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NDRI News

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Fulfilling Nation's Dairy Dreams

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ICAR-National Dairy Research Institute, Karnal

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From the Director's Desk

India is largest milk producer in the World with 187.7 Million MT of milk production per annum. At present, the growth rate of milk production has been around 6%, which is remarkable when compared with world average. Although, milk production has been the focus during last seven decades, the quality of milk has not received due attention. The quality of milk has been a challenge in India due to high ambient temperature and also due to man-made menace of adulteration as well as contamination of milk and milk products with heavy metals, pesticides and antibiotic/veterinary drug residues etc. To address the issues of Quality and Safety in the country, ICAR-NDRI has established "National Referral Centre on milk Quality and Safety" on 19th Feb., 2016 with fund support from NAIP with an amount of Rs. 17.43 crores. Referral centre has been accorded with NABL accreditation as per ISO: 17025: 2017 in Feb., 2020 and further efforts are in process for integrated certification with FSSAI for providing scientific inputs on quality and safety so that safe Dairy Foods can be supplied to the consumers. Referral Centre is working with the mandate of developing rapid sensors for monitoring adulterants, chemical & bacterial contaminants in milk, analytical services to different entrepreneurs in dairy food chain, standard development work with FSSAI and capacity building program in specialized field of Food Safety & Quality Assurance.

The safety of dairy products with respect to food-borne diseases is a great concern especially in developing countries where production of milk and various dairy products takes place under un-sanitary conditions and poor production practices. Further, milk is an ideal medium for the growth of micro-organisms that may originate from the interior of the udder, exterior surfaces of the animal, milk handling equipment and other miscellaneous sources. The health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning & sanitizing the milking and storage equipment are all also key factors in influencing the level of microbial contamination of raw milk.

Further, chemical contaminants, which include antibiotic residues, aflatoxin M1 and pesticide residues and adulterants, are also major quality issues in dairy industry in India. The presence of veterinary drugs residues is a serious concern in milk with special reference to curd setting during preparation of fermented milks and development of antimicrobial resistance (AMR) in pathogenic bacteria. FSSAI has endorsed the harmonization of codex standards on antibiotic residues in milk in FSS act 2016 and now onwards, testing of antibiotics at various points during milk production is mandatory to ensure safe milk supply to the consumers.

Rapid assessment tools are need of the hour for checking the microbial and chemical quality of milk under field and processing conditions. A detection method with similar or better performance, which reduces the time and cost requirements, would, thus, be of great value to the dairy industry. Referral Centre at NDRI has conducted extensive research work in specialized field of Food Safety & Quality Assurance and developed tests for detection of *E.coli*, coliform, Enterococci, *L.monocytogenes*, antibiotics, pesticides and adulterants in milk. Two-stage enzymes assay has been developed for detection of *L. monocytogenes* based on the principle of targeting "enzyme-substrate reaction for specific marker enzyme (s) to release free chromogen that can be visually detected by color change. The assay can confirm the presence of *L. monocytogenes* within real time of 4.30 h after initial pre-enrichment of milk samples in novel selective medium for 24h as against 5-7 days protocol following in conventional method. Similar tests have been developed for *E. coli* & Coliform with detection time of 12-15 hrs.

Paper-based sensor is a new alternative growing technology for fabricating simple, low-cost, portable and disposable analytical devices for addressing quality & safety issues in dairy industry. The major advantages of using paper as a sensing platform include ease of availability, low cost, passive liquid transport, compatibility with chemicals/bio-chemicals and fast response. Referral centre is working extensively and has developed successfully paper based strips for detection of antibiotics, pesticides residues and mastitis in milk. Using spores as biosensor, strips have been developed for detection of antibiotics based on principle of spore germination and release of marker enzyme. Presence of pesticide residues in milk as revealed through survey works carried out in different parts of India as well as in other countries is of serious concern in terms of causing cancer in human beings. Spore based approach was attempted for the first time in India and paper strip assay for detection of pesticides in milk with marker enzyme (s) from prokaryotic system was successfully developed, patented and commercialized in India. Paper strip assay can detect insecticide, herbicide and fungicides group of pesticides at regulatory limits with great degree of repeatability and selectivity. Assay is rapid, cost effective, robust, reproducible, sensitive and selective when compared with conventional chromatographic techniques. Assay can be explored for field application for routine as well as for regulatory compliance of pesticides in milk and other food commodities for organic certification. A potential enzyme marker was also identified in animal suffering from sub-clinical/clinical mastitis and strip based test was developed for its detection in real time. Development of blue color on the strip indicates sub clinical stage in 30 min and clinical stage in 15 min. No colour appearance after 30 min indicates normal milk. Test has been evaluated and validated under field conditions.

Referral Centre has also developed strip based tests for detection of various adulterants in milk. Six different paper based strip methods have been developed for the rapid detection of neutralizers, urea, glucose, hydrogen peroxide, maltodextrin, sucrose and formalin in milk using the concept of dry chemistry. In all cases, sensitivity of the prepared strips was found better than the existing wet chemistry based tests.

The tests have also been developed for the detection of adulteration of milk products such as ghee, khoa etc. The adulteration detection tests developed for the milk have been either validated or modified for their application in products like khoa. For the detection of vegetable oil in ghee, a simple chemical method has also been developed. Recently, a HPLC based method based on the presence of β-siotsterol in milk fat for confirming the presence of vegetable oil has been adopted by FSSAI. Referral centre is further focusing in developing simpler methods for testing quality of milk and milk products, which can be multianalyte, high through-put and can be used at household as well as field level to ensure quality and safety of milk and milk products.

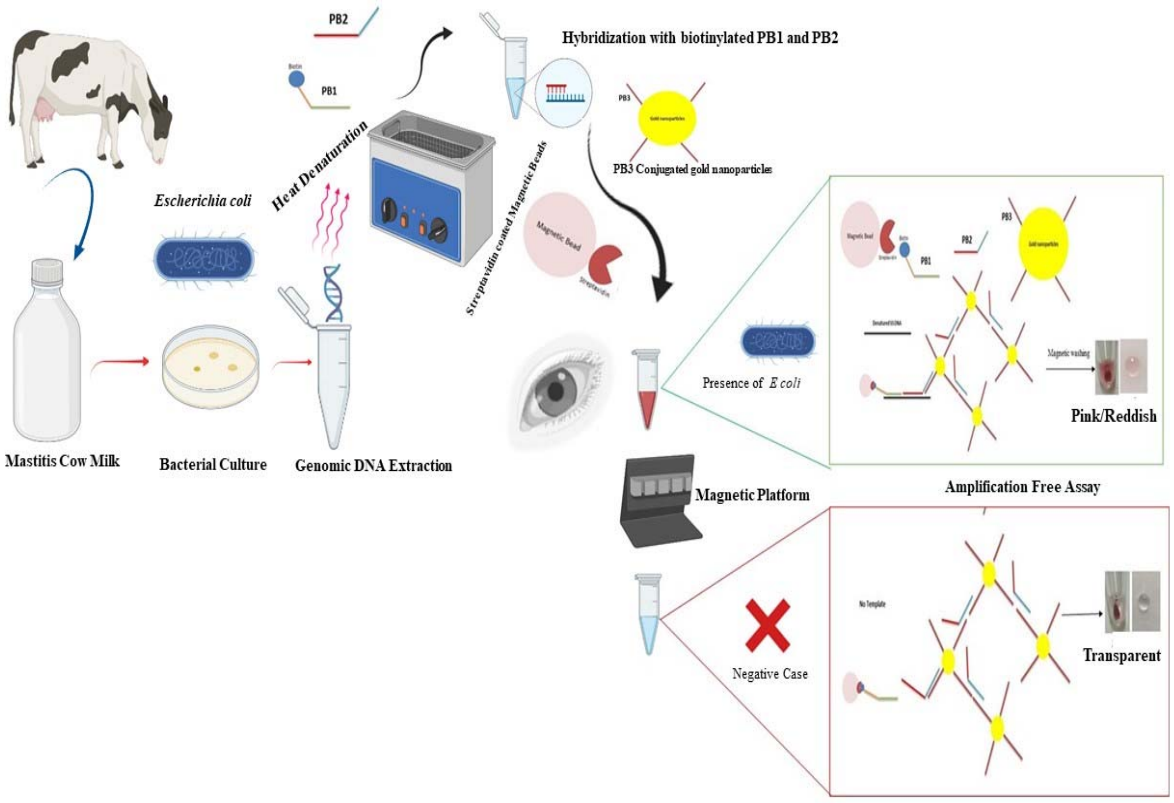
(MS Chauhan)
Director

Amplification free gold nanoparticle aggregation assay for rapid detection of *Escherichia coli* in mastitis cow milk

(Sachinandan De and Rajiv Deb)

Rapid, sensitive and specific identification of microbial DNA from clinical veterinary samples like mastitis cow milk is important for the early detection of pathogens. Amplification free assay for rapid and accurate detection of microbial DNA provides an attractive alternative to the traditional molecular tools for the development of pen-side diagnostics. In the present work, an amplification free visual assay for rapid and sensitive detection of mastitic causing *E coli* based on *uidA* (beta-glucuronidase) gene was developed. To improve the assay sensitivity, the gold nanoparticles based detection was employed to capture the *uidA* gene by using the magnetic microbeads.

The assay can be completed within 1-1.5 h and visualized by naked eyes. The method is able to detect the microbial load as little as 100 bacteria per millilter and bacterial DNA as low as 1 pg by simple visual inspection. The quick and user-friendly assay provides a precise detection tool for identification of *E coli* in clinical samples like mastitis cow milk.



National Referral Centre for Milk Quality and Safety-An Update

National Referral Centre for milk Quality and Safety (NRCMQS) was set up at ICAR-National Dairy Research Institute, Karnal with funding support from National Agricultural Innovation Project Agriculture (NAIP) under project entitled on" Development of Biosensors and Micro-techniques for Analysis of Pesticide residues, Aflatoxin M1, Heavy Metals & Bacterial Contamination in Milk with an amount of **Rs. 17.43 crores**.

Objectives/Activities of National Referral Centre for Food Quality and Safety

1) **Risk Profiling/Scientific investigation related to outbreaks associated with adulteration/ contamination of milk**

   - Risk profiling of Environmental Pollutants and Contaminants-Antibiotic/Pesticides/ Aflatoxin M1/Heavy Metal in milk and standard setting with FSSAI.
   - Surveillance on Zoonotic, Mastitis and AMR pathogens in milk.
   - Profiling of selected major and minor mineral in milk and milk products.
   - Profiling of milk fat of different species with respect to fatty acids, sterols & vitamins.

2) **Rapid Biosensor Based Tools/Assay/Kits for monitoring adulterants & potential chemical & bacterial contaminants in milk**

   - Spores/Enzymes/Polymer based sensors for chemical contaminants/pathogens & hygiene indicators in milk.
   - Paper strip based assay based on Enzymes/new protein bio-markers for detection of sub-clinical mastitis in milk.
   - Development of analytical tools to assess the quality of UHT milk.
   - Development of LC-MS based method for identification and quantitation of veterinary drugs in milk.

3) **Licensing/patenting/commercialization of developed IPs to end user through establishing linkage with regulatory agencies (FSSAI) for field application**

   - Evaluation/Validation of Kits and their approval from regulatory agency and their transfer for field application.
   - Impact assessment of technologies in collaboration with Extension functions.

4) **NABL accreditation and Analytical services to end users for revenue generation, Standard development/capacity building program in specialized field of food safety and Quality Assurance**

   - NABL accreditation of the National Referral Center for Milk Quality and Safety.
   - Imparting Analytical services to industry, farmers and other stake holders as per FSSAI/ regulatory standards.
   - Contribution in standard development programme to FSSAI through expert panel members.
   - Training to Farmers/industry personnel in the field of clean milk and quality assurance and food safety.

Infrastructure Facility at National Referral Centre:

The centre is equipped with state of the art equipment and has capability to do testing in the following areas:

- Microbiological testing of milk and milk products.
- Chemical testing of milk and milk products.
- LC-MS/MS analysis of milk and milk products.

Unit-I (Microbiological Section) of NRCMQS is having two sections namely Microbiological Section (BSL-3) and Biosensor Lab. BSL-3 is equipped with media preparation, autoclaving, inoculation chamber, incubation room, de-contamination room and pathogen detection section, which compiles WHO biosafety standards guideline. BSL-3 facility is equipped with ante room, biosafety cabinet etc.

BSL-3 Containment

Bio-sensor Lab

Unit-I is equipped with following AOAC approved system for testing of pathogens, hygiene indicators and antibiotics residues in milk.

- VIDAS for rapid testing of *L.monocytogenes*, *E. coli* O157:H7, *Salmonella, Staph. aureus Enterotoxin* and *Campylobacte*r in 24 hrs.
- D-Count for testing of Total Viable Count (TVC) in 2 h and Yeast & Mold Count (YMC) in 10 hrs.
- Charm 6602/ROSA for testing different groups of antibiotics within 30 min as per codex standards.

Unit-I (Microbiological Section) is offering analytical testing for following parameters (89 No's) for different categories of milk and milk products as per FSSAI standard requirements:

1) Hygiene indicators: Aerobic plate count/coliform/*Staph aureus*, *E. coli* and Yeast and mold counts).
2) Pathogens testing: *Salmonella*/*Shigella*, *L. monocytogenes*, *B. cereus*, *Enterobacter sakazakii*, Sulphite reducing clostridia (SRC) as per requirement of FSSAI.

Unit–I has developed kits for rapid detection of bacteria ***(L. monocytogenes, E. coli, coliform, enterococci),*** **antibiotics including β-lactam group, pesticide residues, heavy metals and mastitis *in milk*.**

Unit-II (Chemical Section) of NRCMQS has two major sections which include Wet Chemical Laboratory having facilities for routine chemical analytical work, adulteration testing, protein estimation, electrophoresis and Lyophilizer facilities. The other area consists of Chromatographic Laboratory, Protein Laboratory, sample preparation laboratory, hot area etc. Following equipments are housed in Chemical Section:

- HPLC, GCMS, Atomic Absorption Spectroscopy, FTIR, Particle Size Analyzer, Digital Refractometer, PCR, Electrochemical Station, Automatic protein analyzer, Nanodrop etc.

Wet Chemistry Laboratory

LC-MS/MS facility

National Referral Centre for Food Quality and Safety (NRCQS) has been accorded NABL (National Accreditation Board for Testing and Calibration Laboratories) accreditation as per ISO **17025:2017 in February, 2020.** The Analytical Services of Chemical Section are open for Dairy as well as Food Industry for following 187 test parameters.

1) Chemical testing of milk and milk products.
2) Mineral profile of dairy products.
3) Aflatoxin M1 estimation in dairy products.
4) Fatty acid profile of dairy products.

Unit-II has developed technologies on strip based tests for checking adulteration in milk and has been commercialized in India

Unit – III (MS Section): The LC-MS/MS Section has been established with an aim to identify and quantify the proteins, peptides and veterinary drugs in milk and milk products. The LC-MS/MS facility contains following four standalone equipment namely;

1) Ultra-High pressure liquid chromatography (UHPLC) system.
2) Nano-liquid chromatography system (Nano-LC).
3) Electrospray Ionization Quadruple Time of Flight (ESI qTof) Mass Spectrometer.
4) Liquid Chromatography-Triple quadruple system (LC-TQD).

Following testing facilities are offered by LC-MS/MS facility:

1) Fractionation of proteins/peptides.
2) Determination of exact mass of proteins and peptides.
3) Identification and screening of proteins/peptides (Individual/Complex).
4) Relative quantitation of proteins/peptides using high throughput proteomic approaches like TMT, Label free quantitation.
5) LC-MS/MS based screening and quantitation of melamine, veterinary drugs etc.

New methods developed by MS Section:

1) UHPLC-coupled MS based method for A1A1, A1A2, and A2A2 type milk.
2) LC-MS/MS based method for Progesterone β-hydroxy butyrate, melamine, Sulphadimidine, Sulphamethoxazole, Ciprofloxacin, Enrofloxacin, Ampicillin in milk.

Revenue Generation at NRCMQS through Analytical Services, Supply of Testing Kits Training Programme, evaluation/validation of Kits and Technology Development/ Licensing was Rs. 10193979.60 during the period 2018-2021.

Institute Technology Management Unit (ITMU)

Patent Granted:

Sl. No.	Title of the Application	Name of the Inventors	Application Number	Date of Filing	Grant Number	Date of Grant
1)	Peptide sequence and polyclonal antibodies for the detection of cow and buffalo luteinizing hormone	Varij Nayan, Suneel Kumar Onteru & Dheer Singh	1854/DEL/ 2015	23.06.2015	364236	07.04.2021
2)	Development of enzyme-spore based assay (s) for monitoring antibiotic residues in milk.	Naresh Kumar, Alia Khan, Shivani Arora, Falguni Patra, Meenakshi Dahiya, Mandeep Balhara, Pradeep Kumar Sharma & Suleman Shaikh	2213/DEL/ 2014	05.08.2014	365074	21.04.2021
3)	Marker enzymes and spore germination-based assay for detection of *E. coli* in milk and milk products.	Naresh Kumar, Ramakant Lawaniya, Avinash, Bhawna Arora, Raghu H.V.; Mandeep Balhara, Saurabh Kadyan & Vinai Kumar	2214/DEL/ 2014	05.08.2014	367110	20.05.2021

EXTENSION

Krishi Vigyan Kendra

- KVK organized **World Bee Day** at NDRI campus on virtual mode on May 20, 2021. KVK experts delivered lectures on different aspects of bee keeping and farmer's problem. In this event about 35 farmers and farm women participated.

- KVK organized **Awareness programme/ Kisan Goshti on Jal Shakti Abhiyan** on May 28, 2021 at KVK campus in virtual mode. The chief guest of the function was Dr. Anurag Saxena (In-charge, Farm Section) and guest of honor was Dr. Rakesh Kumar (Head, Agronomy). The experts delivered lecture on water shed management, water conservation and crop diversification to save water. In this programme 52 farmers and farm women participated.

- KVK organized **World Milk Day** on June 1, 2021 in which 25 farmers and 12 farm-women participated in the audio-visual room of Krishi Vigyan Kendra, while 220 farmers connected virtually. During the program, social distancing and other guidelines of Ministry were followed.

- KVK organized **World Environment Day** on June 5, 2021 at KVK, campus. In this event, 30 farmers participated. Lecture of Honourable Prime Minister was live telecast.

- KVK organized Field Day on **CFLD summer moong** variety **IPM 205-07** on June 15, 2021 at village-Kamalpur Roda participated by 25 farmers. Experts encouraged the farmers for showing their full participation for carrying out demonstration. They also highlighted the essential role of growing pulses in their own fields for meeting their nutritional security, improving soil heath and increasing their income.

- KVK organized Farmers **"awareness program on "Balanced Use of Fertilizers"** on June 18, 2021 in virtual mode. In this event about 145 persons participated, among which 110 participated through virtual mode and 35 participated physically at KVK. Dr. M S Chauhan, Director, NDRI, Karnal and Dr G P Singh, Director, IIWBR, Karnal presided over the function. Lectures on different aspects of balanced use of fertilizers were delivered by the scientists of NDRI, IIWBR and CSSRI.

- KVK organized Field Day on **CFLD summer moong** variety **IPM 205-07** on June 28, 2021.The program was organized in collaboration with the line department of state. The chief guest of the function was Dr. Pooja Bharti (SDO Gharunda, Karnal). A team of experts from KVK visited the field and encouraged the farmers for showing their full participation in laying down demonstration. They also highlighted the essential role of growing pulses in their own fields, which shall meet their nutritional security, improve soil heath and increase their income. In this event about 65 farmers and farm women participated.

Other activities of KVK

Sl No	Activities	Place	Number of farmers/ farm women/youth	Date
1)	Awareness on Crop residue Management	Vill-Sultanpur	18	06/04/2021
		Village-Sirsi	8	13/04/2021
2)	CRM field Visit	Sirsi	8	13/04/2021
3)	Diagnostic field visit	Nasirpur	12	15/05/2021
		Kamalpur Rodan	10	07/06/2021
		Kunjpura	10	15/06/2021

CRM field visit of Goat Farm on 13/04/2021 at village-Sirsi

Diagnostic field visit of Goat Farm on 15/06/2021 at village-Kunjpura

Off-Campus Trainings

Sl No	Title of training	Place/Village	Number of farmers/ farm women/youth	Date
1)	Off-campus training on nursery raising of Kharif crops	Khulweri	12	22/05/2021
2)	Off-campus training on plant protection in Kharif crops	Kamalpur Rodan	11	15/06/2021
		Khulweri	15	22/05/2021
		Rasin	12	28/06/2021

On-Campus Trainings

Sl No	Activities	Place	Number of farmers/ farm women/youth	Date
1)	मधुमक्खी पालन	KVK	22	19/04/2021 to 22/04/2021
2)	मत्स्य पालन प्रशिक्षण कार्यक्रम	KVK	19	21/06/2021 to 24/06/2021

On-campus training on bee keeping

Frontline Demonstrations (FLDs/OFTs)

1) Pulses/Green Manuring
   - KVK, ICAR-NDRI organized FLDs on summer moong in various villages of Karnal district to encourage the farmers to grow pulses crops. In all, 50 FLDs of summer moong variety **IPM 205-07** in 20ha area were laid under irrigated conditions to generate the data.
   - KVK, ICAR-NDRI organized FLDs on Green manuring (Dhaincha) in various villages of Karnal district to encourage the farmers to grow Green manuring crops. In all, 13 FLDs of Green manuring variety CSD-123 in 5.2ha area were laid under irrigated conditions to generate the data.

Activities on (FLDs)

Sl No	Activities	Place	Number of farmers/ farm women/youth	Date
1)	CFLD Summer moong field visit	Khulweri	12	22/05/2021
		Kamalpur Rodan	15	07/06/2021
		Rasin	25	28/06/2021
2)	Harvesting Day on CFLD Gram	Sultanpur	18	06/04/2021

CFLD Summer Moong field visit on 07/06/2021
at village Kamalpur Rodan

EVENTS

Webinar on World Milk Day-2021 "*Problem and Solutions for Gaushalas and Dairy Entrepreneurs*"

ICAR-National Dairy Research Institute, Karnal organized a webinar on "***Problem and Solutions for Gaushalas and Dairy Entrepreneurs***" to celebrate the "World Milk Day-2021" on June 1, 2021 through online platform. Dr. Trilochan Mahapatra, Secretary DARE and DG, ICAR, was the Chief Guest and Dr. B. N. Tripathi, DDG (Animal Science), ICAR, was the Guest of Honour. The programme was presided over by Dr. M. S. Chauhan, Director, ICAR-NDRI, Karnal. The main objective of the webinar was to understand the problems and concerns of Gaushalas and other dairy entrepreneurs through their experiences. In this Programme, nearly 400 participants including representative of about 70 Gaushalas, 40 dairy entrepreneurs & progressive dairy farmers and more than 290 smallholder dairy farmers including women dairy farmers from various districts of Haryana as well as from other states, personnel from private companies actively participated. Directors of the animal science Institutes of ICAR were also present in this virtual conference.

Dr. M S Chauhan, Director & Vice chancellor, ICAR-NDRI, Karnal congratulated all the dairy professionals for making India proud as the highest milk producing country in the world. He advised the representative of Gaushalas as well as other progressive dairy farmers and entrepreneurs to harness the unparalleled expertise of NDRI for extracting better profits without impairing the production resources. He also assured to extend of NDRI expertise in the areas of advanced reproductive technologies to resolve the reproductive issues. He stressed that NDRI would extend all possible help to the dairy entrepreneurs to achieve nation's dairy development goal.

Dr. Trilochan Mahapatra, Secretary, DARE and Director General of ICAR & Chief guest of this programme thanked Dr. V. Kurien for his untiring efforts which have currently made India the proud producer of 200 MT of milk with commendable 6% annual growth rate. He said that in our country, 95% farmers are rearing 1-5 animals. He also emphasized for giving more attention for the development of the livestock sector especially unorganized dairy sector. He suggested developing a database of gaushalas existing in the country, which may be useful to formulate a development pathway in future. He further stressed that records of good cows, bulls, AI, repeat breeding, growth parameters etc., from calf to cow, should be made available on a "Digital platform" for National Data Base in future. He further stressed upon the feed & fodder scarcity, which is a challenge for productivity enhancement of the dairy cattle and buffalo. He outlined the need for a *Kisan Sarathi* where all the technological knowledge and input related to dairy farming should be available for the farmers. Dr. Mahapatra also advised the scientists of NDRI to study the sustainability issues of the Gaushalas and to work closely with the dairy entrepreneurs for their overall development.

Dr. B N Tripathi, DDG (Animal Science), ICAR and Guest of honour of this programmme in his concluding remarks emphasized that farmers should be provided with latest know–how through skill oriented training, artificial insemination, bio-gas and diversification technologies. He called the farmers to develop the linkages with various agencies and work for the success of the cooperative sector in Haryana. He also suggested making a link/network with Gaushalas and

Government sector for developing Modern Gaushalas with scientific knowledge. He also mentioned that more attention is required for the development of the livestock sector towards profitability and sustainability.

There were two sessions in this webinar i.e. first session was on the gaushala and second session was on the dairy entrepreneurship. These two sessions were coordinated by Dr. S. S. Lathwal, In-Charge, LRC and Dr. A K Singh, Head DT division and list of panelists were present from different disciplines/divisions of ICAR-NDRI, Animal Husbandry Dept, Haryana and NABARD. In the first session, representatives of four Gaushalas presented their achievements and concerns. In the second session, four dairy entrepreneurs promoted by ICAR-NDRI presented their achievements, raised their concerns and extended their willingness to work together in coming days for the dairy development of the country. Towards the end, a question-answer session was organized to address the issues raised by the Gaushala representatives and dairy entrepreneurs.

Webinar on "Role of Rural India in Sustainable Development"

A Webinar on the theme "*Role of Rural India in Sustainable Development*" was organised (in virtual mode) by Dairy Economics, Statistics and Management (DES&M) Division on June 26, 2021. Inaugural address of the Webinar was delivered by Dr. M.S. Chauhan, Director and Vice-Chancellor, ICAR-NDRI, in which he highlighted the major sustainability issues related to agriculture and dairying. Dr. Sudhir Singh, a renowned faculty in the Department of Political Science at Dyal Singh College, University of Delhi was the guest speaker for the event. Dr. Singh shared his rich experience on designing strategies for development of rural community. His talk focused on scope of income generation activities, skill development, and out-migration of rural youth, agricultural diversification, natural resource conservation and role of local rural institutions/administration in implementation of Sustainable Development Goals (SDGs). Around 200 participants from different parts of the country participated in the event.

Dr. M.S. Chauhan, Director and Vice-Chancellor, ICAR-NDRI, welcoming the guest speaker Dr. Sudhir Singh, Department of Political Science at Dyal Singh College, University of Delhi, at the Webinar on "Role of Rural India in Sustainable Development".

National Webinar on "Dairy Vavsaya Hetu Hara Chara Utpadhan evm Sarakshan"

A National webinar on "Dairy Vavsaya Hetu Hara Chara Utpadhan evm Sarakshan" was organized on June 22, 2021. More than 700 participants from 25 states and around 150 research and teaching institutes including Scientists, Professors, Dairy entrepreneurs, Students and Progressive farmers virtually participated in the Webinar. Director NDRI was the chief guest of the programme. Three eminent speakers viz. 1) Dr. Naveen Kumar, Head Agronomy, CSK Palampur,(H.P); Specialist; 2) Dr. Sunil Kumar Tiwari, Head crop production, ICAR-IGFRI, Jhansi (U.P)and) Dr. Rakesh Kumar, In-charge Agronomy section, ICAR-NDRI, Karnal delivered their lectures on fodder production and conservation.

On the behalf of the chief guest, Dr. Dheer Singh (JDR) highlighted the importance of fodder production and conservation for achieving the sustainability in dairy sector in India.

Dr. Naveen Kumar discussed the importance of conventional fodder crop and cropping system including hydroponics approach of fodder production under limited resource condition. Dr. Sunil Kumar, urged for adopting the perennial fodder crops like thorn less cactus and Congo grass which has high biomass potential under stress environment. He accentuated on the Agro-forestry and horti-pasture multistoried cropping system and their role in doubling the farmer's income by using advanced technologies in dairy sector. Dr. Rakesh Kumar outlined the way of fodder conservation and storage for maintaining the availability of fodder and forage during round the year for livestock feeding. Some progressive farmers raised the practical issues and problems related to fodder production and conservation. The experts addressed all the queries to their satisfaction.

PERSONALIA

Joining/ Appointments/ Promotions

- Dr. Dheer Singh, Principal Scientist & Head, Dairy Chemistry Division was appointed as Joint Director (Research,), ICAR-NDRI, Karnal w.e.f. 27.04.2021.
- Consequent upon his transfer from ICAR-NDRI, Karnal to ASRB, New Delhi, Dr. A. P. Ruhil, Principal Scientist was relieved from ICAR-NDRI on March 31, 2021 (AN).
- Consequent upon his transfer from ICAR-Central Sheep & Whool Research Institute, Avikanagar, Rajasthan to ICAR-NDRI, Karnal, Dr. Rajani Kumar Paul, Scientist in Animal Biochemistry discipline joined his duties in the forenoon of dated 8.04.2021 at ICAR-NDRI, Karnal.
- Consequent upon his transfer in public interest Sh. Arvind, Senior Finance & Accounts Officer was relieved of his duties at ICAR-NDRI, Karnal in the afternoon of 23.06.2021 to enable him to join his duties at ICAR-CTRI, Rajamundry-533105, Andhra Pradesh.
- Smt. Meenu Rani, PA joined as Private Secretary in the Pay Level 0-7 (44900-142400) on regular basis at ICAR-NDRI, Karnal w.e.f. from 14.06.2021.

Transfers/Retirements/Relieving

- Smt. Prem Kumari, Private Secretary, Dairy Technology Division retired on attaining the age of superannuation in the afternoon of 30.04.2021 from ICAR Services.
- Dr. Madhu Mohini, Principal Scientist, Animal Nutrition Division retired on attaining the age of superannuation in the afternoon of 31.05.2021 from ICAR Services.
- Dr. Parveen Kumar, Principal Scientist, Animal Physiology Division retired on attaining the age of superannuation in the afternoon of 31.05.2021 from ICAR Services.

Additional Responsibility

- Dr. Dheer Singh, Joint Director (Research) was entrusted with the following responsibilities:-
  - National Referral Lab on Milk Quality and Safety
  - ITMU
  - TBI / BPD
  - Consultancy Cell
  - Human Health Complex
  - ATIC
  - Press

- Dr. Kaushik Khamrui, Principal Scientist, Dairy Technology Division was entrusted with the responsibility of Secretary, SINED (TBI) to in place of Dr. A.K. Singh, Principal Scientist, Dairy Technology Division w.e.f. 17.5.2021
- Dr. A.K. Dang, Principal Scientist, Animal Physiology Division was nominated to handle the twitter / facebook account of the Institute in place of Dr. Gautam Kaul, Principal Scientist, Dairy Chemistry Division.
- Dr. Neelam Upadhyay, Scientist, Dairy Technology Division was nominated as General Secretary of ICAR-NDRI, Staff Club in place of Dr. Gautam Kaul, Principal Scientist, Dairy Chemistry Division.
- Dr. Bimlesh Mann, Principal Scientist, Dairy Chemisty Division was nominated as Chairman, Consultancy Cell in place of Dr. A.K. Singh, Principal Scientist, Dairy Technology Division.
- Dr. Bimlesh Mann, Principal Scientist, Dairy Chemistry Division was nominated as CPIO of the Institute in place of Sh. Abhishek Rana, Senior Administrative Officer.
- Dr. Suneel Kumar Onteru, Principal Scientist, Animal Biochemistry Division was entrusted with the responsibility to act as Officer Incharge, Human Health Complex, ICAR-NDRI, Karnal in place of Dr. Dheer Singh, Joint Director (Research).

Permission granted to the following scientific staff for attending the workshops/ seminars/ symposia/ conferences & training during the period April, 2021 to June, 2021:-

Name & Designation	Title of the seminar/ conference/ workshop/ symposium/ meeting/ training etc.	Period
Dr. A. K. Singh, Principal Scientist	On-Line training Programme on "MDP on Leadership Development" (a Pre-RMP programme) at ICAR-NAARM, Hyderabad.	14.06.2021 to 25.06.2021
Mrs. Khushbu Kumari, Scientist	On-Line training Programme on MOOC on "Theoretical foundations of Education Technology" at ICAR-NAARM, Hyderabad.	01.06.2021 to 30.06.2021
Er. Ankit Deep, Scientist	ऑनलाइन राष्ट्रीय अधिवेशन में भाग लेने हेतु on "कोविड -19 जनित परिस्थितियों में देश के आर्थिक विकास एवं आत्मनिर्भरता में कृषि अभियांत्रिकी की भूमिका" at ICAR-CIAE, Bhopal.	28.07.2021 to 29.07.2021
Dr. Rakesh Kumar, Principal Scientist	Online training on "Management Development Programme on "Leadership Development) (a Pre-RMP programme) at ICAR-NAARM, Hyderabad.	14.06.2021 to 25.06.2021
Dr. K.P. Ramesha, Principal Scientist	A speaker for the OIE Virtual Workshop for Vetey Education Establishments and to participate in the Special Seminar on Antimicrobial Resistance (AMR).	07.06.2021 to 09.06.2021
Dr. Bimlesh Mann, Principal Scientist	To deliver a lecture in the Webinar organized by Indian Dairy Association and submit presentation on "Advances in Health Benefits of Milk and Dairy Products"	01.06.2021
Sh. Brahm Prakash, A.A.O.	Online Training Programme on "Reservation in Service" Organized by ICAR-NAARM, Hyderabad.	28.06.2021 to 30.06.2021

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डा. एम.एस. चौहान, निदेशक, भाकृअनुप–राष्ट्रीय डेरी अनुसंधान संस्थान, करनाल की अध्यक्षता में संस्थान राजभाषा कार्यान्वयन समिति की 91वीं संयुक्त तिमाही बैठक दिनांक 31.05.2021 को अपराह्न 3 बजे से वीडियो कॉन्फ्रेन्सिंग के माध्यम से प्रारंभ की गयी। इस बैठक में संस्थान के करनाल मुख्यालय के पदाधिकारियों के साथ–साथ बेंगलूरू तथा कल्याणी क्षेत्रीय केन्द्रों के अध्यक्ष, राजभाषा अधिकारी व प्रशासनिक अधिकारी भी शामिल हुए। बैठक के मध्य में संस्थान प्रमुख के अन्य अपरिहार्य बैठक से जुड़ने के कारण उनके द्वारा डा. धीर सिंह, संयुक्त निदेशक (अनुसंधान) को समिति की अग्रिम कार्यवाही की अध्यक्षता करने के लिए प्राधिकृत किया गया। इस बैठक में सर्वसम्मति से सेवा–पुस्तिकाओं के सभी इन्दराज हिन्दी में करने, सभी कर्मचारियों के द्वारा ईमेल के डिफाल्ट हस्ताक्षर को द्विभाषी में प्रयोग करने, हिन्दी अग्रेषण पत्र लगाकर अंग्रेजी पत्र आदि जारी करने, राजभाषा एकक के द्वारा प्रत्येक तिमाही में कुछ प्रभागों का हिन्दी संबंधी निरीक्षण करने, ई–ऑफिस पर छोटी–छोटी टिप्पणियां केवल हिन्दी में लिखने, पुस्तकों के लिए आबंटित बजट का 50 प्रतिशत हिन्दी पुस्तकों की खरीद पर व्यय करने, बैनर, प्रमाणपत्र, प्रशिक्षण सामग्री, नामपट्ट तथा वेबसाइट सामग्री आदि को द्विभाषिक या त्रिभाषिक रूप में तैयार कर प्रदर्षित करने, डाक प्रेषक के द्वारा लिफाफों पर द्विभाषी में पते लिखकर डाक प्रेषित करने तथा अधिकारियों एवं कर्मचारियों की सेवा पुस्तिकाओं में प्रविष्टियां हिंदी में ही करने का निर्णय लिया गया। इसके अलारवा, संस्थान के सभी अधिकारियों एवं कर्मचारियों के हिंदी ज्ञान से सम्बन्धित रोस्टर को अद्यतन कर उन्हें व्यक्तिशः आदेश जारी करने, धारा 3 (3) के सभी दस्तावेजों को अनिवार्यतः द्विभाषी में यथावत् जारी करने, प्रभागों व अनुभागों तथा क्षेत्रीय केन्द्रों के अध्यक्षों के द्वारा अपने स्तर पर हिन्दी जांच बिन्दु बनाये जाने, राजभाषा नियम 5 के अनुसार हिन्दी पत्रों के उत्तर यथावत् हिन्दी में जारी करने व भारत सरकार, राजभाषा विभाग, गृह मंत्रालय के द्वारा समय समय पर जारी किये जाने वाले वार्षिक कार्यक्रम तथा सभी निर्देशों की अक्षरशः अनुपालना सुनिश्चित करने बावत् भी निर्णय लिये गए।

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संस्थान के द्वारा संस्थान प्रमुख डा. एम.एस. चौहान की अध्यक्षता में नगर राजभाषा कार्यान्वयन समिति के तत्वावधान में दिनांक 25.05.2021 को नगर स्तरीय वर्चुअल हिन्दी कार्यशाला मय चर्चा संगोष्ठी का आयोजन किया गया। संगोष्ठी के मुख्य अतिथि डा. ए.पी.राय, उप महानिदेशक, भारतीय भूविज्ञान सर्वेक्षण, कोलकाता ने 93 प्रतिभागियों को संसदीय राजभाषा समिति की संरचना, समिति की निरीक्षण की कार्यप्रणाली, निरीक्षण हेतु की जाने वाली तैयारियों, माननीय संसद सदस्यों की गरिमानुसार संबंधित व्यवस्थाएं करने, निरीक्षण प्रश्नावली भरने, कार्यालय में कार्यालय प्रमुखों के द्वारा राजभाषा नीति, नियमों व व्यवस्थाओं तथा संवैधानिक नियमों के अक्षरशः अनुपालन के लिए महत्वपूर्ण मार्गदर्शन प्रदान किया। उन्होंने पिछले संसदीय निरीक्षण के दौरान दृष्टिगत अपेक्षाओं पर विस्तारपूर्वक चर्चा डालते हुए प्रतिभागियों की शंकाओं का भी समाधान किया। करनाल के क्षेत्रीय भविष्य निधि आयुक्त श्री अमित सिंगला तथा रा॰डे॰अनु॰सं॰, करनाल के संयुक्त निदेशक (प्रशासन) श्री विवेक पुरवार ने उनके कार्यालय के संसदीय राजभाषा निरीक्षण के दौरान अर्जित अनुभवों को साझा भी किया।

RESEARCH HIGHLIGHTS

Integrated -omics analyses for bull fertility prediction

(Thirumala Rao Talluri and A. Kumaresan)

Bull fertility is of paramount importance in bovine industry because semen from a single bull is used to breed several thousands of cows; however, currently, bulls are selected based on breeding soundness evaluation that mainly focuses on examination of phenotypic characteristics and evaluation of few characteristics of spermatozoa. In an approach to develop bull fertility prediction tools, spermatozoa from high- and low-fertile bulls were subjected to high-throughput transcriptomic, proteomic and metabolomic analysis. Using an integrated multi-omics approach the molecular differences between high- and low-fertile bulls were identified. A total of 18,068 transcripts, 5041 proteins and 3704 metabolites were identified in bull spermatozoa, of which the expression of 4766 transcripts, 785 proteins and 33 metabolites were dysregulated between high- and low-fertile bulls. At transcript level, several genes involved in oxidative phosphorylation pathway were found to be downregulated, while at protein level genes involved in metabolic pathways were significantly downregulated in low-fertile bulls. It was found that metabolites involved in Taurine and hypotaurine metabolism were significantly downregulated in low-fertile bulls. Integrated multi-omics analysis revealed the interaction of dysregulated transcripts, proteins and metabolites in major metabolic pathways including Butanoate metabolism, Glycolysis and gluconeogenesis, Methionine and cysteine metabolism, Phosphatidyl inositol phosphate, pyrimidine metabolism and saturated fatty acid beta oxidation. These findings collectively indicated that the bull fertility is potentially influenced by the molecules governing sperm metabolism.

Integrated proteogenomic annotation of the whole genome and identification of novel protein-coding gene in Malnad Gidda cattle

(K .P. Ramesha, N. Azharuddin and T. S. Keshava Prasad)

Aimed to identify the genetic diversity among Malnad Gidda, Deoni, and Hallikar breeds of cattle, studies were carried out to assemble their whole genome and discover sequence variations with reference to the genome of *Bos taurus*. The short paired-end (25X) and mate-pair reads (5X) from Illumina HiSeq 2500 platform for all three breeds and further the long reads from PacBio for Malnad Gidda were used for genome analysis. A total of 58,795; 61,366 and 62,198 genes were predicted, out of which 171, 132 and 144 unique genes were found with reference to *Bos taurus* in Malnad Gidda, Deoni and Hallikar breeds respectively. A total of 24,645; 24,109 and 23,772 sequence variations specific to Malnad Gidda, Deoni, and Hallikar breeds of cattle respectively, with 69,868 variations common to these three breeds were identified in protein-coding genes. Integrating with a proteogenomics annotation pipeline, genome annotations of Malnad Gidda were refined and a novel protein-coding gene found in Malnad Gidda, which is not yet known to be expressed in any of the cattle breed data in the public repositories. Based on the known QTL/SNV variant associations mainly with the exterior, health, meat and carcass, milk, production and reproduction traits, the current knowledge-based trait associations were also provided.

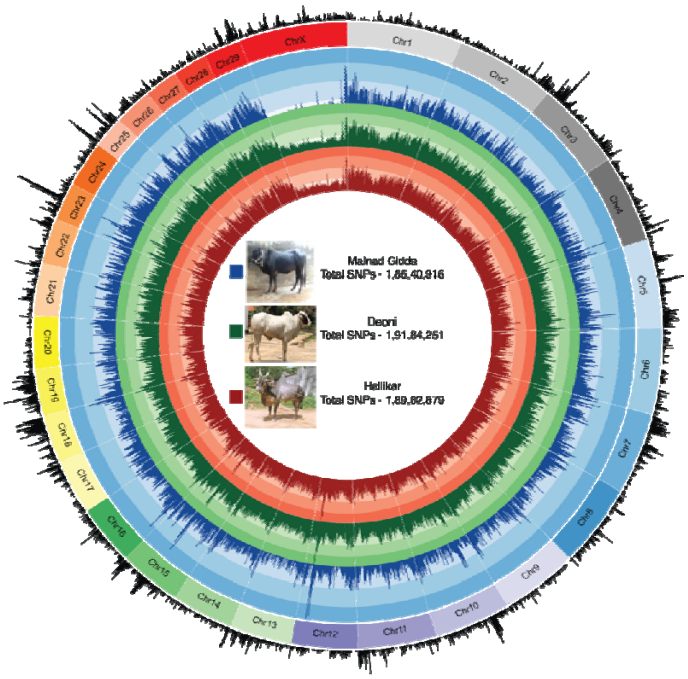


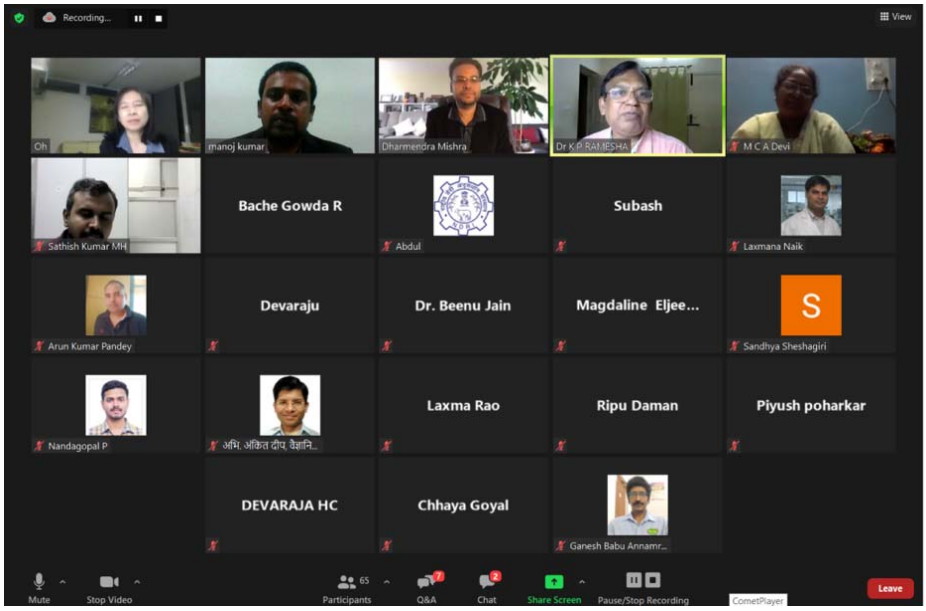
Figure depicting SNP density in Malnad Gidda (blue), Deoni (green), and Hallikar (red) breeds of cattle per 1Mb base pairs. The outer circle (black) represents the gene density per 1Mb base pairs. SNP density of chromosome X in Malnad Gidda is comparatively less with respect to the other two breeds, Deoni and Hallikar

TECHNOLOGY DEVELOPED

Technology for preparation of protein rich cheese squeeze

Protein rich cheese squeeze (27% protein on DM) spreadable at refrigeration temperatures was developed using cheddar cheese, milk solids and stabilizing salts. The product was packed in stand up pouch with spout, which is convenient to use and carry. The product was launched by honorable Director General ICAR Dr. Trilochan Mohapatra and released at the NDRI milk parlor.

International Webinar



A webinar on the topic entitled "Advances in Aseptic Processing and Packaging" was conducted on 31.05.2021 under IDP-NAHEP project for the benefit of students and faculty members of BDRI and other educational institutes. The webinar was delivered by Dr. Dharmendra K Mishra, Department of Food Sciences, Purdue University, USA and Dr. Patnarin Benyathiar, Food Technology Division, Mahidol University, Thailand. The speaker Dr. Dharmendra K Mishra delivered a lecture on "Recent developments in particulate aseptic processing" and Dr Patnarin Benyathiar delivered a lecture on "Packaging materials and filling machines for aseptic packaging".

EASTERN CAMPUS, KALYANI

Research

Effect of bypass fat supplementation on reproductive parameters in dairy heifers
M. Karunakaran, Kinkar Debbarma, Mohan Mondal, Ajoy Mandal and S.K. Das

The study was conducted on 12 prepubertal heifers of >16 months of age. Heifers were divided into two groups viz. Control (C) without bypass fat supplementation and Treatment (T) with bypass fat supplementation @50 g/day/animal for a period of 150 days. There was no significant difference (P>0.05) in mean age (509.00±15.15 vs 501.17±16.64 days), and body weight (180.25±10.54 vs 188.83±11.25 Kg) between the control and treatment groups at the beginning of the trial.

The reproductive tract of heifers (RTS) were scored based on the size of uterine horn, ovaries and ovarian structures in a 1-5 scale (1 being immature and 5 being mature and cyclic) based on a score card by Andersen *et al.* (1991). The RTS ranged from 2.67±0.21 to 4.00±0.37 and 3.00±0.45 to 4.00±0.45 in control and treatment group, respectively during the trial period. The

overall mean RTS during the trial period of 150 days was similar (P>0.05) in both treatment (3.42±0.08) and control (3.32±0.11) group. On the final trail day (150th day) of the experiment, two heifers in the control group and three heifers in the treatment group had an RTS of 5, suggesting cyclicity with a functional corpus luteum. Onset of puberty was confirmed by appearance of estrus signs and development of corpus luteum. The difference in percentage of heifers that attained puberty was insignificant (P>0.05) between treatment (50.00%) and control group (33.33%). The age at onset of puberty (days) was insignificantly (P>0.05) lower in treatment group heifers (605.67±22.67 days) than in control heifers (626.50±28.50 days). The estrus signs exhibited by each animal during the trial period was noted down and assigned a score as per the scale of Layek *et al.* (2011). The estrus intensity score (P>0.05) in heifers attaining puberty was similar in both treatment 56.00±1.15 and control group 55.50±1.50. Mucus discharge was observed as the most commonly expressed estrus behavior in heifers attaining puberty. The duration of estrus (hours) in treatment group (21.33±4.81 hours) and control group (18±6.00hours), did not differ statistically. Ultrasound examination was carried out at fortnightly intervals to monitor the follicular growth and classified according to size (small<4 mm; medium 4-8 mm and large>8 mm).The overall mean number of small follicles was 6.05±0.46 and 5.67±0.36 in control and treatment groups, respectively. There was no significant (P>0.05) effect of bypass fat supplementation on the number of small follicles. The number of medium (4-8 mm) follicles ranged from 3.83±0.60 to 6.00±1.83 and 3.33±1.02 to 7.67±0.95, in control and treatment groups, respectively. The overall mean number of medium follicles was 4.83±0.24 and 4.98±0.37 in control and treatment groups, respectively. There was no significant (P>0.05) difference in number of medium follicles between the groups. The number of large (>8 mm) follicles ranged from 0.67±0.21 to 1.33±0.56 and 0.67±0.21 to 1.33±0.42, in control and treatment groups, respectively. The average number of large follicles was 0.91±0.07 and 0.92±0.08 in control and treatment groups, respectively. There was no significant (P>0.05) difference in number of large follicles between the groups. It can be concluded that supplementation of bypass fat @ 50g/day to pre-pubertal heifers did not have any significant effect on onset of puberty and development of reproductive structures.

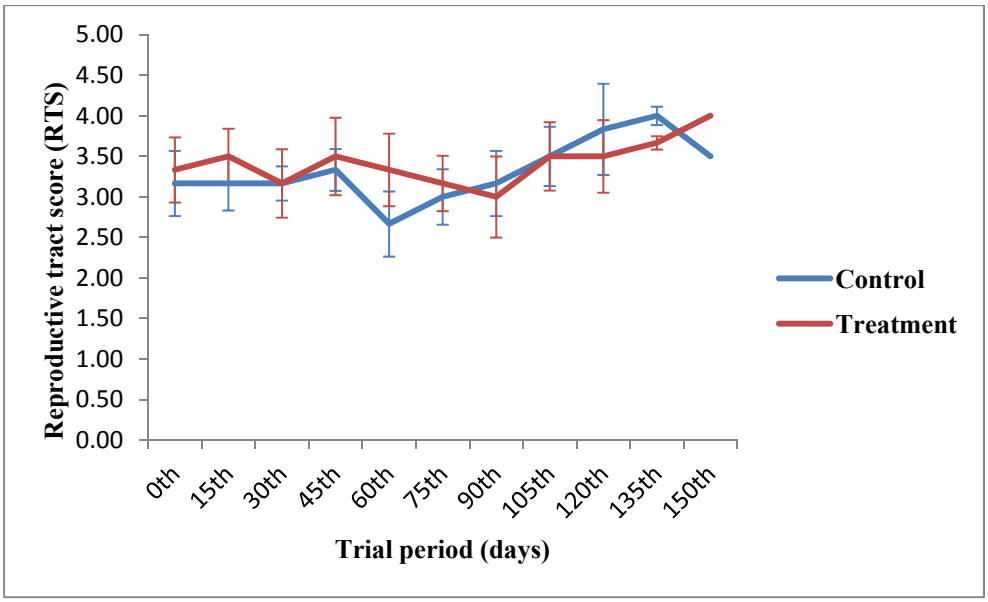


Fig. Reproductive tract score (RTS) in experimental heifer groups (Mean ±SEM)

Tree leaves as herbal feed additive to reduce rumen protozoal population in growing crossbred calves

A. Santra, S K Das and T K Dutta

Ficus hookeri

Manipulation of rumen microbial ecosystem for reducing rumen protozoal population for efficient utilization of dietary energy and protein is the useful strategy to improve production efficiency of ruminant animals. Recently, there is increasing interest in the use of tree leaves as natural herbal rumen manipulators to reduce rumen protozoal population for efficient utilization of dietary protein and energy. Daqingshu (*Ficus hookeri*) tree leaves were collected from Shilong, Meghalaya during the month of November and sun dried grounded (2 mm size) leaf meals were used as feed additive for conducting the experiment. Twelve numbers of growing Jersey male cross-bred calves (about four months of age) were randomly divided in to two equal groups (G1 and G2) and were fed individually under stall feeding on a mixed ration containing 50 % paddy straw and 50 % concentrate mixture for 140 days. Two types of iso-nitrogenous concentrate mixtures (C1 and C2) were prepared. Wheat bran in concentrate mixture (C2) of test group (G2) was partially replaced (4 parts w/w) with *Ficus hookeri* leaf meal.

Dietary supplementation of sun dried *Ficus hookeri* leaf meal as herbal feed additive did not have any effect on voluntary feed intake of the experimental calves. On an average the calves of G1 and G2 group consumed 3.3 and 3.1 kg DM per 100 kg body weight per day as well as 112.7 and 108.2 g DM/kg$W^{0.75}$/d, respectively. Plane of nutrition in terms of DCP and TDN intake was also similar in the calves of both the experimental groups. Calves of both the experimental groups consumed on an average 8.1 g DCP and 67.3 g TDN per kg metabolic body size per day, respectively. The rumen protozoal population present in the rumen of the experimental calves in bothe the experimental groups were B type due to presence of *Epidinium* sp, and the absence of *Polyplastron multivesiculatum*. Numerically spirotrich protozoa comprised more than 90 % of total protozoal population in the calves of all the two experimental groups. The lowest number of rumen protozoal count was observed just before feeding (0 h) with increased in total protozoal number at 6 h (peak value) followed by a gradual decrease at 8 to 10 h post-feeding in the calves of all the experimental groups (Fig. 1). Rumen total protozoal number reduced ($P<0.01$) drastically due to feeding of *Ficus hookeri* tree leaves as herbal feed additive to the experimental calves (G2 group). Rumen protozoal number in the calves of G1 and G2 groups was 40.2 and 26.5 x 10^3 per ml rumen liquor, respectively. There was about 34% reduction of rumen protozoal population due to feeding of *Ficus hookeri* leaf meal as herbal feed additive in growing crossbred calves. The result of the study showed that Daqingshu (*Ficus hookeri*) leaf meal may be used as herbal feed additive to manipulate rumen fermentation to reduce rumen protozoal population for efficient utilization of dietary protein and energy in growing crossbred calves.

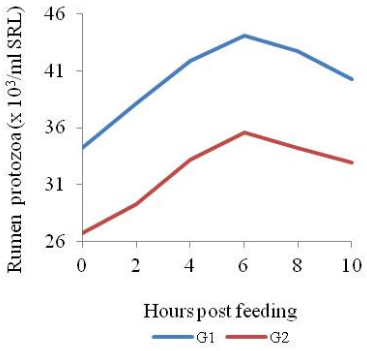


Fig 1. Rumen protozoal numbers