुआ। यह पुरस्कार श्री जे. के. केवलरमानी, संयुक्त निदेशक द्विप्रकासनक तथा राजभाषा एकक के स्टाफ ने डा. ए.के.श्रीवास्तव निदेशक/अध्यक्ष नराकास के करकमलों द्वारा प्रदत्त किया गया।

SOUTHERN REGIONAL STATION, NDRI, BANGALORE

RESEARCH HIGHLIGHTS

Malnad Gidda – Unique Dwarf Cattle of Karnataka

The Malnad Gidda are unique dwarf cattle of Karnataka state. Malnad gidda cattle was registered as a breed in July 2012 with Accession Number INDIA_CATTLE-



Elite Malnad Gidda cow with calf

0800MALNADGIDDA-03037. Under the Karnataka Livestock Development Agency funded project, survey was conducted in the breeding tract of Malnad Gidda cattle. The coat colour in majority of Malnad Gidda cattle is black with light shades of fawn on thigh and shoulder region. Cows have bowl shaped small sized udder with funnel shaped teats with pointed tips. They mostly thrive on grazing and play a unique role in the farming systems of the heavy rain fall Malnad and coastal regions of Karnataka. Malnad Gidda cows calve for the first time at the age of around 3 years and give milk for 8-9 months. The average lactation milk yield, daily milk yield, peak yield and inter-calving period among the elite cows under field condition were 522.33 ± 69.40 liters, 2.17 ± 0.29 liters, 3.42 ± 0.39 liters and 14.91 ± 0.93 months respectively. There is a huge demand for Malnad Gidda cow milk and other products particularly ghee due to preference for usage in ayurvedic medicine preparation.



Malnad Gidda bull at NDRI Farm

The average fat and SNF in milk was 4.18 ± 0.20 and 8.66 ± 0.13 percent respectively. They have the reproductive uniqueness of regular calving (inter calving period of 396+33 days) under low input regime.

They are invariably bred by natural service when they go for grazing. The scientists of NDRI have introduced bull exchange programme to avoid inbreeding and selected elite Malnad Gidda males for semen collection, propagation and conservation. It is possible to develop this breed into small sized cattle thriving on low input regime and giving 3-4 liters of quality milk per day with adaptability to heavy rainfall conditions through selective breeding and scientific management practices.

Utilization of Whey in Dairy Whitener

Concentrated paneer or cheese whey was utilized for dairy whitener preparation. The whiteners were prepared by blending concentrated whey (11.5% TS) and toned milk (11.5% TS) in 75:25, 50:50 and 25: 75 proportions and homogenizing them. These were evaluated for their ability to whiten tea and coffee decoctions. Based on the results of colour, mouthfeel and flavour of tea and coffee whitened with the developed whiteners, it was concluded that the homogenized blend of 25:75 yielded a dairy whitener with a satisfactory whitening ability. The experimental whitener had a shelf life of two days at $7 \pm 2^\circ\text{C}$.

Utilization of Whey Solids in Cheese Manufacture

Whey proteins in the form of whey solids (WS) and whey protein concentrates (WPC) were used in cheese manufacture. These were incorporated into fresh cheese @ 1 and 3% of the curd at milling stage. Results of ripening parameters studied in comparison with control cheese indicated that decrease in pH, increase in titrable acidity and FFA, breakdown in proteins as indicated by electrophoresis, decrease in instrumental hardness were all higher in case of WS or WPC added cheese than in control cheese. Sensory evaluation showed that addition of whey solids resulted in deterioration of sensory quality of cheese while addition of WPC improved the sensory attributes during ripening.

Consultancy

Screening of Males for Genetic Diseases and Cytogenetic Abnormalities

(K. P. Ramesha, M. A. Kataktaaware, D. N. Das and S. Jeyakumar)

It is absolutely necessary to screen all animals for genetic diseases, especially bulls used in the artificial insemination programmes, to minimize the risk of spreading of genetic diseases to next generation. In all 262 males belonging to Bos Taurus (Holstein Friesian, Jersey), Bos indicus (Deoni, Amrithmahal, Khillar, Hallikar) and buffaloes (Surti and Murrah) either under semen collection or intended for

semen collection from 4 semen stations in Karnataka were screened for four genetic diseases viz. BLAD, Dumps, Citrullinemia and Factor X1 using molecular genetic tools and for karyotypic abnormalities. Two BLAD carrier Holstein Friesian males from one of the semen station were identified. All except one male had normal chromosomal complement of 60 XY in cattle and 50 XY in buffaloes. Revenue of ` 16.203 Lakhs was earned as consultancy charges towards screening for genetic diseases and cytogenetic abnormalities.

Training Programmes

- One week training was conducted on "Commercial Dairy Production" from 18.03.2013 to 23.03.2013.
- One week training was conducted on 'Maintenance and Propagation of Starter Cultures' for the personnel of Winner Dairy (P) Ltd., Puducherry from 29.04.2013 to 04.05.2013.
- One week training was conducted on 'Commercial Dairy Production' from 27.05.2013 to 01.06.2013.
- Ten days training on "Dairy Cattle Feed Analysis and Feed Formulation" was conducted for four personnel from Hatsun Agro Product Limited, Chennai from 25.04.2013 to 04.05.2013.
- One student of MOP Vaishnav College for Women, Chennai joined for In-lab Training in Dairy Engineering from 15.05.2013 to 30.06.2013.
- Two students of Amity Institute of Food Technology, Amity University, Noida joined for In-lab training in Dairy Technology from 15.05.2013 to 14.06.2013.
- One M. Tech (Food & Nutritional Biotechnology) from SRM University, Kattankulathur (TN) completed her In-lab training in DT Section from 01.02. 2013 to 27.04.2013.

Extension Activities

- **Visitors:** During the period under report, 197 visitors in seven batches comprising of students from various educational institutes, farmers and entrepreneurs from various parts of southern region, visited the institute. The visitors were taken round the Institute to various

sections as per their needs and were explained the ongoing research and extension activities.

- **Advisory Services:** Technical advice was rendered to seven of the needy clientele during their personal visits to the Institute. The profile of advisory included information regarding training programme on dairy farm management, technical know-how for dairy business projects, developing model dairy farm and availability of skilled manpower for dairy farm management.
- **Extension Literature:** Extension literature on dairy production and processing were distributed to the needy clientele groups, visitors, students and trainees during their visits to the institute.
- **Orientation Programme:** An orientation programme was organized for the student of management from Sri Satya Sai Institute of Higher Learning, Anantapur, Andhra Pradesh, from 22nd to 25th April 2013. Theoretical



Farmers training programme

orientation was provided on dairy production and processing aspects and needed discussion with concerned faculties.

- **On-farm Interactive Meeting:** An interactive meeting with clientele was organised at Marasarahalli and Aurohalli villages of Kanakapura Taluk, Bangalore Rural



Students of NDRI Deemed University displaying their sporting mettle during the competitions organized at the Sports Meet 2013

District to identify the need-based focus areas for Rural Extension Programme.

Training Programme

- One day training programme on, “Scientific Dairy Farming Practices” was organized on request for the progressive dairy farmers of Kasargod, Kerala under ATMA programme on 10th April 2013.
- An exposure visit cum training programme at the institute was organized on 4th April 2013 for the IIHR trainee farmers from Thrissur, Kerala under ATMA programme, Department of Agriculture, Govt. of Kerala.

Events

- Director visited SRS of NDRI on 26th April 2013 and addressed the staff and students of SRS highlighting the salient achievements of NDRI during the preceding five years and the contemplated activities for the coming five years. He appreciated the strong support and co-operation extended by the staff and students of SRS during his tenure and sought the continued co-operation and dedication for the future in realizing the set goals of the Institute.
- World Milk Day was celebrated at SRS of NDRI by students and staff of NDRI on 1st June, 2013 and awareness campaign was organized for the public on importance of milk and milk products.

- The farmers / trainees were provided with needed information on scientific dairy farming by brief-up sessions on clean milk production, animal health care, animal housing and milk processing/products followed by discussion with subject matter specialists and visit to dairy production units and to Mega Dairy unit of Karnataka Milk Federation as per their needs.
- A refresher programme was organized on 31st May, 2013 for the NIANP trainees on Trainers' Training programme facilitated for the Animal Husbandry Officers/Directors of Department of Animal Husbandry, Dairying and Fisheries from various Central and State Govt. from different states.



SRS of NDRI celebrated World Milk day in association with IDA (SZ)

EASTERN REGIONAL STATION, KALYANI

Research News

Supplementation of fresh *Azolla microphylla* to Lactating Crossbred Cattle

(A. Chatterjee, Puneet Sharma, M. K. Ghosh, A. Mandal, S. K. Das, P. K. Roy and T. K. Dutta)

Azolla is a floating aquatic macrophyte belonging to the family of Azollaceae. The fern *Azolla*, hosts a symbiotic blue green algae *Anabaena azollae*, which is responsible for the fixation and assimilation of atmospheric nitrogen. *Azolla*, in turn, provides the carbon source and favourable environment for the growth and development of the BGA

symbiont. It is this unique symbiotic relationship, which makes *Azolla*, a wonderful plant. *Azolla* has been reported to be a very good source of protein, essential mineral elements and vitamins for livestock. There are several species of *Azolla*, out of which, *Azolla microphylla* has been reported to be better for livestock feeding and also best suitable for hot humid climate of eastern India.

An experimental *azolla* production unit was established at ERS campus. Fresh yield, DM content and nutrient composition of *Azolla* was regularly monitored. The fresh Yield of *Azolla* was around 200-230 g/ sq m/ day. Chemical



analysis indicated that it was a fair source of plant protein. The mean concentration (% of DM) of organic matter, crude protein, crude fibre, ether extract, total ash, NFE, NDF, ADF and ADL in Azolla microphylla meal were also studied.

The effect of fresh Azolla microphylla supplementation in crossbred cattle was studied in a lactation trial of 90 days. Ten crossbred jersey cattle were distributed into two groups. The animals in control group (T0) were fed on conventional ration of paddy straw, green fodder and concentrate mixture as per NRC recommendation. In the treatment group (T1) 2 kg of fresh Azolla was supplemented over conventional ration

There was no significant difference in total DMI and CP intake between the two groups. The average milk yield (kg/d) over 6 fortnights was significantly higher in T1 than T0. Fat corrected milk (FCM) yield also showed similar trend. These results showed that supplementation of Azolla caused an increase in milk yield by 11.2% and FCM yield by 12.5%. Total dry matter intake per kg FCM yield was significantly lower in the treatment group than in control group indicating better feed conversion efficiency. The average percentage of milk fat, milk protein, SNF, total solid and ash showed no significant difference between two

groups. Fresh Azolla microphylla has been found to have potential to serve as a green supplement for the dairy cattle for the improvement in milk yield especially where/when green fodder availability is scanty.

World Milk Day -2013 Celebrations

World Milk Day was celebrated on 1st June, 2013 by the Eastern Regional Station of National Dairy Research Institute (ERS-NDRI), Kalyani, Nadia, India to create awareness on the importance of milk on human health, clean milk production from dairy animals etc. and to publicize the importance of livestock and livestock products in socio-economic upliftment of resource-poor dairy farmers in the region. Dairy farmers from different parts of West Bengal with more women representation, NGO, progressive farmers, experts participated in the programme.

Extension Activities

Training organized

One 15 day refresher course was organized at ERS of NDRI, Kalyani on 'Artificial Insemination and veterinary First Aid' for Pranibandhu workers. The course was conducted from 14.06.13- 28.06.13 in which 8 trainees from different districts participated.

FEATURE ARTICLE

Animal Proteomics: Way Forward Beyond Animal Genomics (A. K. Mohanty, J. K. Kaushik and Sudarshan Kumar)

Animal Biotechnology Centre, NDRI, Karnal

Genes are transcribed into mRNA, which is translated into proteins. The translated proteins might be further modified by glycosylation, phosphorylation or proteolysis to produce mature proteins. Therefore, the gene sequence, transcription, translation, and post translational modifications are all processes in a gene product's expression. Proteins are the end products of gene expression that control the cellular behaviour. Recent advances in genomics and bioinformatics has led to great advancements in proteomics. Proteomics is the study of the proteome, the protein complement of the genome. Proteomics refers to the branch of science focusing on discovery proteins. The term 'proteome' is used to describe the complete set of proteins that is expressed, and modified following expression, by the entire genome in the lifetime of a cell. Proteomics is now a scientific discipline that promises to bridge the gap between our understanding of genome sequence and cellular behaviour. It can be viewed as a biological assay system or tool for determining gene function. Unlike other methodologies that analyze a few proteins at a time, proteomics can analyze thousands of proteins in a single experiment. This ability to analyze thousands of proteins gives the field of proteomics a unique capability to demonstrate how cells can dynamically respond to changes in their environment. Therefore, goal of proteomics is to identify changes in protein expression, interaction, or modification as a result of an experimental treatment. Generation of large

proteomic data sets is expected to demonstrate the interdependence of cellular processes important for normal cell growth or a cell's response to abnormal or disease conditions.

Proteomic Strategies for the Identification and Analysis of Proteins

Protein Separation: The complexity of biological material makes the proteomic experiment quite tedious sometimes. The abundance of a particular protein/s many a times masks the identification of low abundant proteins. Therefore, suitable protein separation strategies are essential to make the sample less complex. These separation strategies include chromatographic procedures such as ion exchange and gel filtration chromatography to separate the proteins using high performance chromatography (HPLC). The proteomics can be broadly classified into two categories such as qualitative and quantitative proteomics. Qualitative proteomics helps us to identify the proteins while quantitative proteomics conveys quantity. Both are essential components for any successful proteomics experiment. The classical method of quantitative and qualitative expression proteomics combines protein separation by high-resolution 2D gel electrophoresis with MS (Mass Spectrometer) or MS/MS identification of selected protein spots.

2D Proteomics: Two-Dimensional Gel Electrophoresis (2D-GE) and Mass Spectrometry: This separation method has become synonymous with proteomics and remains the single best method for resolving highly complex protein mixtures. Two-dimensional SDS-PAGE is a combination of two different types of separations. In the first, the proteins are resolved on the

basis of isoelectric point by isoelectric focussing (IEF). In the second, focused proteins are then further resolved on the basis of difference in molecular weight by electrophoresis on a polyacrylamide gel. Thus 2D-SDS-PAGE resolves proteins in the first dimension by isoelectric point and in the second dimension by molecular weight. Dedicated 2D-SDS-PAGE systems are available that use immobilized pH gradient (IPG) strips to facilitate the transfer of proteins from the IPG strip into the SDS-PAGE slab gel. The IPG strip is based on the use of immobilized pH gradients, in which polycarboxylic acid ampholytes are immobilized on supports to reproducibly create stable pH gradient. The use of narrow pH ranges facilitates the separation of proteins with highly similar isoelectric points. Proteins separated by 2D gels are visualized by conventional staining techniques, including silver, Coomassie, amido black stains and fluorescent staining. Silver-staining and newer fluorescent dyes are the most sensitive.



Fig. 2D-GE Workflow

The visualized spots in the protein gel are picked up and subjected to trypsin digestion for generation of peptides. The peptides so produced are subjected to Mass Spectrometer (MALDI-TOF-TOF / LC-MS-MS) for their identification.

The mass spectrometer will record all the peptide in terms of m/z ratio generated from each protein spot on gel. Identification of a protein is based on the measurement of multiple peptides that are generated from that protein. For example, after a mass spectrometer has determined the m/z value for the peptides from a gel spot, this information is matched to a protein database. A successful protein match will be based on the number of peptides matched to the protein and the accuracy of the matches. The second method to identify proteins involves use of tandem mass spectrometers that allow for sequencing of individual peptides.

Shotgun Proteomics: Shotgun proteomics refers to the identification of all the proteins in a protein mixture. The proteins may either be in solution or in 1-Dimensional SDS-PAGE. The protein mixture or the protein containing sliced SDS-PAGE gel pieces are digested with trypsin and the peptides are extracted. These peptides are passed through strong cation exchange chromatography and LC-MS-MS to generate peptide spectrum followed by their identification using search algorithms such as Mascot search / Sequest / Masswiz etc. Multiple separation methodologies are employed in a proteomic experiment to maximize the number of proteins identified.

Differential Display Proteomics for Biomarker Discovery: A fundamental aspect of proteomic research is the

determination of protein expression levels between two different states of a biological system (e.g. relative quantification of protein levels), such as that encountered between a normal and diseased cells or tissues. This is referred to as differential display or comparative proteomics. This can be done in two ways such as running and comparing samples in 2D-Differential in gel electrophoresis (2D-DIGE) or using Isobaric Tags for Relative and Absolute Quantitation (iTRAQ). The 2D-DIGE has become a powerful quantitative technique for differential-display proteomics where the individual abundance changes for thousands of intact proteins can be simultaneously monitored in replicate samples over multiple variables with statistical confidence. This technology is widely used enabling the detection of more subtle changes in protein expression than conventional 2D PAGE. The iTRAQ technique is another powerful and is a non-gel based multiplexed protein quantitation technique that provides relative and absolute measurements of all peptides from different samples/treatments. iTRAQ is ideally suited for comparing normal, diseased and treated samples, time course studies, biological replicates and relative quantitation. The quantitation of the differences in protein amount between different samples can be done by iTRAQ isotope tagging of peptides and 2D-LC separations (up to 8 separate samples with 600-1200 proteins can be identified and quantitated in a single experiment).

Advances in Animal Proteomics: Worldwide farm animal proteomics is at its infancy unlike its counterpart in human being. Various groups have been trying to apply proteomics techniques to understand functions of various systems in farm animals. Specific emphasis has been given on production, reproduction and milk safety aspects by different groups.

Proteomics of farm animal production and reproduction systems: The mammary gland provides an excellent system to study questions pertaining to organogenesis, cell differentiation and oncogenesis. Intensive efforts have been made to understand the development of the mammary gland, particularly in terms of lactogenesis, cell apoptosis and tissue remodelling.

Mammary gland proteomics: Mammary gland is made up of a branching network of ducts that ends in alveoli. Terminally differentiated mammary epithelial cells (MECs), which constitute the innermost layer of alveoli are involved in the synthesis and secretion of milk proteins during lactation. NDRI has deciphered the proteome of MECs in lactating cows using 2-DE MALDI-TOF/TOF MS and 1-D-Gel-LC-MS/MS. We have been able to identify around 600 proteins, which reflect their role during physiological lactation. Extensive bioinformatics analysis has revealed involvement of novel proteins that works at cellular level for milk synthesis and secretion. To understand the role of various proteins in high vis-a-vis low producing cows and also expression of various proteins at different time points in lactation, 2D-DIGE was performed, which identified 28 and 44 differentially expressed proteins, respectively. The proteins may have potential role in the regulation of milk production and lactation persistency. We have also identified ~90 proteins in lactating buffalo mammary gland tissue that gives exclusive reflection of association of various proteins in milk synthesis and secretion. Few groups have profiled the protein expression in cow mammary gland and identified more than 200 proteins. To understand the milk fat globule secretion mechanism, proteomics of milk fat globule membrane (MFGM) have been reported by various groups and pathway of

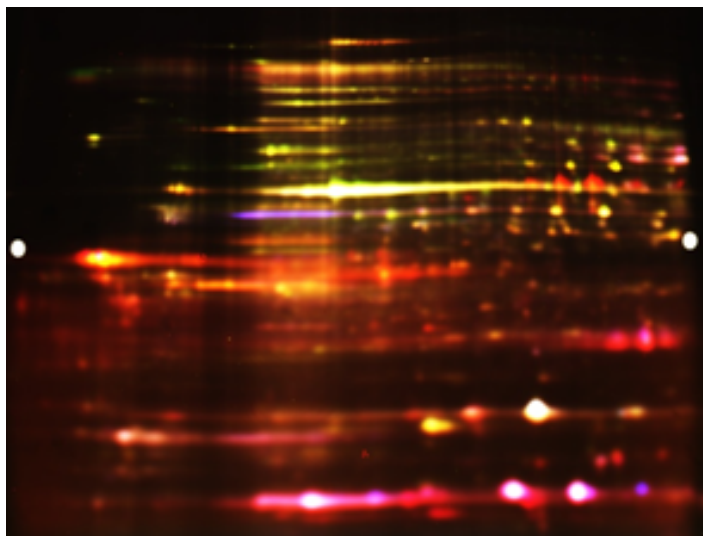


Fig. 2D-DIGE of MEC protein in High vs Low producing cows

milk fat synthesis and secretion has been deciphered. By using high resolution Mass Spectrometry, composition of milk or whey in cow and buffalo has been profiled.

Bull Fertility: High reproductive performance and factors that influence it have a significant impact on the economics of animal production. Proteomics has been used to show that variation in protein types and amounts in seminal fluid and sperm that regulates fertility indexes in dairy bulls. These findings can be used to identify potential biomarkers to select dairy bulls of high fertility. Research at NDRI, Karnal is also focussed to find potential biomarkers in spermatogonial cells for early selection of high fertile bulls.

Biomarkers for early detection of pregnancy and estrus: Few groups have attempted to identify biomarkers for early detection of pregnancy from serum and urine of cows following proteomic approaches. However, no real biomarker has been identified so far. NDRI has recently undertaken identification of early pregnancy and estrus biomarkers in cows and buffaloes by employing proteomics or metabolomics approaches.

Dairy Food Production & Bacterial Proteomes: A large number of bacterial species are used in the production of dairy products. Milk processing methodologies are highly dependent on the total repertoire of milk proteins. The presence of bacteria in milk may change the quality of milk in many that might critically impact the manufacturing processes. Bacterial behaviour itself changes drastically when it is moved from laboratory to manufacturing environment presenting challenges to the manufacturing strategies. It becomes necessary that bacterial proteomics be known and understood so that suitable adaptation plan can be laid in position. A proteomic approach can yield information regarding the global changes in protein expression to understand the adaptations that these bacteria undergo. The proteomes of *Lactobacillus bulgaricus*, *Lactococcus lactis*, and *Streptococcus thermophilus* have been examined for protein changes resulting from growth in laboratory medium or in milk. These data provide food scientists with new information to modify/select these bacteria for optimum dairy food production practices. Research on probiotics is currently one

of the potential areas of food biotechnology where friendly bacteria are in use. But looking at the rate of their application in industry, it is felt that a screening and identification of probiotics is needed. The latest innovation and development in proteomics will enable rapid characterization of a large number of candidate bacterial species to qualify as probiotic which can be then characterized chemically and phenotypically. It is essential that a thorough proteome data base be generated for friendly as well as pathogenic bacteria so that future research attempts for quality milk product and processing could find an informative platform to move on.

Proteomics for infectious diseases and mastitis biomarkers:

Infectious diseases of dairy animals are again another side of whole animal health care strategies which warrants attention. Mastitis and FMD like diseases are always menace to any farm and with time we have realized that a continuous efforts will always be needed to deal with these infectious diseases where latest technologies could be applied as best diagnostic and therapeutic strategy. Protein data base is important because they help us to focus the attention of our approach on the point of problem. Only 2-3% of genome results in functional proteins. If we could understand the product of this small fraction of whole genome probably we will find solution to many of the existing problems if not all. These data will equally be utilized for getting a more complete picture of bacterial pathogenesis in mastitis by examining changes in the bacterial proteome of common mastitis causing bacteria when grown in milk. Mastitis is the inflammation of mammary gland, which is mostly commonly caused by bacteria. Recent development in Mass Spectrometry based techniques has promised crucial advancement in diagnosis of subclinical mastitis. Milk contains large number of proteins, which are inherent to its composition. In bacterial infection, the overall protein profile changes which includes some bacterial origin proteins and some endogenous proteins from animal body whose normal expression level is altered. By using 2D-proteomics and MS-based techniques many proteins have been identified. Some of them are Lipocalin-type prostaglandin D synthase, transferrin, microsomal triglyceride protein, apolipoprotein A-1, cathelicidin-1, heat shock protein 70kD protein, peptidoglycan recognition receptor protein (PGRP), calgranulin B and C, and serum amyloid A (SAA) are present in excess in mastitic milk. Animal Biotechnology Centre at NDRI is in the process of optimizing the use of a discovered molecule, which can be used as a potential subclinical mastitis diagnostic biomarker.

Future perspective in farm animal proteomics: Animal proteomics has major implications in understanding the physiological process in a better way. That will allow the researcher to identify various proteins which are expressed inside a cell and may not be same as in the transcriptome profile. The major challenge in the field of proteomics includes an incomplete genomic sequencing of some species and an incomplete understanding of protein function in all species. Genome sequencing programs are enabling molecular understanding of various species, whereas determination of the functions of many proteins is likely to take years. The importance of proteomics based studies in animal science

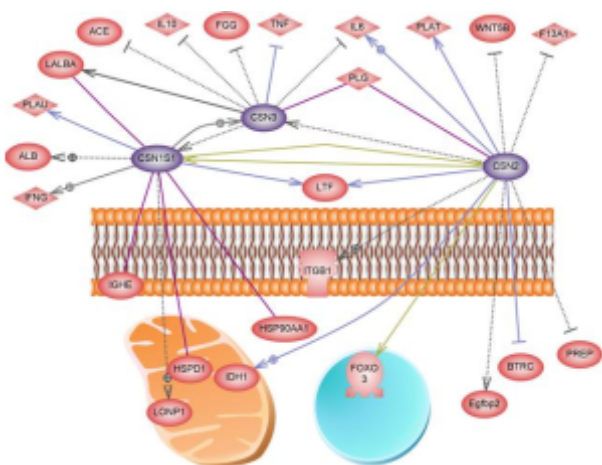


Fig. Interactome map of a, b & κ casein in Mammary Epithelial Cell

becomes manifold when compared to other species. In other species thorough information on genome is available in a highly curated form. But the same has not been possible yet in animal science. Many regions on genome remain unnotated. The detailed proteome of dairy animals will serve as a bottom up approach toward curation of genomic database. The same can be called as emerging area of proteogenomics. Proteomics is likely to play an important role in determining functions of many proteins. The promise of proteomics is onerous that will enable researchers to understand the complex interplay of hundreds of potential proteins involved in cellular processes and to identify and quantitate these complex protein networks as a cell response to its environment. Proteomics will enable the exploration of post translational modification in proteins which otherwise is nearly difficult through any other approaches.

Some of the potential areas which need to be pursued in Animal Science are:

- Building proteome map of different indigenous breeds of livestock species. Animal proteome project can be initiated for cataloguing various proteins in indigenous cows and buffaloes in line with
- The human genome project. Major emphasis must be given on the generation of bovine protein reference data base (BPRD) which will lay foundation to the future research endeavours towards understanding various animal science specific problems.
- Differential expression proteomics will help to estimate quantitative expression of various proteins in different species for identification of protein biomarkers in production and reproduction systems and their beneficial effects can be assessed by functional genomics studies.
- Health promoting attribute of various animal proteins can be assessed by proteomics studies.
- Climate change effects can be studied in terms of expression of bad and good proteins and their future application as biomarkers of heat stress.
- Adulteration of milk with non-milk source based proteins like plant, hide, melamine and proteins from pathogenic microorganisms can be identified.
- Specialized attention needs to be given towards immunoproteomics where the much talked genetic resistance of indigenous animals against stress and pathogens is crucial for understanding the genetic basis.

Forthcoming Events

- Course on Innovative Approaches in Processing and Packaging of Milk and Milk products from 19th July to 9th August, 2013 (21 days).
- Course on Advances in Manufacturing and Quality Aspects of Traditional Indian Dairy Products from 8th – 28th October, 2013 (21 days).
- National Training on Climate Resilient Livestock and Production System from 18th November to 1st December, 2013.
- National Training on Stem Cell Biology from 3rd – 23rd December, 2013 (21 days).

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