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INVITED REVIEW

Ethnoveterinary medicine for responsible dairying

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Abstract: India is the largest milk producer in the world with 22 percent of the global share and engages around 6.3 crore rural households. The cost and availability of animal health services for small dairy holders in a country like India has always been a cause of concern. There is a growing apprehension about the presence of drug residues in milk and milk products as well emergence of drug resistant bacteria and its associated health risks due to indiscriminate use of these products. The cost of therapeutic medicines, antimicrobial resistance and the residues in milk, meat and other animal products have forced the animal health sector to look for alternate forms of therapy to overcome these deficiencies. The new alternatives should be affordable, farmer friendly and lead to minimal or no residues in milk and other products. Practice of Ethno-Veterinary Medicine (EVM) is an approach that fulfils the above criteria. However, animal health service providers have very limited knowledge about EVM which in India is largely based on Veterinary Ayurveda. EVM has been used throughout the world by traditional animal rearing communities. EVM has been used for most common ailments in almost all domestic animal species. While there are efforts to document these practices, such documents are not widely available. Only recently, scientific scrutiny of these treatments for efficacy, mode of action and active ingredients has been initiated.

National Dairy Development Board (NDDB) along with Sabarkantha District Cooperative Milk Producers Union, Sabarkantha, Gujarat and Trans-Disciplinary University, Bengaluru, evaluated the use of EVM initially for management of bovine mastitis and subsequently for management of other common ailments of dairy animals. It was observed that EVM was helpful in successfully managing mastitis (85%), diarrhoea (86%), fever (85%), indigestion (87%), anoestrus (77%), worm infestation (89%), wound healing (79%) and ecto-parasite infestation (87%) with high clinical cure rate. It was economical compared to conventional allopathic treatment. A reduction in residues of common antibiotics (quinolones, tetracycline, sulphonamide, ß-lactam) in milk was observed in the area of operation. Drug resistant bacteria (*S. aureus* including methicillin resistant *S. aureus* – MRSA; and *E. coli* including Extended Spectrum Beta Lactamase producing – *E. coli* - ESBL), were isolated from the milk of bovines suffering from mastitis. EVM was able to cure even these animals. However, awareness about EVM is limited among animal health service providers and other stakeholders. EVM needs to be given adequate focus and mainstreamed. This would entail concerted efforts and initiatives in policy, research and development and infrastructure.

Keywords: Antimicrobial resistance; Antibacterial residues; Drug residues; Ethnoveterinary medicine; Ethnoveterinary practice; Mastitis;Veterinary Ayurveda; Veterinary herbal medicine

Introduction

Globally, dairying has a significant role as it generates employment and provides livelihood to about 1 billion people through 150 million dairy farms and provides healthy and nutritious milk and milk products to billions of customers every day. India –the largest producer of the milk in the world, with a share of around 22%, engages about 6.3 crore rural households in dairying. The value of milk group output was at ₹ 7,01,530 crore in 2017-18. With such large social and financial engagement, dairying needs to be responsible and sustainable in terms of product quality for the customer and animal health and welfare for the producer. The cost and availability of animal health services for small dairy holders is a concern. At the same time, there is a growing concern

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about the presence of drug residues in milk and milk products and its associated health risks (Bacanli and Basaran, 2019). Further, there is an emergent alarm regarding the development of drug-resistant bacteria with enormous health consequences. Recognising the importance, the World Health Organization (WHO) has developed the global action plan (WHO, 2015), guidelines for developing national action plans (WHO, 2016) and is monitoring the global progress (WHO, 2018). India has also prepared the national action plan (GOI, 2017), but has made little progress on appropriate use of antibiotics in animals and on following the guidelines on milk withholding period, subsequent to administration of drugs. While, the current status requires us to develop plans for judicious use of medicines at one end, it also calls to search for alternate forms of practices where such precautions may not be required. Application of Ethno-Veterinary Medicine (EVM) could play a pivotal role in addressing these issues and help in delivering safe milk and milk products to consumers while making animal health affordable to small holders.

What is EVM

Ethno-Veterinary Medicine is defined as "*The holistic, interdisciplinary study of local knowledge and its associated skills, practices, beliefs, practitioners, and social structures pertaining to the healthcare and healthful husbandry of food, work, and other income-producing animals, always with an eye to practical development applications within livestock production and livelihood systems and with the ultimate goal of increasing human wellbeing via increased benefits from stockraising*." (McCorkle, 1998)

However, the general understanding of EVM is that it is a compilation of people's traditional practices, knowledge, and beliefs to keep their animals healthy, productive and performing, using local resources in a sustainable manner. While in a few countries EVM uses animal origin products also, a significant part of EVM is based on the use of locally available herbs or plants and other local natural ingredients. Most of the pastoral and animal rearing communities in different countries have generated this knowledge over generations.

In India, EVM is largely the practice of Veterinary Ayurveda. Early written accounts describing the medicinal use of plants are found in the ancient Vedic texts. These writings originated in the period circa 3147 BC (Silver, 2007). Chapters that discuss animal husbandry appear in *Skanda Purana*, *Devi Purana* and other lesser known texts. Palkapya, around 1000 BC and Shalihotra, around 2350 BC specialized in the treatment of horses and elephants respectively. Shalihotra compiled an Indian *Materia Medica* which provided step-by-step descriptions of methods of administration of herbs, including instructions on preparing injectables. The first book on veterinary medicine was written by Shalihotra in Sanskrit and describes specific techniques in veterinary medicine, including the use of indigenous herbs in the treatment of working animals.

EVM has been used in most of the domestic and zoo animals – for example in cattle, buffalo, sheep, goat, pig, dog, horse, camel, elephant and birds. Practice of EVM is documented in many developing countries and some developed countries like- Argentina, Botswana, Cameroon, Canada, India, Italy, Kenya, Pakistan, PR China, Romania, South Africa, Switzerland, Turkey and Zimbabwe.

EVM has been used for management of most common diseases like mastitis (Nair and Punniamurthy, 2017), parasitic infestations (Sanhokwe et al. 2016), liver fluke (Jeyathilakan et al. 2012), gastrointestinal nematode (Ademola et al. 2006), ecto-parasite (Nyahangare et al. 2015), ticks (Nimbalkar et al. 2020), wound healing (Marume et al. 2017), respiratory disease (Ayrle et al. 2016), reproductive disorders (Dey et al. 2020) and fungal infections (Dikhoba et al. 2019).

EVM has also been used for specific viral diseases, e.g. Rabies, Bovine herpesvirus-1, Rotavirus, Bovine viral diarrhoea (Zitterl-Eglseer and Marschik, 2020), Foot and mouth disease (Kpodékon et al. 2015), African swine fever (Fasina et al. 2013), Infectious bursal disease (Ganguly et al. 2020), and Porcine reproductive and respiratory syndrome (Kaewprom et al. 2015).

In India, ethno-veterinary practices have been documented in almost all parts of the country since many decades – Andhra Pradesh, Assam, Arunachal Pradesh, Bihar, Chhattisgarh, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Nagaland, Odisha, Punjab, Rajasthan, Sikkim, Tripura, Uttar Pradesh, Uttarakhand and West Bengal.

Some of the widely used herbs for EVM in India are *Aloe vera*, *Curcuma longa*, *Azadirachta indica, Murraya koenigii*, *Cissus quadrangularis*, *Mimosa pudica*, *Acalypha indica*, *Lantana camara*, *Acorus calamus*, *Leucas linifolia*, *Adhatoda vasica*, *Moringa oleifera*, *Gentiana chirayita*, *Ocimum sp*., *Ocimum basilicum*, *Papaver somniferum*, *Piper betle*, *Tamarindus indica*, and *Lawsonia inermis*. Spices commonly used are cumin, fenugreek, pepper, coriander, bay leaves, asafetida and chilly. Vegetables and fruits commonly used are garlic, ginger, onion, lemon, bitter gourd, radish, lady finger, coconut and mustard. Other ingredients include limestone, coconut oil, sesame oil/ gingelly oil, salt, jaggery, ghee and butter. Based on scientific studies, pharmacopoeial monographs for 57 crude herbs and 47 processed herbs/excipients were published in Indian Pharmacopoeia 2014. (Rastogi et al. 2015).

EVM, Drug residues and Antimicrobial Resistance (AMR)

The emphasis on enhancing the productivity of dairy animals has, besides enhancing milk production, also resulted in an

increased use of agro-chemicals, mainly antibiotics and anti-parasite drugs. The residues of these drugs, could leak into the environment including milk. Public health can also get affected due to residues in milk. Antibiotic residues may cause various toxic effects such as allergy, immunopathological effects, carcinogenicity (sulphamethazine, oxytetracycline, furazolidone), mutagenicity, nephropathy (gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol), anaphylactic shock and development of antimicrobial resistance in bacteria which may cause serious infection in humans (Darwish et al. 2013).

Withdrawal periods reflect the duration of time necessary for an animal to metabolize an administered drug and the amount of time necessary for the drug/ product concentration level to decrease to a safe, acceptable level in milk. Treatment with antimicrobials in dairy animals usually requires withdrawal periods of 6-7 days for collection of milk. This practice is not genuinely followed by all farmers due to economic reasons and allows milk with residues to reach customers. However, in large dairies the residues in milk get substantially diluted after getting mixed with the normal milk.

Considering the health hazards associated with various drugs, maximum permissible limits for their residues are fixed internationally by Codex (CODEX, 2015) and Food Safety and Standards Authority of India (FSSAI) for India. India is among the leading producer and consumer of antibiotics (Van Boeckel et al. 2014).

Studies documenting the presence of antibiotic residues in milk in India are limited. A national milk survey in 2018 in India recorded 1.2% samples with antibiotic residues with Oxytetracycline being the main antibiotic (FSSAI, 2018). A compilation of antibiotic residues identified in milk in India by various authors is mentioned in Table 1.

The use of EVM for treatment of ailments of animals reduces the use of contemporary allopathic drugs to a considerable extent resulting in reduced presence of drug residues in animal products. Use of EVM for reducing the drug residues in animal products is a sensible approach and has been described by various workers (Groot and van't Hooft, 2016; Nair, 2019). In fact, use of medicinal plants is considered important for organic livestock farming in Europe (Mayer et al. 2014).

EVM and Antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) is a complex, multifaceted problem that threatens human and animal health, the global economy, as well as national and global health security. WHO has included AMR as one of the top ten threats to global health in 2019 (WHO, 2019). Current 'One Health' approaches to AMR focus primarily on reduction in antibiotic usage in food animals.

With a few exceptions where certain classes of antibiotics are restricted for human or animal use, most antibiotics are used both in human and animal (FAO, 2016). Unregulated, indiscriminate use of antibiotics as well as under or excessive dosing is the leading cause of AMR. Use of veterinary antimicrobials without the prescription of a veterinarian was reported in 87% and 38% among urban and rural farmers, respectively (Sudershan and Bhat, 1995).

Various types of antibiotic resistant bacterial agents have been isolated from bovine mastitis milk. Among them, methicillin

Table 1 Presence of antibiotic residues in milk, reported from India

Study location	Year	Antibiotic residues detected in milk								Author
		ß Lactam	Tetracycline	Oxytetracycline	Enrofloxacin (Quinolones)	Neomycin	Sulphonamide	Chloramphenicol	Macrolide & gentamycin	
Andhra Pradesh	2015	P	P	P						(Kalla et al. 2015)
Assam	2015	P	P		P	P	P	P	P	(Lundén, 2015)
Bihar	2017		P	P			P			(Nirala et al. 2017)
Punjab	2014		P							(Gaurav et al. 2014)
Punjab	2019	P		P	P		P			(Moudgil et al. 2019)
Kerala	2018	P	P		P					(Kumarswamy et al. 2018)
Kerala	2020			P						(Hebbal et al. 2020)
Kerala	2017	P	P							(Lejaniya et al. 2017)
Telangana	1995			P						(Sudershan and Bhat, 1995)

P = Presence of antibiotic residue detected in milk

resistant *S. aureus* and Extended Spectrum Beta-Lactamase (ESBL) producing *E. coli* are considered serious human health hazard.

In India several studies on the antibiotic resistance pattern of the circulating bacteriological agents have been undertaken which indicated varying degree of resistance to frequently used antibacterial agents.

In West Bengal, 54.5% *E. coli* strains isolated from milk showed the capability of producing ESBL, both phenotypically and genotypically with the presence of bla_{CTX-M} gene. The strains exhibited varying degree of resistance to drugs such as cefotaxime, ceftazidime, amoxicillin/clavulanic acid, tetracycline, and gentamicin (Batabyal et al. 2018). In Uttar Pradesh and New Delhi all *E. coli* strains isolated were resistant to ceftriaxone, cefepime, cefotaxime, ceftazidime, aztreonam, ampicillin and carbenicillin. The resistance was varying from 97.61% to 14.28% for Sulfadiazine, cefpodoxime, nalidixic acid, tetracycline, ciprofloxacin, sulphamethoxazole and trimethoprim, doxycycline, kanamycin, chloramphenicol, amoxicillin–clavulanic acid and gentamicin (Sivakumar et al. 2020).

In another study in West Bengal, a total of 450 composite milk samples both from cattle with or without mastitis were collected. Nine (9.6%) *S. aureus* isolates were detected as MRSA and all the MRSA isolates were multidrug resistant (Mahanti et al. 2020).

In Tamil Nadu, a total of 89 positive isolates of *S. aureus* were obtained from 258 raw milk samples screened (34.49%), of this 24 (24/258; 9.3%) samples and (24/89; 26.96%) isolates were positive for MRSA (Deepak, 2020).

In Jharkhand, both MRSA and non MRSA were isolated from milk samples. Based on the antimicrobial sensitivity results, MRSA isolates were found resistant to, cloxacillin, penicillin, methicillin and oxacillin. Similarly, non MRSA isolates were found resistant to penicillin, cloxacillin and cefazolin (Kumari et al. 2020). In another study in Tamil Nadu, from the milk samples of 35 cows with mastitis, *S. aureus* was isolated from 40% samples and all samples were MRSA (Manimaran et al. 2020).

In Rajasthan, 73 *S. aureus* strains were obtained from bovine raw milk and 90% strains were multidrug resistant, of which 15 were methicillin resistant (Sharma et al. 2017).

In Kashmir, a total of 80 (53.33%) *S. aureus* isolates were recovered from cases of bovine mastitis of which 20 (25%) were methicillin (*mecA*) gene positive. *In vitro* antibiotic sensitivity testing of MRSA revealed complete resistance towards methicillin and other penicillin group of antibiotics (Shah et al. 2019).

In view of the above, EVM is advocated for combating antimicrobial resistance (Nair et al. 2015; Ranganathan, 2017).

EVM is farmer friendly and less resource intensive

Most preparations of EVM are made from the ingredients that exist either in kitchen of the animal owner or in nature as vegetable, fruits or herbs. These ingredients are easily available and are very affordable. The method of preparation of EVM is also simple and uncomplicated. It avoids cost and efforts taken to go to town to consult the veterinarian and visit the pharmacy to get the medicine. EVM has been suggested as a sustainable approach for mastitis control (Rana et al. 2019). EVM is a tool for poverty reduction through increasing productivity of small ruminants by using locally available economical remedies (Iqbal et al. 2005).

India has a shortage of veterinary and para-veterinary professionals. A study in 2013 estimated the number of veterinary graduates at around 41,000, and forecasted additional annual requirement of 5000 veterinary graduates and thrice this number the para-vets to meet the requirement (Agrawal et al. 2013). The policy paper of National Academy of Veterinary Sciences, highlights acute shortage of veterinary personnel in India and lists various suggestions for meeting the shortfall (NAVS, 2015a). Use of EVM by farmers for common ailments of animals will considerably reduce the workload of veterinarians enabling them to focus on serious medical cases where their professional knowledge and expertise is most needed.

NDDB's Experience with Ethnoveterinary Medicine

Mastitis is one of the leading economically important diseases of bovines in India with an annual loss of ₹ 7165.51 crore estimated in 2009. More losses were recorded in sub-clinical mastitis (₹ 4151.16 crore) than clinical mastitis (₹ 3014.35 crore) (Bansal and Gupta, 2009). The contemporary management of mastitis includes testing of milk samples by California Mastitis Test (CMT) and antibiotic treatment usually infused in the udder through teats.

Information Network for Animal Productivity and Health (INAPH) is a software developed by NDDB that identifies each animal with a unique identification number printed on the ear-tag and records individual animal-wise various events related to breeding, feeding and health (Harikumar et al. 2015). Sabarkantha District Cooperative Milk Producers' Union (Sabar Dairy) in Gujarat was the first milk producers' cooperative to ear-tag all the cattle and buffaloes in their area and record diseases and treatment data. The data collected during 2013 indicated that mastitis was the leading problem. In year 2014, NDDB initiated a project to control mastitis at Sabarkantha district in association with Sabar Dairy. Testing of milk by CMT and administration of Tri Sodium Citrate (TSC) to CMT positive animals was the initial approach. TSC has been used for treating animals suffering with mastitis (Dhillon et al. 1995; Manzoor et al. 2020). Of the 19,259 SCM CMT positive animals given TSC oral regimen, 69.92 % became CMT negative after the first TSC dose. In another study, in 218 animals, when two consecutive TSC regimens were provided, recovery rate was

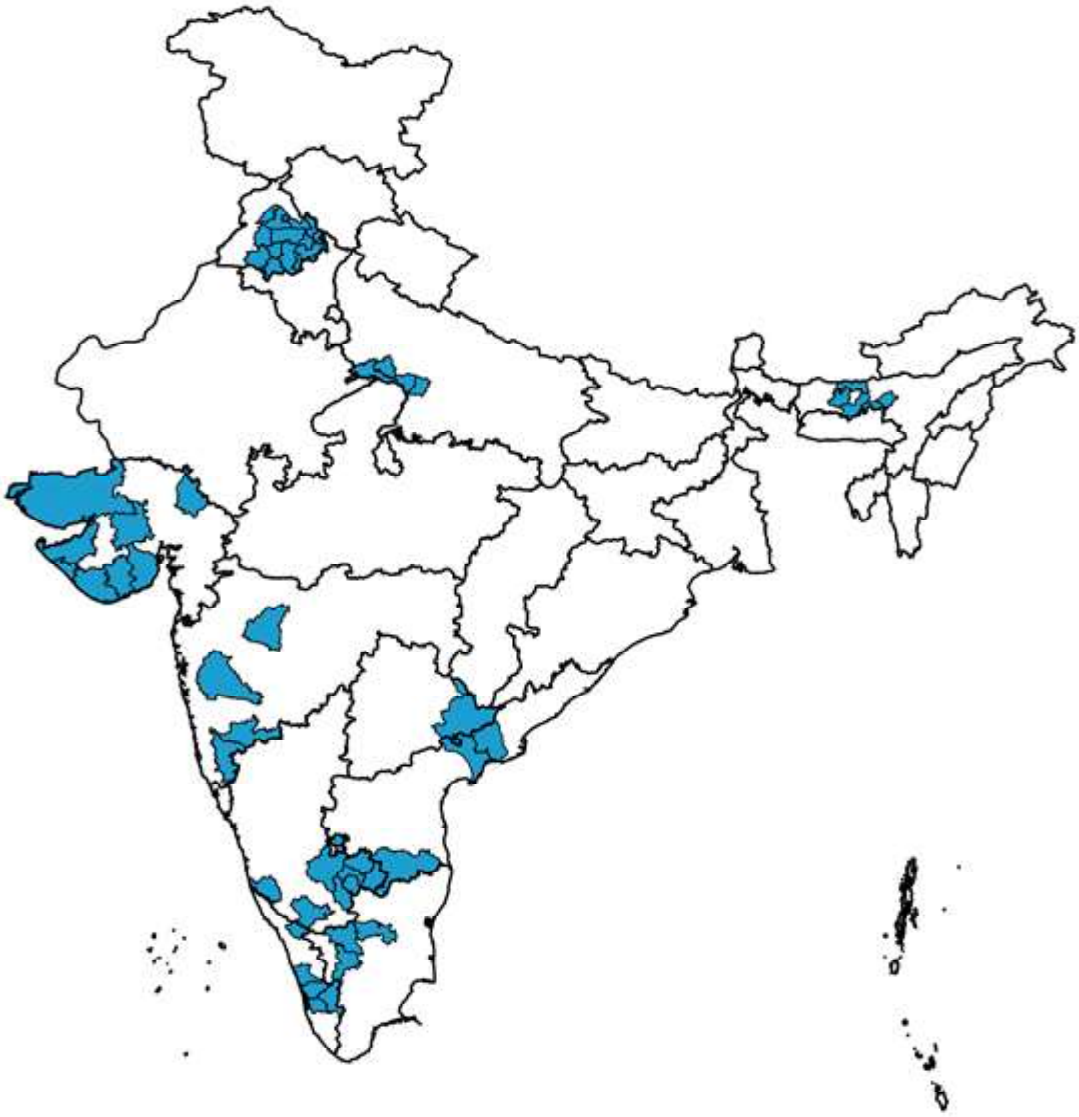

Fig. 1 Representative map of India displaying various districts included in Mastitis Control Popularization Project (MCPP)

recorded as high as 89 % (Dutta et al. 2017). Use of EVM preparation made from medicinal plants, spices and other locally available traditional ingredients for treating clinical mastitis was initiated in 2016 in collaboration with Trans-Disciplinary University, Bengaluru (TDU). The veterinary doctors of the Sabar Dairy were trained at TDU who later became the trainers for other local veterinarians, and in turn delivered the EVM to the ailing animals at farmer's doorstep and demonstrated to farmers how to deliver EVM preparation so that the farmers can perform it without any help from the veterinarian. The EVM for mastitis consisted of a paste prepared fresh from *Aloe vera*, *Curcuma longa* and calcium hydroxide. The use of *Aloe vera*, C*urcuma longa* and calcium hydroxide for mastitis treatment has been evaluated (Nair and Punniamurthy, 2017). EVM formulations containing *Curcuma longa* for treating mastitis have been tried by other workers also (Aruna et al. 2019; Bhatt et al. 2014).

In total, in Sabar Dairy, during 2017-2020, around 67,833 mastitis cases were treated with 85% recovery rate. The NDDB study found that the cost of treatment of mastitis through EVM was much lower than that of conventional treatment using antibiotics. This study indicated that the alternate approach employing the use of TSC for treatment of sub-clinical mastitis and EVM for treatment of clinical mastitis was very effective. Encouraged with these results, a Mastitis Control Popularization Project (MCPP) was launched by NDDB in 2017 which now covers more than 1500 villages (dairy cooperative societies) in 24 dairy cooperatives organizations spread in 9 states as shown in Figure 1.

Some 235 veterinarians have been trained in practicing EVM at TDU, Bangalore who have now trained around 680 fellow veterinarians and 5220 field milk testing personnel. For creating awareness and interest among farmers, 344 demonstration plots have been established by planting various herbs used in EVM. From about 7.3 lakh CMT done under the project on pooled milk samples, about 21% samples were recorded positive for sub-clinical mastitis. Of the 92,917 clinical mastitis cases treated with EVM, 72,129 got clinically cured providing a recovery rate of 79.78%.

In NDDB Study, presence of antibiotic residues in bulk milk samples was tested with the use of rapid antibiotic residue kits (Unisensor, Belgium) – test suitable for field testing. The total number of samples tested during 2017-18, 2018-19 and 2019-2020 were 790, 617 and 1124 respectively. It was observed that there was reduction in milk samples having presence of Quinolones group of antibiotics in milk from 13 % to 2 %; Tetracycline from 1 % to 0.4 %; Sulphonamides from 15 % to 3 % and Beta Lactams from 25% to 9 % during the observation period.

NDDB study on bacterial agents isolated from the clinical and sub-clinical mastitis cases revealed the presence of *Streptococcus uberis* (19%), *S. aureus* (14%), *Streptococcus dysgalactiae* (14%), *Streptococcus agalactiae* (4%), *Klebsiella sp*. (7%) and *E. coli* (5%). Studies on anti-microbial resistance with isolated strains of *S. aureus*, *Klebesiella sp.* and *E. coli* revealed that 14, 50 and 18 % isolates respectively were multidrug (three or more antibiotics) resistant to various classes of antimicrobials like penicillins, cephalosporins, tertracyclines, gentamicin, trimethoprim/sulfamethoxazole etc. Around 30% *S. aureus* isolates were resistant to methicillin (Rana et al. 2019). These Livestock-associated *MRSA (LA-MRSA)* strains were first described in the initial years of the 2000s and after a decade, now pose a risk of transmission to animal handlers and veterinarians (Cuny et al. 2015). In NDDB study, based on *pvl* gene analysis, two isolates recovered from mastitis milk were typed as *community-acquired S. aureus*. The presence of *ESBL- E. coli* was 11% of the total *E. coli* isolates recovered from mastitis. Based on gene profiling study by NDDB of 17 virulence and toxin producing genes, all *E. coli* isolates were expected to be less virulent/potent in causing human infection and food poisoning. Apart from the resistance to antibiotics in these organisms, the other impediment is the ability of these bacteria to form biofilm. Bacterial biofilms are highly recalcitrant to antibiotic therapies due to multiple tolerance mechanisms (Olivares et al. 2020). A preliminary study by NDDB revealed that around 90% of isolated organisms namely *S. aureus* (Rana et al. 2019) and *E. coli* from mastitis milk had biofilm forming genetic determinants.

In NDDB study, the EVM was able to successfully treat even those mastitis cases in cattle and buffaloes where the causative agents like *S. aureus, E. coli* and *Streptococcus sp.* having varying degree of AMR and biofilm forming ability were isolated.

NDDB expanded the scope of EVM to treat diarrhoea and fever in addition to mastitis. The clinical cure rate was 97.41 % (1958 cured from 2240 treated) for diarrhoea and 97.79 % (1691 cured from 1627 treated) for fever (Dutta et al. 2020). NDDB further expanded the scope of EVM for management of other common health conditions of dairy animals, namely indigestion, anoestrus, infestation with worms and ticks and wound healing, besides mastitis, diarrhoea and fever. NDDB is currently studying the efficacy of EVM in management of these common health conditions in the MCPP. The comprehensive results of clinical recovery are shown in Table 2 and are very promising.

Future outlook

Field studies by NDDB clearly demonstrate that EVM is very effective in management of mastitis, diarrhoea, fever, indigestion, anoestrus, infestations of worms and ticks and healing of wound. EVM is an efficacious, affordable and farmer-friendly clinical intervention for common health problems of dairy animals. EVM may be considered as a first-line-of-action in bovine health management and those not responding to EVM may be treated with allopathic drugs. Since, most EVM preparations could be delivered by the farmer himself with local resources, treatment would start early and would not be dependent on availability of a veterinarian. As India and many other developing countries face a shortage of qualified veterinary graduates, practice of EVM by farmer to manage minor animal health problems would help organize animal health delivery with reduced veterinary infrastructure and much lesser cost. This will also avoid delays in treatment of animals thus improving the health for continued productivity.

EVM usage suffers from low level of adoption by farmers as it is not recommended as first choice by the veterinary practitioners. It is also not very popular among scientific community and policy planners. Therefore, Research and Development efforts, to find *in-vitro* and *in-vivo* efficacy, active ingredients, mode of action,

Table 2 Various common health conditions of bovines treated by EVM in Mastitis Control Popularization Project (MCPP)

	Diarrhoea	Fever	Indigestion	Anoestrus	Worm infestation	Wound	Ecto-parasites
Cases treated	65508	64223	14129	13655	7979	3629	2749
Cases clinically cured	56302	54512	12239	10538	7080	2882	2381
Cure percent (%)	85.95	84.88	86.62	77.17	88.73	79.42	86.61

dosage, safety, formulations for convenient delivery, shelf life, setting of quality standards etc. are required to be established and widely circulated. The Policy paper of National Academy of Veterinary Sciences (India) also makes several recommendations which include capacity building of veterinary graduates in herbal medicine, strengthening of R&D and short as well as long term policy and development issues (NAVS, 2015b). Veterinary Council of India and Indian Council of Agricultural Research have a significant role to play in mainstreaming EVM.

EVM needs a big push for its popularization among farmers through demonstrations and vocational training. Regional compilation of EVM practices should be encouraged and authenticated. Good practices for herbal cultivation, harvest, processing and storage must be documented.

Conclusions

EVM is a practical, effective, affordable and sustainable approach for management of common animal health problems of bovine. This will also reduce the usage of antibiotics and thus their residues in livestock products and emergence of drug resistant bacteria – both of which have severe human health implications. However, EVM needs mainstreaming and focus. By practicing EVM not only we can revitalize our age old knowledge of Veterinary Ayurveda, we also protect our animals, humans and the environment which is the hallmark of responsible dairying.

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RESEARCH ARTICLE

Effect of cardamom powder and rosemary extract on textural, sensory, microbiological and colour properties of *pinni* during storage

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Abstract: *Pinni* is an immensely popular milk cereal based composite sweet having dark brown colour with granular texture. The shelf life of *pinni* was studied by using natural preservatives *i.e.,* cardamom powder and rosemary extract. Two packaging techniques *viz.*, cardboard boxes and polystyrene tray were compared for sensory, textural, microbiological and colour quality of *pinni* during storage for 21 days and 28 days respectively at 30±1°C. The extent of loss of most sensory attributes was rapid in control samples packed in both packaging materials as compared to cardamom and rosemary extract added *pinni* samples. Based on the results obtained in the study it was concluded that *pinni* could be best preserved up to 28 days at room temperature (30±1°C) without appreciable quality loss and should be packed in polystyrene trays with rosemary extract and have shelf life 14 days more than control sample.

Keywords: Cardamom, Pinni, Rosemary extract, Shelf-life

Introduction

Conversion of raw milk into a wide variety of milk products has been practiced in our country since time immemorial. Amongst various categories of Indian milk based sweets available, heat desiccated variants presents a pleasing and healthful variety of products compared to others because whole milk is used for producing those products. India has the largest repository of such products and out of them *pinni* is an immensely popular traditional milk-cereal based sweet of Northern states of India mainly Punjab, Haryana and Delhi. The product is a rich source of nutrients derived from milk solids as well as goodness of wheat or gram flour, nuts, dry fruits etc. It is also a concentrated source of fat, protein, minerals and energy. Considering its high nutritive value, traditionally *pinni* is considered as an ideal food for young ones in their grow-ing stage, pregnant and lactating women (Talwar and Brar, 2015).

Milk products undergo changes during storage, showing adverse effects on quality, ranging from minor sensory defects to complete spoilage (Kotsianis et al. 2002). Several physico-chemical and microbiological changes may take place making them unfit for human consumption. Food products, especially dairy products prepared under strict hygienic conditions are likely to be spoiled quickly if they are not stored properly. Like other milk products *pinni* also has limited shelf life about 10 days packed in high density polythene pouches, (Saxena et al. 1996). Because of its high fat content and non-acidic nature, spoilage in *pinni* is mostly caused by growth of surface yeasts and moulds and manifests as fermentative and acidic as well as rancid odour also observed during storage. This is a recurrent problem in humid and warm atmospheric conditions that are prevalent in tropics.

With the increasing demand of cheap, non-toxic and natural food preservatives globally, it was possible to extend the shelf life of many perishable and non-perishable foods by using cardamom and rosemary extract (Jessica et al. 2015). However, commercial application of cardamom and rosemary extract for Indian milk products is still at a nascent stage. Several studies have been reported to extend the shelf life of different food products using cardamom; Palit and Pal (2005) observed that vacuum packaging and addition of cardamom and potassium sorbate, each at the rate of 0.1% of *khoa* (w/w) increased the shelf life of *burfi* upto 60 days at 30±1ÚC. Prasad et al. (2017a) reported that the flavour score of cardamom added *burfi* was not significantly ($p>0.05$) different from that of control *burfi*. Cardamom incorporated yoghurt (Vijaylakhshmi et al. 2014) and rosemary extract (RME) enriched ghee showed significantly higher anti-oxidant activity.

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The presence of antioxidants reduces oxidative changes of fats and enhances shelf-life. Quality and properties of packaging material affects shelf life of a food product. Several studies have been reported on increased shelf-life of traditional dairy products using spices and herbs (Londhe et al. 2012 and Ghayal et al. 2015). Sweets like *kalakand,* milk-cake and other sweets have been packaged in paper cartons or duplex board boxes as reported by Goyal and Rajoriha (1991) to enhance shelf life. The present study was conducted to study the effect of two natural preservatives *i.e.,* cardamom powder and rosemary extract on physico-chemical, sensory and microbiological attributes of *pinni* packed in two different packaging materials *viz*., cardboard boxes and polystyrene trays.

Materials and Methods

Raw materials

Freshly pooled buffalo milk obtained from NDRI Karnal Cattle Yard, was standardized to 6.0% fat and 9.0% SNF. Food grade cane sugar (sucrose) and cardamom powder were obtained from the local market. Wheat flour of Aashirvad brand was procured from local market; it contained 11.8 % protein, 1.7% fat and 75.1 % carbohydrate. Ghee prepared from buffalo milk was procured from the Experimental Dairy of the Institute and it contained 99.5% fat and 0.5 % moisture. Free sample of rosemary extract was supplied by M/s Zakinl Biotech private limited (Chamrajnagar, Karnataka, India).

Preparation of *khoa*

Khoa samples were prepared from buffalo milk using the method of Bhatele (1983) with some modification such as open double jacketed stainless-steel kettle at a steam pressure of 2 kg/cm^2 instead of a karahi over a brisk non - smoky fire.

Preparation and compositional analyses of *pinni*

A batch of approximately 2 kg of *pinni* was prepared in triplicate for each experiment. Ghee (540 g) was taken in a heavy bottom pan then heated to 90°C and wheat flour (682 g) was added. Roasting of wheat flour was done at 90°C till typical roasted flavour and color was developed. The *khoa* (400g) was then added accompanied by continuous stirring to break the large lumps into small granules. Roasting was continued and sugar (360 g) was added and stirred to properly mix the ingredients. After this stage heating was discontinued and the mixture was allowed to cool to room temperature (30°C). Cardamom powder was added @ 2.5 % (of *pinni*) and rosemary extract 200 mg and then using a hand operated cylindrical mould the product was moulded into 25 g cylinders each. The proximate composition of the *pinni* in terms of moisture (IS: SP: Part 18 XI, 1981), fat (IS, 1981), protein (Meneffee & Overman, 1940), sucrose (IS: SP: Part 18 XI, 1981) and ash IS: SP:18 (Part XI, 1981) was estimated.

Packaging of *pinni*

Packaging materials for *pinni* i.e. cardboard boxes of 15 x 12 x 15 (L x B x H) cm were procured from the local market of Karnal, Haryana and Polystyrene (PS) trays (thickness: 1.50 mm; WVTR: 1.96 g/m^2 - 24 h; OTR: 26 mL/m^2 - 24 h) were procured from M/s Elixir Technologies, Bangalore. Trays were sealed with Polyamide-Polyethylene films.

Sensory evaluation

Sensory evaluation of fresh and stored samples of *pinni* was carried out in terms of flavor, body and texture and colour and appearance using a 9-point Hedonic scale. Seven members of semi-trained sensory evaluation panelists constituted from the faculty of Dairy Technology Division, National Dairy Research Institute, Karnal performed the sensory evaluation. Panel members carried out sensory evaluation in individual booths where an intact piece of *pinni* (tempered to 15ÚC) was presented with random three-digit codes in closed glass containers. Samples were served monadically with de-ionized water. Each panelist offered independent observation on randomized samples of *pinni.*

Colour measurement

Colour measurements were conducted as described by Nalwade et al. (2014) using Color Flex (Hunter Associates Laboratory, Inc., Reston VA, USA) colour measurement system equipped with dual beam xenon flash lamp and universal software. The instrument was calibrated prior to sample measurements with standard black, white and green tile as prescribed by the supplier. The results were represented by the Hunter L*, a*, b* notation.

Texture Profile Analysis (TPA)

Stable Micro System Texture Analyser, Model No: - TAXT2i; as described in Khamrui and Solanki (2010) fitted with a 25 kg load cell was used to measure the textural attributes of cylindrical samples of *pinni* measuring 15mm height and 20mm diameter. Samples tempered to 25°C were compressed to 70% of their initial height using an aluminium 75mm diameter compression plate (P-75), maximum force exerted by the sample, *i.e*., the peak of the force-time curve was measured as hardness (N) and fracturability is a force at the first significant break at the TPA curve. The texture analyzer settings that were employed to determine the textural attributes were; program: return to start, load cell: 25 kg, probe type: P-75 compression plate; pre-test, test, and post-test speed: 2 mm/s trigger type: auto, trigger force: 5 g, threshold: 50 g, time: 2 S data acquisition rate: 250 pps. This measurement was performed in triplicate on the three random samples of a batch.

Statistical analysis

The data generated from present study was analyzed using ANOVA for determining difference in mean using SAS ver. 5.3

(SAS Institute Inc., Cary, NC). Wherever, required in the study, the overall mean and standard error of the data was calculated.

Results and Discussion

The proximate composition of the *pinni* was found to be moisture 5.2% ± 0.34 fat 36.74% ± 0.33, protein 9.13% ± 0.5, sucrose 40.67% ± 0.45 and ash 1% ± 0.5.

Changes in sensory quality of *pinni* during storage

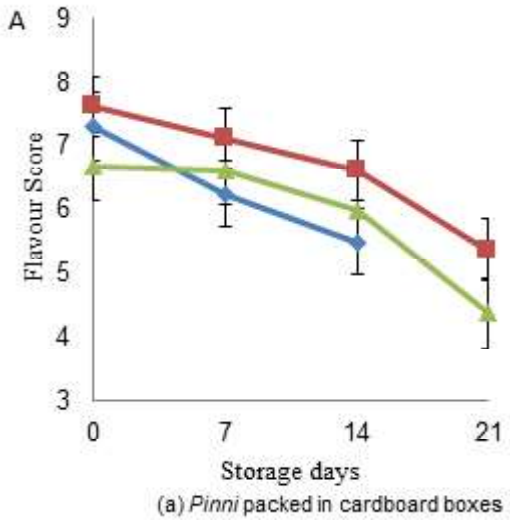
The sensory scores showed a decreasing trend during storage in all samples of *pinni* irrespective of packaging techniques (Fig. 1). Significant ($p<0.05$) effect of addition of cardamom and rosemary was noticed on flavour score of *pinni* in both the packaging materials at 30°C. Maximum score was recorded in rosemary added and polystyrene tray packed samples since PS has better moisture and oxygen barrier properties. In present study *pinni* was criticized for lack of freshness at the end of storage period of 28 days. Similar observations with respect to flavour degradation were reported for *doda burfi* by Chawla et al. 2015b. During storage of *pinni* in cardboard boxes and polystyrene trays at 30±1ÚC significant ($p<0.05$) decrease in body and texture score was observed. (Fig. 1). The stored samples progressively became brittle and showed decrease in cohesiveness. Londhe et al. (2012) reported rapid reduction in body texture score for control samples of brown *peda* at 30ÚC as compared to vacuum packed samples which were acceptable upto 40 days. Decreasing trend in colour and appearance score was observed in case of control, cardamom and rosemary extract added *pinni* samples respectively (Fig. 1). Different treatments showed significant ($p<0.05$) effect on colour and appearance score of *pinni* in both the packaging materials at any particular storage interval. Probable reason for reduction in colour and appearance score could due to change in surface appearance of *pinni* caused by loss of moisture from surface and darkening of samples with progress of storage period. Decrease in colour scores was also observed during storage of brown *peda* by Londhe et al. (2012. During storage of *pinni* in cardboard boxes as well as in PS trays at 30±1°C significant ($p<0.05$) decrease in sweetness score was recorded except in rosemary added samples in both the packaging material. Addition of rosemary extract and cardamom had significant ($p<0.05$) effect on overall acceptability score of *pinni* samples packed in both the packaging materials. The reduction in overall acceptability score of *pinni* was possibly due to formation of stale flavour, and increased brittleness with storage. Controlled samples stored at 30°C were found to have mould growth after 14 days of storage in both packaging materials which resulted in reduced acceptability. However, cardamom added samples were found to have mould growth after 21 days of storage in both packaging materials and rosemary added *pinni* samples have shelf life 21 days in cardboard boxes and 28 days in PS trays that results indicated antioxidant effects of rosemary. Despite decreasing trend, the samples were found to score well over the minimum acceptable limit (*i.e.*, a score of 7). The overall acceptability of *doda burfi* was reported to decrease and caused dark colour formation with storage (Chawla et al. 2015b). These results are in agreement with the finding of Prabha (2006) and Londhe (2006). These changes in sensory scores during storage are reflection of the predominant flavour reduction of *pinni*, with increased staleness as storage preceded, and followed by deteriorating colour and appearance and body and texture due to drying, dark colour formation and brittle texture development of the product.

Textural changes of *pinni* during storage

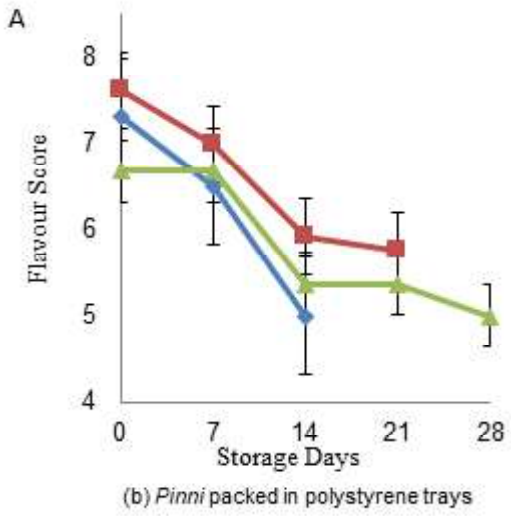
The changes in textural quality of *pinni* in terms of texture profile analysis (TPA) during storage packaged under different packaging conditions are given in Fig. 2. In the present study, a gradual increase in hardness in all *pinni* samples irrespective of the packaging material was observed, which could be attributed to the decrease in moisture content in the product (data not reported) This result was in accordance with the earlier findings of Saxena et al. (1996) who reported that increase in hardness of *pinni* with increase of storage period. While some worker likes Jha et al. (2014b), Gupta et al. (1990) and Suresh et al. (1994), found that increase of hardness of *khoa* correlates with the increase in the total solid content and that the moisture content of *peda* had direct relationship with hardness of the product.

Fracturability of fresh *pinni* decreased significantly ($p<0.05$) in both packaging materials. In general, as the moisture content increased (data not reported) fracturability of *pinni* indicating that the samples having higher moisture content was softer, hence broke on application of lower amount of force. Springiness refers to a food's ability to return to its original form after compression. It is the height that the sample recovers between the first and second compression. Cohesiveness is the ratio of area under the second bite curve before reversal of compression to that under the first bite curve. It is the measure of the extent to which the food structure is disrupted during the first compression (Bourne, 2002). Cohesiveness value of fresh *pinni* decreased non-significantly ($p>0.05$) in both packaging material (Fig. 2).

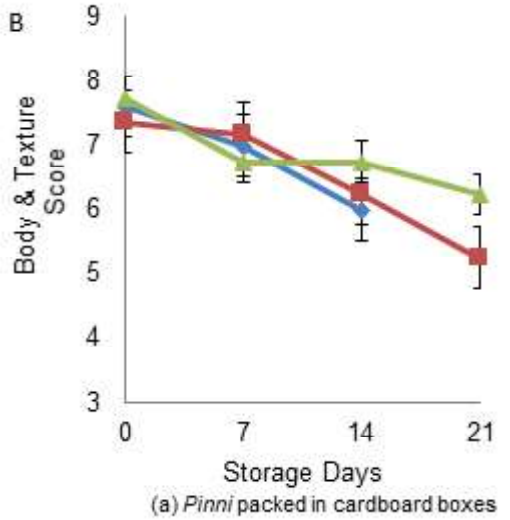
Gumminess is related to the primary parameters such as hardness and cohesiveness. Gumminess of freshly prepared *pinni* increased non-significantly ($p>0.05$) at the end of storage period in both packaging materials. Gumminess of *pinni* showed a trend similar to one noticed for hardness and cohesiveness the former being a secondary parameter based on hardness. Gumminess of brown *peda* (12.56 N) increased to 20.51N after 40 days in vacuum packaging (Londhe et al.2012). Chewiness refers to the energy required to masticate food into a state ready for swallowing and is the product of gumminess and springiness. Chewiness value of *pinni* increased significantly ($p<0.05$) in both packaging materials. Chewiness of fresh cow, buffalo and mixed milk *rasogolla* was reported to be 28.70, 73.70 and 34.50 N by Adhikari



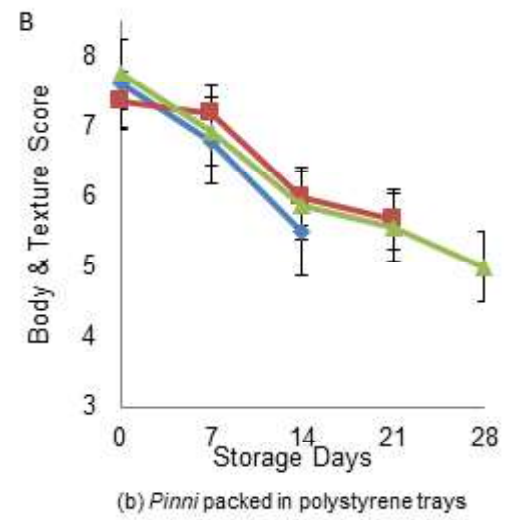
(a) *Pinni* packed in cardboard boxes



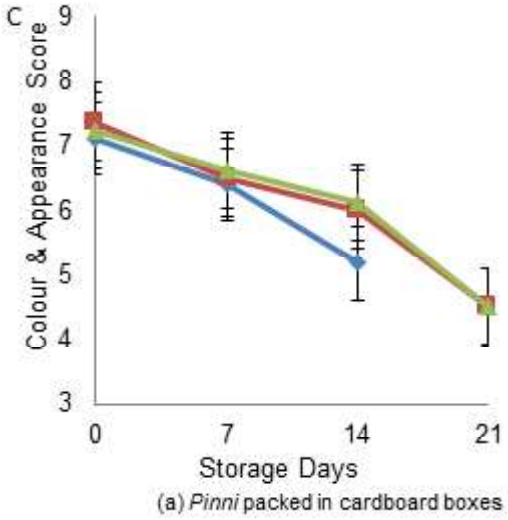
(b) *Pinni* packed in polystyrene trays



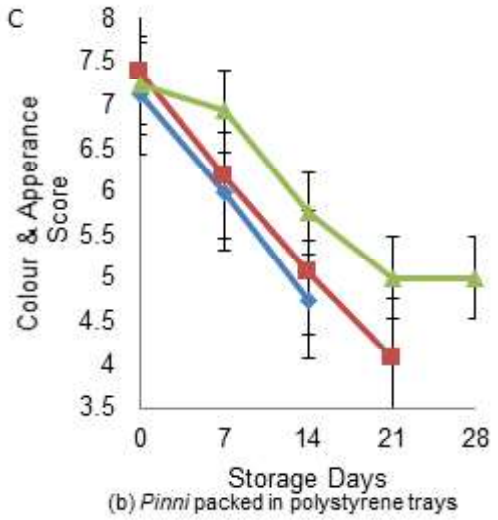
(a) *Pinni* packed in cardboard boxes



(b) *Pinni* packed in polystyrene trays



(a) *Pinni* packed in cardboard boxes



(b) *Pinni* packed in polystyrene trays

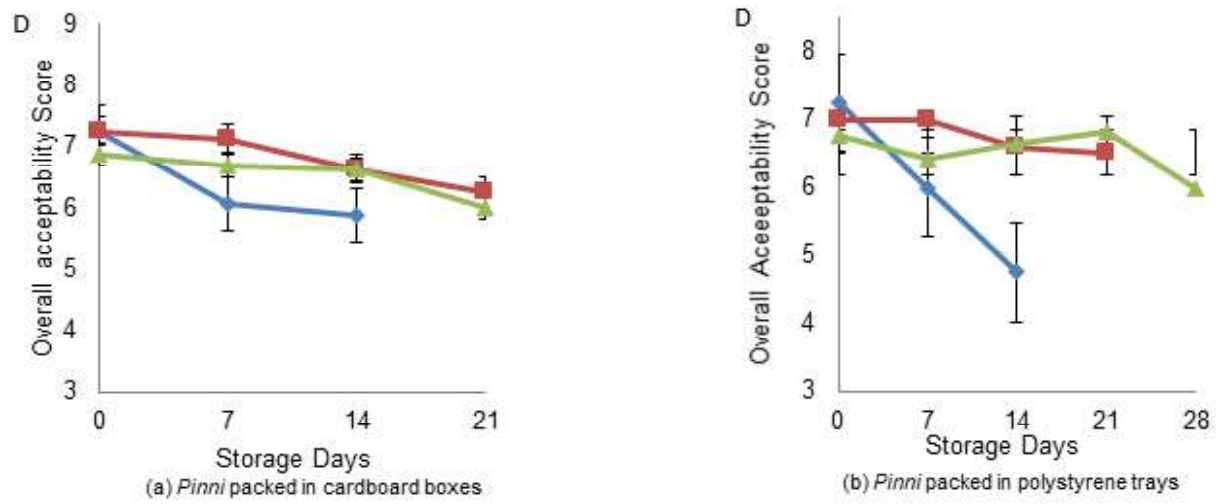
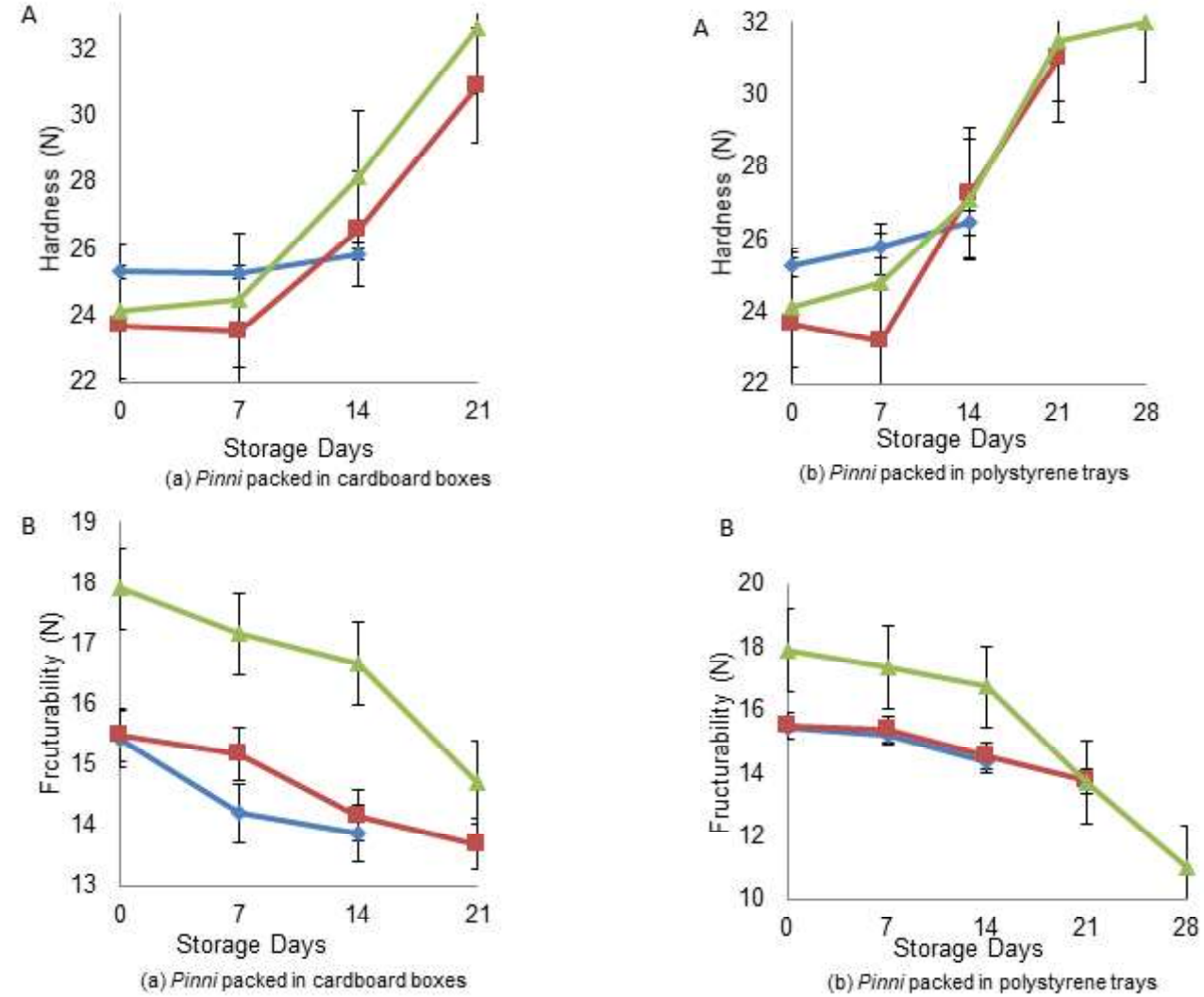


Fig. 1 Effect of natural preservatives and packaging techniques on sensory attributes of *pinni* during storage at 30±1ÚC (A: Flavour, B: Body & Texture Score, C: Colour & Appearance Score and D: Overall acceptability)
(Control sample, Cardamom added and Rosemary added *pinni* sample



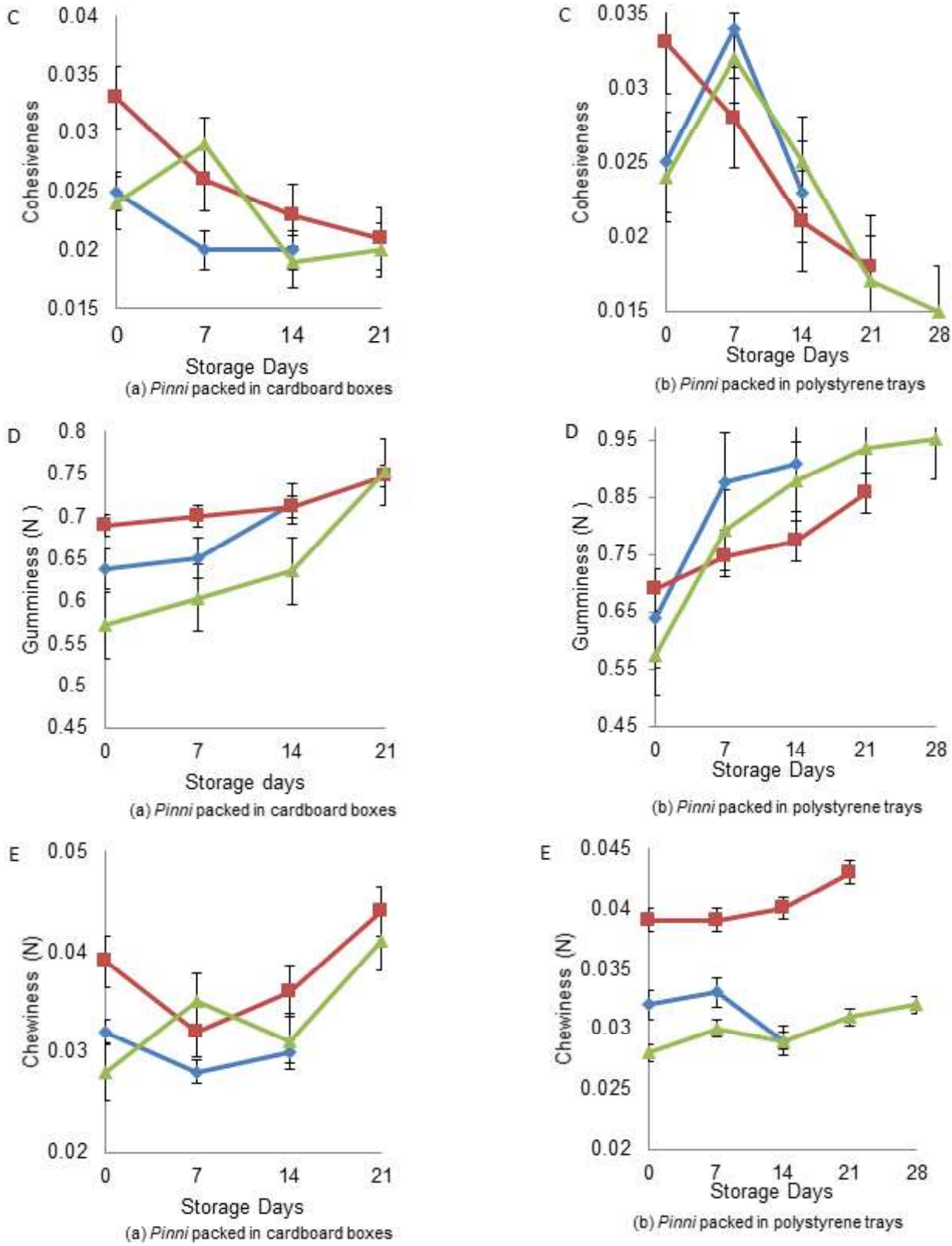


Fig. 2 Effect of natural preservatives and packaging techniques on textural parameters of *pinni* during storage at 30±1°C (A: Hardness; B: Frcuturability; C: Cohesiveness D: Gumminess; E: Chewiness) (◆Control sample, ■Cardamom added and ▲ Rosemary added *pinni* sample)

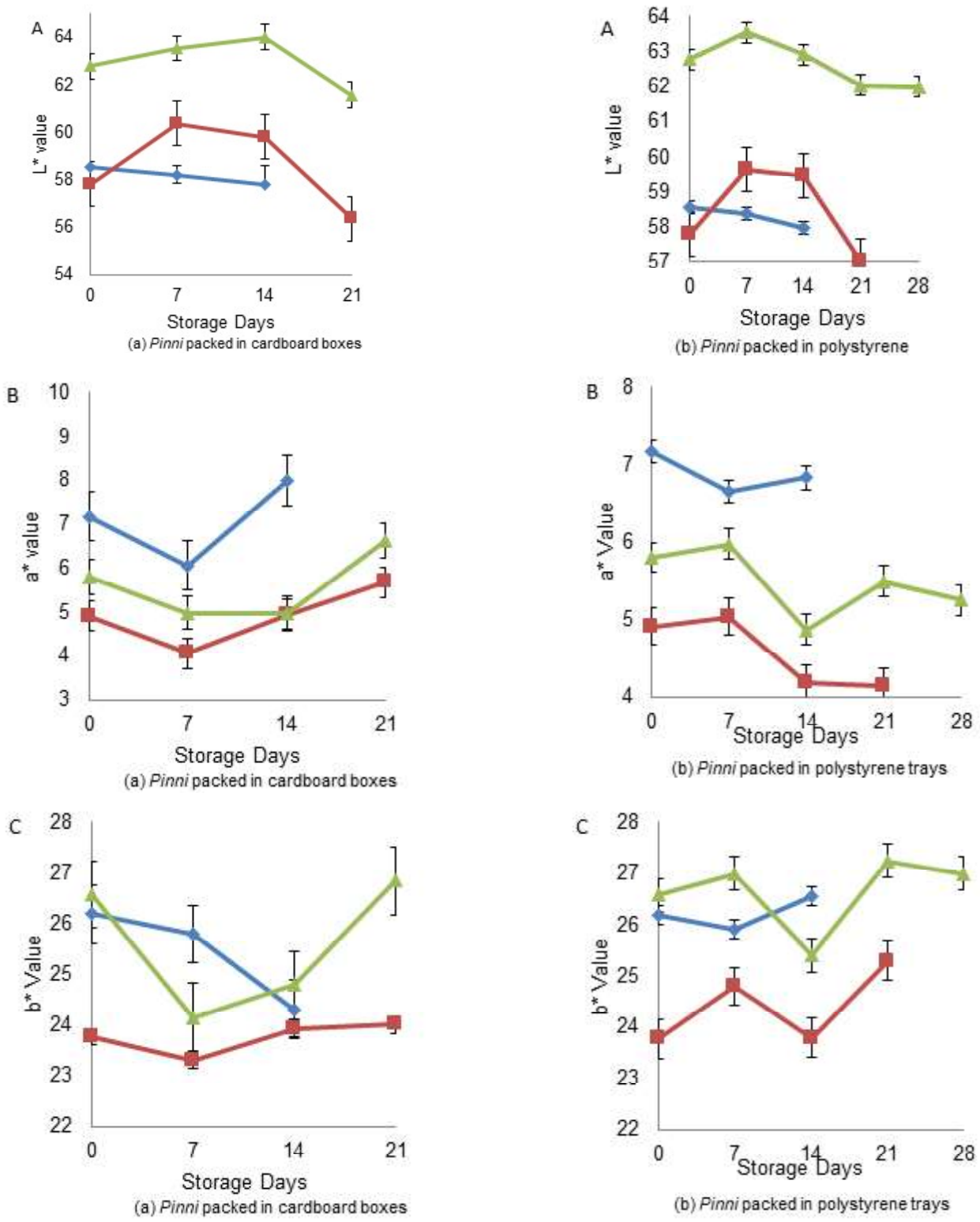


Fig. 3 Effect of natural preservatives and packaging techniques on colour attributes of *pinni* during storage at 30±1°C (A: L* value; B: a* value; C: b* value) (◆Control sample, ■ Cardamom added and ▲ Rosemary added *pinni* sample)

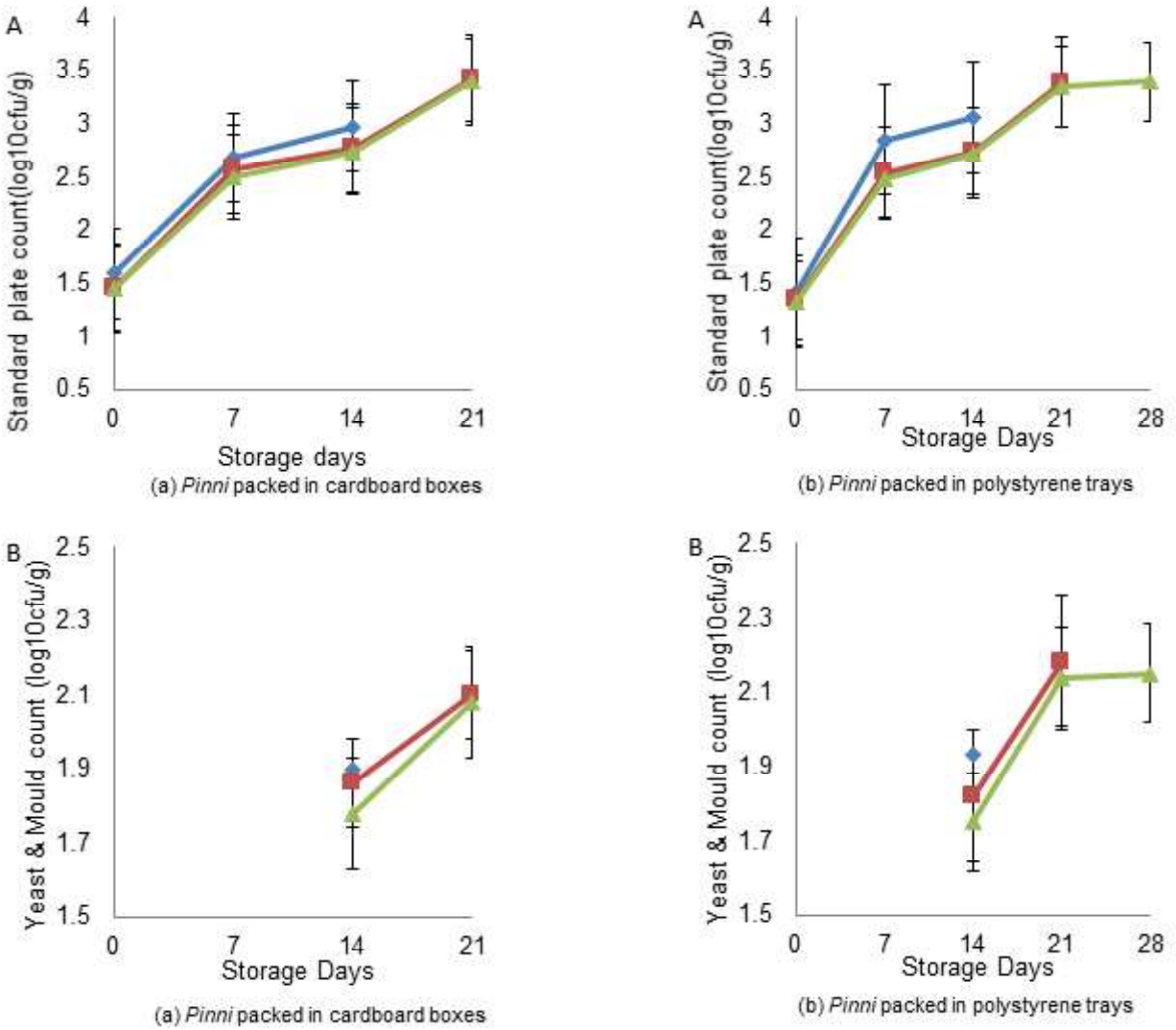


Fig. 4 Effect of natural preservatives and packaging techniques on microbiological parameters of *pinni* during storage at 30±1°C (A: Total standard plate count; B: Total yeast mould count) (◆Control sample, ■Cardamom added and ▲ Rosemary added *pinni* sample)

et al. (1992a). Khamrui and Solanki (2010) reported increase in chewiness of *sandesh* during storage. As because cardamom and rosemary extract added in ppm levels their addition had no effect on any of the textural attributes of *pinni* in both the packaging materials.

Changes in colour attributes of *pinni* during storage

Colour of any food product assumes a great significance from consumers' acceptance point of view. Changes in L* value of *pinni* packed in cardboard boxes and PS trays store at 30±1°C is presented in Fig. 3. The average L* value of control and cardamom added *pinni* samples decreased significantly ($p<0.05$) in cardboard boxes after 21 days and in rosemary added *pinni* samples PS tray packed samples after 28 days. Gothwal and Shukla (1995) reported the effect of refined wheat flour and sugar on the browning of *khoa*-based sweets such as *burfi* and *kalakand*. The average a* value of rosemary added *pinni* samples increased significantly ($p<0.05$) in cardboard boxes after 21 days and significantly ($p<0.05$) decreased after 28 days when *pinni* packed in PS trays Prasad et al. (2017b) also observed increased redness value of control and herbal *burfi* during storage at 37±1ÚC and 4±1°C when packed in cardboard and high-density polyethylene (HDPE) boxes. Samples packaged in PS trays had significant ($p<0.05$) increase in b* value after 28 days of storage in case of rosemary added *pinni* samples and 21 days in case of cardamom added *pinni* samples. Londhe et al. (2012) reported increase in b* value of *peda* when packaged in cardboard boxes and found decrease in the b* value of samples packed in vacuum packed 5-layer nylon from initial value of 34.28 to 33.48 after 40 days of storage.

Microbiological changes in *pinni* during storage

The microbiological changes in *pinni* packaged under different packaging conditions during storage are given in Fig. 4. During storage all the samples showed an increasing trend in standard plate count in both the packaging materials. Total plate counts were significantly higher in control product than cardamom and rosemary added *pinni* samples indicating preservation effect of cardamom and rosemary extract. Increase in SPC of *burfi* and brown *peda* during storage had been reported by earlier workers (Reddy, 1992; Londhe 2006). However, Kumar and Srinivasan, (2010) did not observe any microbial growth in *peda* packed under MAP and with oxygen scavengers. Yeasts and moulds in fresh *pinni* were not detected during initial period of storage however it significantly ($p<0.05$) increased controlled *pinni* samples after 14 days of stored at 30±1ÚC in both packaging materials (Fig 4). Lower extent of increase in yeast and mould count in PS trays was probably due to higher barrier properties of polystyrene tray as compared to cardboard boxes. Results are in agreement with the earlier finding of Palit and Pal (2005) and Rajarajan and Lango, (2006). Germicidal effect of cardamom has also been reported by Gaikwad and Hembade (2012) in *Ujani Basundi*.

Conclusions

The present study attempted for enhancement of shelf life of *pinni* by adding two natural preservatives *viz.,* cardamom powder and rosemary extrcat and by using different packaging interventions. It was observed that control samples of *pinni* were organoleptically unacceptable after 14 days in both the packaging materials when stored at 30°C. However, cardamom powder added *pinni* samples have a shelf life 21 days when packed in cardboard boxes as well as when packed PS trays. Total plate counts and yeast and mould counts were significantly higher in control sample than cardamom and rosemary extract added *pinni* samples indicating preservation effect of cardamom and rosemary extract. *Pinni* could be best preserved up to 28 days at room temperature (30±1ÚC) without much quality loss when packed in polystyrene trays and added with rosemary extract.

Acknowledgments

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RESEARCH ARTICLE

Physico-chemical analysis of control and optimized 'Banana Enriched Ice Cream' during storage period (60 days) at 15 days intervals

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Abstract: Ice cream is a frozen product and the popularity of ice cream and frozen desserts is attributed to their refreshingly cool and delightfully sweet characteristics. The present research work was conducted to study the effect of storage periods on different physico-chemical properties of optimized (banana pulp enriched ice cream) and control ice cream. The storage study was done at 15 days interval up to 60 days. On the basis of findings, it was observed that the overrun, hardness, first dripping time, melting rate, complete melting time and fat were significantly ($P<0.05$) changed over the storage period but total solids, protein and titrable acidity content of optimized and control ice creams did not differ significantly throughout the storage period. The physico-chemical composition of optimized ice cream observed were 36.57% total solids, 8.66 % fat, 4.15 % protein, 0.22% acidity and 48.50% overrun and of control ice cream observed were 36.48% total solids, 9.86% fat, 4.45% protein, 0.22% acidity and 44.69% overrun.

Keywords: Emulsifiers, First dripping time, Hardness, Melting rate, Overrun, Stabilizer

Introduction

Ice Cream means the frozen milk product obtained by freezing a pasteurized mix prepared from milk or other products derived from milk, or both, with or without addition of nutritive sweeteners (e.g. sugar, dextrose, fructose, liquid glucose, dried liquid glucose, maltodextrin, high maltose corn syrup and honey) and other permitted non-dairy ingredients (FSSAI, 2019). It may also contain chocolate, and bakery products (e.g. bread, cake or cookies) as a separate layer and/or coating. It may be frozen hard or to a soft consistency. Ice cream has categorized under 'Dairy based desserts' by Food safety and standard authority of India. The popularity of ice cream and frozen desserts is attributed to their refreshingly cool, delightfully sweet characteristics, besides their nutritive value (Marshall et al. 2003). Now a days, use of some ingredients having nutritional and functional properties such as some fruits, probiotics, alternative sweeteners, dietary fibers, natural antioxidants in ice cream manufacture has increased due to interest of consumers for their healthier life (Erkaya et al. 2012).

In recent years, fruit based ice cream has attracted attention due to presence of different functional properties. Several works on incorporation of fruits in ice cream as an ingredient have been reported but, ice cream prepared with banana pulps have not been done so far. India is world's largest producer of banana. Considering the nutritional aspect, it is the world's leading fruit crop, and in terms of economic value it is the number five agricultural crop in world trade (Aurore et al. 2009). Banana has also been shown to be rich in vitamins A, B (thiamine, riboflavin, niacin, and B_6) and C, and in Mg, P and K when fully mature (Kanazawa and Sakakibara 2000; Aurore 2009). Keeping this view, banana enriched ice cream was prepared. So, the present investigation was planned with an objective to study the effect of storage period (60 days) at -18±1°C on physico-chemical properties of optimized 'Banana Enriched Ice Cream' which can meet the demand of modern health conscious consumers, so that a value-added dairy product could be offered to the consumers as a convenience food.

Materials and Methods

Fresh milk was collected from Dairy farm of Banaras Hindu University; Varanasi was used as the base material for preparing ice cream. The cream (25% milk fat) was purchased from local market and pasteurized at 80 °C for 2 minutes and cooled to 7°C and used as the fat source in the preparation of ice cream mix. Skim milk powder of 'Lord Krishna' brand, manufactured by Markendeshwar foods and allied products (Kurukshetra), purchased from local market of Varanasi was used in the

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preparation of ice cream mix as the source of milk solids not fat (MSNF). Sugar of commercial grade obtained from the local Varanasi market was used as the sweetener. CREMODAN SAMPOORNA (Danisco India Pvt. ltd., Haryana) was used as stabilizer and emulsifier in the preparation of ice cream. It includes Guar gum, Mono & Diglycerides of fatty acids, Sorbitan esters of fatty acids and Carrageenan. Banana pulp which was prepared from banana fruits used for flavoring. The process optimization for manufacturing of Banana enriched ice cream was done by using RSM (Response Surface Methodology) on the basis of sensory score (Saloni et al. 2019).

Testing of ice cream

Overrun was calculated according to the equation [(volume of ice cream)"(volume of mix)/volume of mix × 100] given by Jimenez Florez et al. (1993) using a standard 100 mL cup. First dripping and complete melting times of samples were determined as seconds by Giri et al. (2014). Melting rate was calculated by placing 50 g of ice cream on a wire net (4 pores per 2.5 square inch) which was positioned over a funnel and measuring cylinder. The volume of melted Ice-Cream after a period of 30 minutes was measured. Then the melting rate of ice cream was expressed as ml per 30 min.

Moisture and total solids were determined in milk and ice cream as per AOAC (2000). Fat content of milk was determined by Gerber's method (BIS, 1981), while that of ice cream was determined using Soxhlet apparatus. Total nitrogen content of milk and ice cream was estimated by Kjeldahl method (AOAC, 2000). Percentage of protein was calculated by multiplying total nitrogen content by a factor of 6.38 for milk and ice cream. Total acidity was determined by volumetric-potentiometric titration with 0.1 N NaOH.

Statistical analysis

All experiments were performed in triplicate. Data is expressed as mean value. The means were compared using Duncan's multiple range test (DMRT) at $P < 0.05$.

Results and Discussion

Changes in overrun during storage period

The overrun of optimized ice cream and control sample was 48.50 % and 44.69 %, respectively on day zero. The overrun values of the optimized ice cream and control ice cream was decreased from 48.50 to 46.70% and 44.69 to 43.28% during entire storage period, respectively. The changes in overrun score of Banana Enriched Ice cream against control during storage is shown in the table 1 and Fig. 1.a. The difference between the values of overrun of control and optimized was significant ($P<0.05$). This depletion was may be due to loss of air and moisture from ice cream. These observations are similar to those reported by Guzeler et al. (2012) that explained the overrun values decreased significantly in ice cream during their storage.

Changes in hardness (N) during storage period

The changes in hardness (N) of optimized and control ice cream sample is shown in table 1 and Fig.1.b. The value of hardness of optimized and control sample on day zero was 24.70 and 28.15, respectively and hardness continuously increased during the entire storage period in both cases. The observed score of control and optimized ice cream at 15 days interval showed significant changes ($P<0.05$). Difference observed in hardness could be due to difference in overrun of optimized and control sample. Similar study of Prindiville et al. 1999 and Muse et al. 2004 explained that the lower overrun leads to increase in hardness. They also reported that hardness is also influenced by ice phase volume, ice crystal size, overrun, fat destabilization, and the rheological properties of the mix.

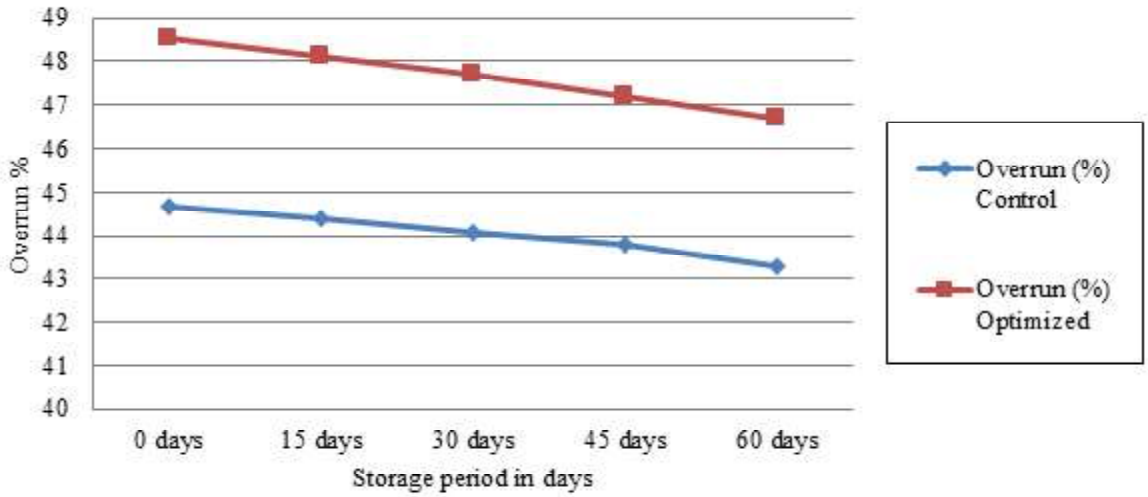
Effect of storage period on first dripping time (S)

The first dripping time for control and optimized was ranged from 474 to 683 and 450 to 610 seconds, respectively during entire storage period. The change in first dripping time of banana enriched ice cream against control during storage period is shown in table 1 and Fig. 1.c. The result shows that the first dripping time of control was higher than the optimized during storage period. It was found that 'Banana enriched ice cream' had the lower dripping time than control ice cream and the difference between sample at 15 days intervals was significantly high ($P<0.05$). The difference in the values could be due to addition of banana pulp extract. Similarly, Guzeler et al. (2011) reported that

Table 1 Physico-chemical analysis of control and optimized 'Banana Enriched Ice Cream' during storage period (60 days) at 15 days intervals

S. No.	Property	Group	0 days	15 days	30 days	45 days	60 days
1.	Overrun (%)	Control	44.69	44.38	44.05	43.80	43.28
		Optimized	48.50	48.10	47.70	47.20	46.70
2.	Hardness (N)	Control	28.15	31.50	32.25	32.20	31.85
		Optimized	24.70	25.75	25.35	26.76	26.85
3.	First dripping Time (S)	Control	474	631	678	680	683
		Optimized	450	593	598	605	610

a.



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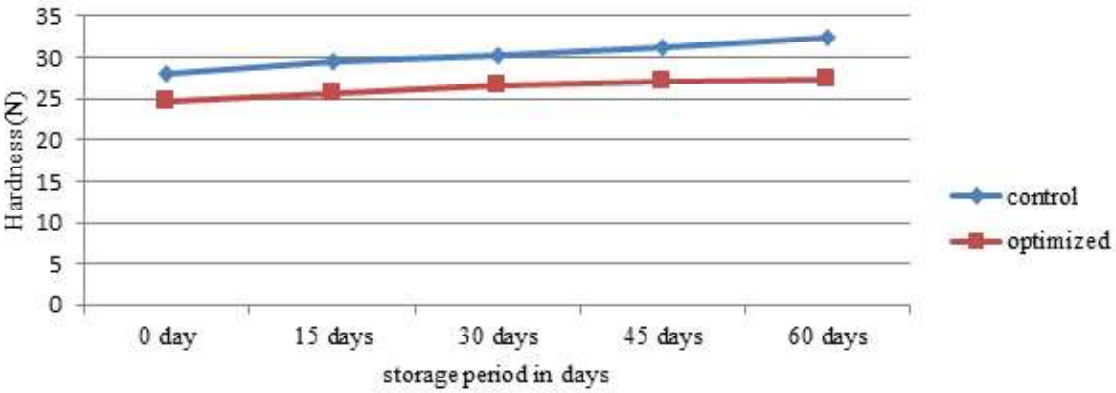

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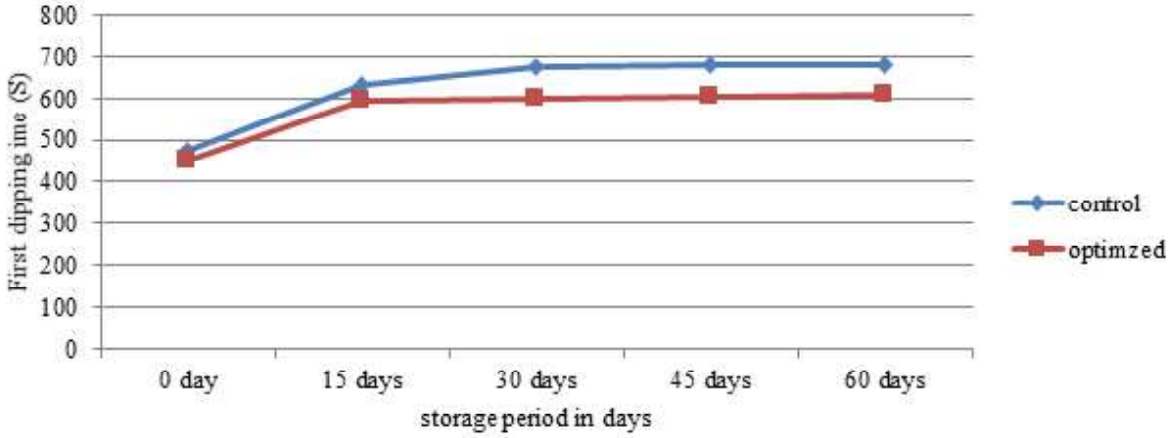


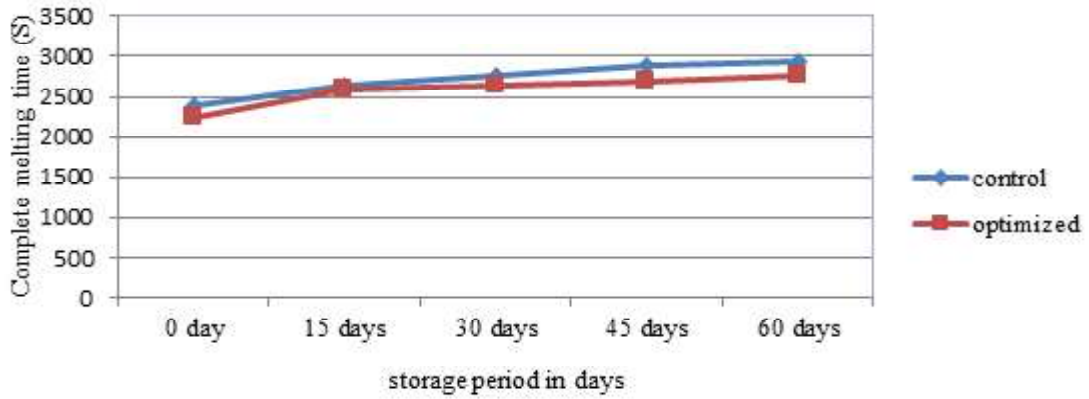
Fig.1 Changes during storage period **(**a. Changes in Overrun b. Changes in hardness (N), c. Effect of storage period on first dripping time (S)

there is significant ($P<0.05$) increase in first dripping time of ice cream during three months of storage period.

Effect of storage period on complete melting time (S)

The complete melting time of control and optimized ice cream was ranged from 2378 to 2945 and 2230 to 2769 seconds, respectively during entire storage period. The complete melting time of optimized against control ice cream is shown in table 2 and Fig. 2.a. The table 2 and Fig. 2.a. clearly depicts that the complete melting time of control sample was significantly high than the optimized one during the entire storage period. The lower value of complete melting time of banana enriched ice cream compared with control ice cream could be due to addition of banana fruit pulp. Similar observation was reported by Erkaya et

a.



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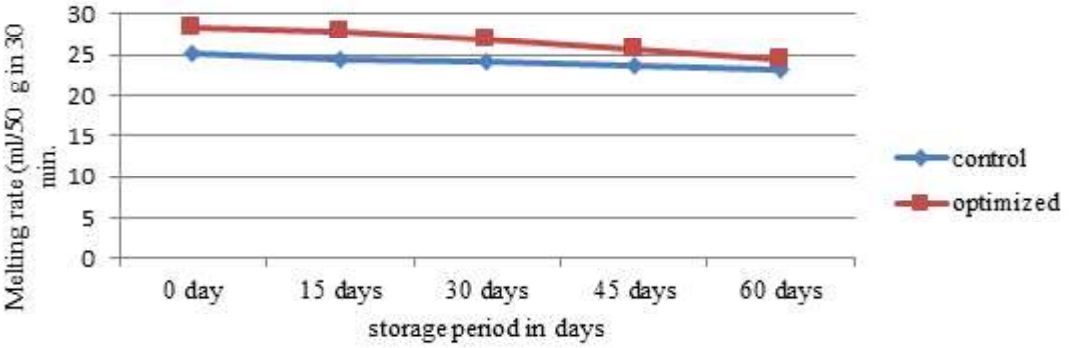

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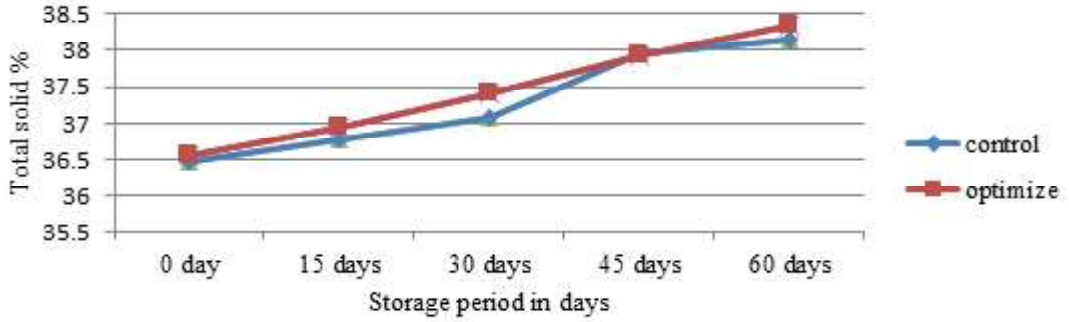

Fig. 2 Effect of storage period (a. on complete melting time (S) b. on melting rate (ml/50 gm) c. on total solid content (%))

Table 2 Physico-chemical analysis of control and optimized 'Banana Enriched Ice Cream' during storage period (60 days) at 15 days intervals

S. No.	Property	Group	0 days	15 days	30 days	45 days	60 days
1.	Complete meltingTime (S)	Control	2378	2629	2750	2891	2945
		Optimized	2230	2584	2645	2692	2769
2.	Melting rate (ml/50 g in 30 min.)	Control	25.13	24.44	24.10	23.65	23.13
		Optimized	28.36	27.89	26.77	25.59	24.41
3.	Total solids (%)	Control	36.48	36.78	37.09	37.96	38.16
		Optimized	36.57	36.93	37.40	37.94	38.35

al. (2012) where they observed that the ice cream with 15% Cape gooseberry had the longer complete melting time (4515 S) as compared to the control sample (4005 S).

Effect of storage period on melting rate (ml/50 gm)

The average melting rate for control and optimized ice cream was decreased from 25.13 to 23.13 ml and 28.36 to 24.41 ml, respectively during entire storage period. The melting rate of control and optimized ice cream during storage period is shown

a.

b.

c.

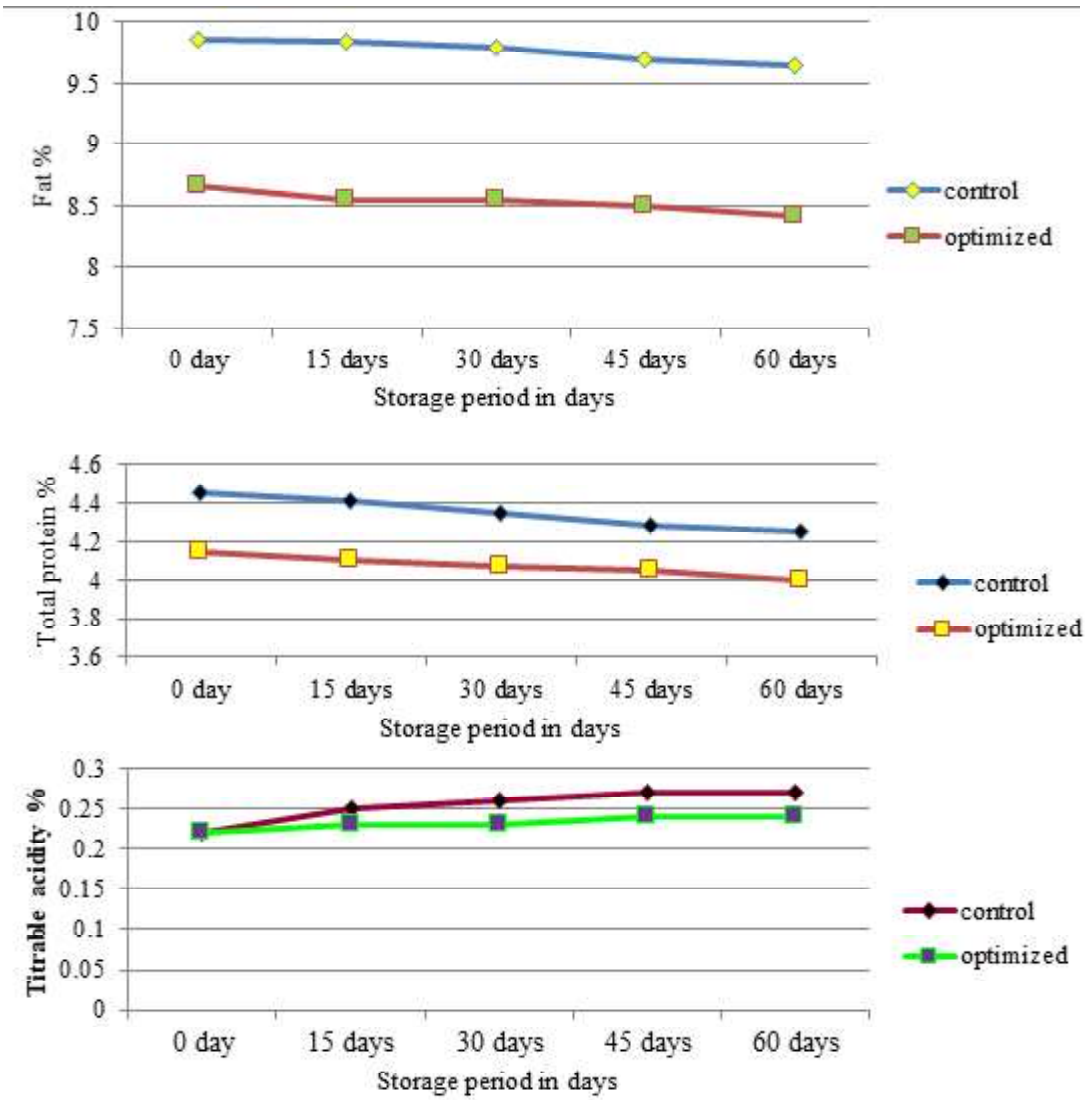

Fig. 3 Effect of storage period on (a. fat% b. titrable acidity (% Lactic acid) c. total protein %

in table 2 and Fig. 2.b. The result shows that the difference between melting rate of optimized ice cream and control was significant ($P<0.05$) up to 60 days of storage. The difference in the values could be due to addition of banana pulp. Melting rate decreased with progress in storage period because it is influenced by its composition and amount of air incorporated in it. Similar study of Singh et al. (2014) showed that melting rate decreased with progress in the storage period. The per cent decrease in melting rate ranged from 2.32 to 9.12 per cent being lowest in plain cookie and highest in chocolate cake ice cream.

Effect of storage period on total solid content (%) in control and optimized ice cream

The total solid content of optimized and control ice cream sample at zero day was 36.57% and 36.48%, respectively and it was increased from 36.57% to 38.35% and 36.48% to 38.16%, respectively during the entire storage period. The optimized ice cream analyzed for total solid against control sample are shown in table 2 and Fig.2.c The changes between total solids content of optimized ice cream and control ice cream was not significant ($P>0.05$). Similar results were observed by Guven et al. (2002) where they reported the positive relation between the total solid content and fruit concentration of ice cream.

Effect of storage period on fat%

The average fat percent for control and optimized ice cream on day zero was 9.86% and 8.66%, respectively and it was varied from 9.86% to 9.65% and 8.66% to 8.42% in control and optimized ice cream sample, respectively during entire storage period. The effects of storage period on fat per cent of 'Banana enriched ice cream' against control sample are shown in table 3 and Fig. 3.a. It was observed that control sample has more fat% as compared to

Table 3 Physico-chemical analysis of control and optimized 'Banana Enriched Ice Cream' during storage period (60 days) at 15 days intervals

S. No.	Property	Group	0 days	15 days	30 days	45 days	60 days
1.	Fat (%)	Control	9.86	9.84	9.79	9.70	9.65
		Optimized	8.66	8.55	8.54	8.50	8.42
2.	Total Protein (%)	Control	4.45	4.41	4.35	4.28	4.25
		Optimized	4.15	4.10	4.07	4.05	4.00
3.	Titrable acidity (%)	Control	0.22	0.25	0.26	0.27	0.27
		Optimized	0.22	0.23	0.23	0.24	0.24

optimized product but the changes within the fat percent of optimized and control sample separately was not significant ($P>0.05$) from 0 to 60 days while the level of fat % between control and optimized sample varied significantly from 0 day to 60 days ($P<0.05$) with the progression of storage period. It might be due to low fat content in fruit pulp than the milk powder.

Effect of storage on total protein %

The average milk protein percent for control and optimized ice cream was varied from 4.45% to 4.25% and 4.15% to 4.00% during entire storage period. The effect of storage period on milk protein % of optimized ice cream against control sample is shown in table 3 and Fig. 3.b. The result shows that the total protein content of optimized and control on day zero was 4.15% and 4.45 %, respectively. It clearly indicates that total protein content was higher in control than optimized group ice cream group but the differences within control and optimized ice cream at 15 days interval was not significant ($P>0.05$). While optimized ice cream had lower protein content than control, it might be due to addition of more amount of SMP in control ice cream. Since protein content of SMP is more as compared to banana pulp. It was also observed that change in protein content in ice cream samples was greatly influenced by incorporation of fruit pulps. Protein content in ice cream samples decreased with the increase of pulp content. Low protein content in fruit pulp than raw milk might be major cause of low protein content in fruit pulp incorporated ice cream. Similar study of Roy et al. (2015) explained that significant difference ($p<0.05$) in protein content were found in control sample of yoghurt as compared to the banana pulp incorporated yoghurt.

Effect of storage period on titrable acidity (%Lactic acid)

The titrable acidity of optimized and control ice cream were analyzed at an interval of 15 days storage. It was observed that the titrable acidity for control and optimized on day zero was same i.e, 0.22%. The changes in titrable acidity of 'Banana enriched ice cream' against control during entire storage period were mentioned in table 3 and Fig.3.c. It clearly depicts that the difference between control and optimized was not significant ($P>0.05$) till 60 days. The result also shows that the titrable acidity was found higher in control than optimized up to 60 days storage. It might be due to addition of banana pulp results in decrease in acidity, especially at higher levels of banana pulp addition.

Conclusions

Storage conditions were known to bring about some physico-chemical changes in frozen foods. The present investigation was carried out to examine the effect of storage period on the physico-chemical quality of ice cream by keeping it for 60 days storage periods at 15 days intervals. Under observations, it was found that the physico-chemical property that showed significant changes were the overrun, hardness, fat, first dripping time, melting rate and complete melting time. But total solids, protein and titrable acidity content of these ice creams did not differ significantly from each other. The composition of optimized showed 36.57% total solids, 8.66 % fat, 4.15 % protein, 0.22% acidity and 48.50% overrun. So, addition of banana pulp may increase the functional value of ice cream but animal or human trials need to be conducted to evaluate its effect on the health. Apart from this, consumer acceptability can be evaluated for the commercialization of the product.

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RESEARCH ARTICLE

Selection of stabilizers and processing aids to encapsulate bitter gourd extract in a stable double emulsion

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Abstract: The aim of the present investigation was to assess the effect of three formulation variables and their levels on the stability of bitter gourd encapsulated double emulsion using a 3^3 full factorial design. The ingredients used were i) NaCl (3-5%, w/w), ii) polyglycerol polyricinoleate (2-4%, w/w) and iii) protein:polysaccharide complexes (sodium caseinate-β-pectin / WPC-80-β-pectin, 1.5-4.5%, w/w). The dependent variables were apparent viscosity, zeta potential, sedimentation stability and turbidity. The optimized formulation consisting of bitter gourd extract (55.2%, w/w), 3% NaCl, 4% PGPR and 4.5% sodium caseinate-pectin as complex had higher apparent viscosity (2060±17.32 cP), zeta potential (-30.33±0.18 mV), lower turbidity (1.18±0.03 cm^{-1}), and was stable for 29 days at 37°C, when compared to other combinations ($p< 0.001$). The study resulted in an optimized emulsion containing bitter gourd extract as a potential delivery vehicle, to incorporate the antidiabetic herbal component in functional foods.

Keywords: Dairy protein-polysaccharide complex; NaCl; PGPR; Bitter gourd extract; Stability

Introduction

Over the past few decades, with increased awareness and interest among consumers in health-enhancing bioactive food components, people have shifted to better alternatives such as functional foods over medicine. The quality and quantity of different dietary components are determining factors for overall health. Dietary habits and life style are associated with various metabolic diseases such as diabetes mellitus. Bitter gourd is fast gaining attention as a potential management for diabetes, hence increasing its use in foods and as a dietary supplement (Wu and Ng 2008; Habicht et al. 2011). Incorporating bitter gourd extract in foods could be a convenient way to combat diabetes mellitus. Utilization of bitter gourd extract needs a medium by which its sensorial aspects can be made acceptable or masked. Double emulsion is one such technique which is used to mask the unacceptable flavor and odor of plant extracts by compartmentalizing it in inner phase, which is then protected by layer of another immiscible liquid. $W_1/O/W_2$ emulsions can replace conventional O/W emulsions, not only by reducing the oil content, but also having similar sensorial perception in mouth (Jiménez-Colmenero 2013; Oppermann et al. 2016). In addition, by way of encapsulation, they can protect heat- and light-sensitive ingredients such as flavor compounds, vitamins and bioactive compounds and facilitate their controlled release within food (Pawlik and Norton 2014).

Instability is the key issue in double emulsion which limit their applicability as food matrices. The type and concentration of surfactants, electrolytes, the nature of encapsulants, ratio of dispersed phase to continuous phase, osmotic balance between inner and outer phase, various processing regimes such as formulation methods, shear applied, temperature of operation and conditions, final desired droplet size and storage conditions are the predominating factors which affect the stability of the double emulsion (Shima et al. 2004). Instability can be reduced by balancing the osmotic pressure between the two aqueous phases (Sameh et al. 2012) and by using protein-polysaccharide electrostatic complexes (Salminen and Weiss 2014) as secondary surfactant. Two different types of surfactants are usually required to stabilize a double emulsion ($W_1/O/W_2$) i.e. primary emulsion surfactant for the inner aqueous droplets and secondary emulsion surfactant for intermediate oil phase droplets. Polyglycerol polyricinoleate (PGPR) is used as an oil-soluble surfactant, which prevents coalescence of newly formed water droplets by the formation of tiny water droplets in the oil phase and facilitates droplet break-up by reducing the interfacial tension (Landfester

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2006; Bahtz et al. 2016). Salt (NaCl) is an electrolyte that generates the osmotic pressure to counterbalance the Laplace pressure and stabilizes the W/O emulsions against Ostwald ripening (diffusional ripening) (Landfester 2006; Capek 2010; Colman et al. 2014). Therefore, the addition of electrolytes along with appropriate hydrophobic emulsifiers and hydrophilic emulsifiers is essential to prepare coalescence-stable $W_1/O/W_2$ double emulsion.

Biopolymers which includes proteins and polysaccharides may improve the stability of double emulsions by increasing the viscosity of inner and outer water phase of the double emulsion (Surh et al. 2007). Thus, change of particle size is controlled through formation of interfacial layer surrounding droplets (Lutz et al. 2009b; Sameh et al. 2012) and controlling osmotic pressure (Pawlik et al. 2010). Proteins are less effective emulsifiers near their iso-electric pH, which limits their application in acidic foods. Hence, electrostatic protein-polysaccharide complexes gave better functional properties: emulsifying, stability, encapsulation efficiency, texture/rheology, surface hydrophobicity and mouth feel, than that of the proteins and polysaccharides alone even near the iso-electric pH of the protein (Li et al. 2012; Hernández-Marín et al. 2013; Klein et al. 2013; Salminen and Weiss 2014). However, the stability of the emulsions stabilized with protein-polysaccharide complexes is influenced by several factors such as protein-to-polysaccharide molar mass ratio, pH, ionic strength, temperature and processing methods of the complexes (Lutz et al. 2009a,b).

The overall objective of the current investigation was to assess the effect of PGPR, the hydrophobic emulsifier as primary surfactant, NaCl as an electrolyte and dairy protein-polysaccharide complexes as secondary surfactant on stability of the bitter gourd extract encapsulated double emulsion ($W_1/O/W_2$). The formulated double emulsion was characterized for its physicochemical properties *viz*., apparent viscosity, zeta potential, sedimentation stability (by visual appearance) and turbidity.

Materials and Methods

Materials

PGPR Grinsted® PGPR-90, β-pectin [GENU® β-pectin with ~ 18% degree of acetylation and > 50% degree of esterification (DE)] were supplied free of cost by M/s DuPont Danisco India Pvt. Ltd. (Gurgaon, India) and CP Kelco, Huber India Company (Mumbai, India), respectively. Dairy proteins such as whey protein concentrate-80 (WPC-80) with 79.86% protein and 8.09% lactose and sodium caseinate (SC) with 88% protein were purchased from M/s Mahaan Proteins Ltd. (New Delhi, India). Aqueous extract of bitter gourd consisting 5.95% w/w total solids (TS) and 5% bioactive compound (Charantin) was purchased from M/s Ambe Phytoextracts Pvt. Ltd. (New Delhi, India). Rice bran oil, vinegar (4% acetic acid) and sodium chloride (NaCl) were purchased from local market of Karnal (India). Reverse osmosis (RO) water was used as an aqueous phase.

Experimental factorial design

The double emulsion ($W_1/O/W_2$) composed varied concentrations of NaCl in inner aqueous phase (W_1) containing bitter gourd extract (55.2%), PGPR in middle oil phase (O) and dairy protein-polysaccharide (WPC-pectin, SC-pectin) complexes (WPC-P/SC-P, 1:2) in outer aqueous phase (W_2). All the variables are presented in percentage (%), w/w and their influence on the apparent viscosity, sedimentation stability (at 7 and 37°C) by visual observations, zeta potential and turbidity of the functional mayonnaise were evaluated. The experimental design consisted of 54 formulations (27 each for the two dairy protein-polysaccharide complexes) as shown in Table 1. A full factorial design approach was adopted to study the influence of the variables and their interaction on the properties of the double emulsion to optimize the double emulsion.

Preparation of dairy protein-polysaccharide complexes

The formulation of protein-polysaccharide complexes was done according to Salminen and Weiss (2014) with slight modifications. Dairy protein (WPC-80 and SC) and polysaccharide (β-Pectin -P) were used in the ratios 1:2 (Kumar et al. 2020). The protein and polysaccharide were weighed into separate beakers for solubilization with RO water according to Table 1. Protein solutions were prepared by magnetic stirring constantly at ambient temperature for 1-2 h, whereas polysaccharide solutions were prepared by magnetic stirring for 6-10 h at moderate speed to ensure complete dissolution. Protein and polysaccharide solutions were initially adjusted to pH 7.0 using 1.0 N NaOH before further mixing. Then, they were mixed together and pH was reduced up to 4.0 using 4% acetic acid (vinegar) and stored at ambient temperature for overnight.

Preparation of double emulsion ($W_1/O/W_2$)

Inner aqueous phase (W_1) was prepared with an aqueous soluble bitter gourd extract, (55.2%, w/w) and varying levels of NaCl (3.0-5.0%) in RO water. Middle oil phase (O) consisted of rice bran oil containing PGPR as hydrophobic emulsifier (2-4%, w/w). Outer aqueous phase (W_2) was added with either WPC-P or SC-P prepared as explained in the previous section. Both W_1 and O were pasteurized at 72°C for 15 sec, while outer aqueous phase heated at 85°C for 20 min to prepare biopolymer particles because of thermal denaturation and aggregation of the proteins followed by cooling to 4-7°C in ice water bath.

A primary water-in-oil (W_1/O) emulsion was premixed by mixing the inner aqueous phase (W_1) (30%, w/w) with the oil phase (O) (70%, w/w) at room temperature, using a magnetic stirrer at moderate speed for 5 min. The mixture was then homogenized using an Ultra-Turrax [IKA Ultra-Turrax T25 (IKA®) India Pvt.

Ltd., Bangalore, India] operating at 22,000 rpm for 5 min to form primary W_1/O emulsion. The primary (W_1/O) emulsion (30%, w/w) was gradually added to the outer aqueous phase (W_2) (70%, w/w) and mixed with magnetic stirring at moderate speed for 5 min. The pre-mix was finally homogenized using Ultra-Turrax at 15,000 rpm for 5 min to produce final double emulsion ($W_1/O/W_2$) (9:21:70::$W_1/O/W_2$).

Apparent viscosity

A controlled stress rheometer (RHEOPLUS/32 (M/s Anton Paar, GmbH, Ostfildern, Germany), using a cone and plate geometry (CP-75, D=0.149mm) was used for the measurement of apparent viscosity (cP) in the shear rate range 0.01-100 s^{-1} at 25°C (Lupi et al. 2011).

Zeta potential

The zeta potential of the double emulsions were measured using Zetasizer Nano-ZS90 (Malvern Instrument Ltd., Malvern, Worcestershire, UK) to determine the stability of the emulsion. The freshly prepared emulsions were diluted 100 times with RO water and the experiment was carried out at 25°C temperature. Zeta potential measurements were carried out in triplicate for each emulsion and the results were expressed in mV.

Turbidity

The turbidity of the double emulsions were determined in triplicates according to Pearce and Kinsella (1978). Aliquots (1 mL) of the emulsion were diluted serially with water and sodium dodecyl sulfate (SDS) solution to give final dilutions in the range 1/1000 to 1/5000 and an SDS concentration of 0.170%. The absorbance of the diluted emulsion was then determined in a l-cm path length cuvette at a wavelength of 500 nm in a spectrophotometer (M/s Thermofisher Scientific Inc., Rochester, New York, USA). Identical cuvettes were used for all samples and rinsed with a jet of distilled water between determinations. Absorbance of triplicate aliquots of each emulsion was measured in each case.

$$T = \frac{2.303A}{l}$$

where A is the observed absorbance and l is the path length of the cuvette.

Sedimentation stability

The sedimentation stability (by visual appearance) of the double emulsion was determined in triplicates according to Sapei et al (2012). Freshly made double emulsions were poured into 40 mL glass vials (internal diameter = 21 mm; length = 90 cm; Corning India, Gurgaon, India) to a height of 4 6 cm and stored at 37°C. The emulsion was observed daily until the phase separation occurred.

Statistical data analysis

The data obtained were analyzed by two-way analysis of variance (ANOVA) using SPSS (IBM SPSS Statistics 21) software. Significant difference between variables and attributes (apparent viscosity, zeta potential, sedimentation stability and turbidity measurements) of the functional mayonnaise was determined at $p< 0.001$. Two-way ANOVA results table of 3^3 full factorial designs are shown in Table 2-5. All experimental attribute measurements were done in triplicate.

Results and Discussion

Effect of different variables on physicochemical properties of the double emulsion as presented below:

Apparent viscosity

The effect of NaCl (3-5%, w/w), PGPR (2-4%, w/w) and two different (WPC-P and SC-P) complexes on the apparent viscosity (at 100 s^{-1} shear rate) immediately after preparation of the double emulsion ($W_1/O/W_2$) shown in Fig. 1. The double emulsion stabilized with SC-P complex had a very highly significantly ($p< 0.001$) higher mean value of apparent viscosity than that with WPC-P complex (Table 2). It is clear from Fig. 1 (a,b) that the apparent viscosity increased ($p< 0.001$) with increase in concentration of both the complexes. Further, as PGPR and NaCl concentrations increased, apparent viscosity of emulsions stabilized with both complexes (WPC-P and SC-P) increased. The differences in the mean apparent viscosity values were very highly significantly affected by all the variables and their interactions (Table 2).

In the present study, the viscosity of the double emulsions was influenced mainly by relationships between the concentrations

Table 1 3^3 full factorial designs to investigate the influence of variables on double emulsion

Variables (%, w/w)	Levels		
	Low level (-1)	Medium level (0)	High level (+1)
NaCl	3	4	5
PGPR	2	3	4
Dairy protein-polysaccharide complexes (1:2)	1.5	3	4.5
WPC-P			
SC- P			

of the protein-polysaccharide complexes and NaCl in the outer aqueous phase and inner aqueous phase, respectively, and the degree of mobility of the water in the external aqueous phase. The effect of concentration of the protein-polysaccharide complexes on the apparent viscosity of the double emulsions ($W_1/O/W_2$) was the same as observed by Lutz et al. (2009a).

Table 2 Analysis of variance of apparent viscosity, zeta-potential and turbidity of double emulsion stabilized with WPC/SC-P complex at 37°C

Attributes	Components	Type III Sum of Squares	Degree of freedom (Df)	Mean Square	F value
Apparent viscosity	NaCl	517864.778	2	258932.389	884.343***
	PGPR	10414484.11	2	5207242.056	17784.522***
	Complex	997813.556	1	997813.556	3407.876***
	Level of Complexes	34429487.44	2	17214743.72	58794.267***
	PGPR*NaCl	449568.556	4	112392.139	383.858***
	Level*PGPR	2713007.556	4	678251.889	2316.463***
	NaCl*Level	264671.556	4	66167.889	225.986***
	NaCl*Complex	7991.148	2	3995.574	13.646***
	Complex*Level	502617.815	2	251308.907	858.306***
	Complex*PGPR	207872.259	2	103936.130	354.978***
	Complex*NaCl*Level	193465.926	4	48366.481	165.188***
	Complex*NaCl*PGPR	99229.148	4	24807.287	84.725***
	Complex*Level*PGPR	127744.370	4	31936.093	109.073***
	NaCl*Level*PGPR	988721.333	8	123590.167	422.103***
	Complex*NaCl*Level*PGPR	109522.444	8	13690.306	46.757***
Zeta-potential	NaCl	295.678	2	147.839	91.085***
	PGPR	86.445	2	43.222	26.630***
	Complex	116.366	1	116.366	71.694***
	Level of Complexes	522.680	2	261.340	161.014***
	PGPR*NaCl	645.063	4	161.266	99.357***
	Level*PGPR	45.391	4	11.348	6.992***
	NaCl*Level	180.934	4	45.233	27.869***
	NaCl*Complex	37.710	2	18.855	11.617***
	Complex*Level	364.239	2	182.120	112.206***
	Complex*PGPR	0.711	2	0.356	0.219***
	Complex*NaCl*Level	68.927	4	17.232	10.617***
	Complex*NaCl*PGPR	285.325	4	71.331	43.948***
	Complex*Level*PGPR	85.474	4	21.369	13.165***
	NaCl*Level*PGPR	123.520	8	15.440	9.513***
	Complex*NaCl*Level*PGPR	146.819	8	18.352	11.307***
Turbidity	NaCl	40.174	2	20.087	1419.210***
	PGPR	193.374	2	96.687	6831.230***
	Complex	5.296	1	5.296	374.157***
	Level of Complexes	49.793	2	24.897	1759.026***
	PGPR*NaCl	104.094	4	26.024	1838.639***
	Level*PGPR	9.174	4	2.293	162.038***
	NaCl*Level	0.259	4	0.065	4.579***
	NaCl*Complex	54.875	2	27.438	1938.540***
	Complex*Level	0.696	2	0.348	24.589***
	Complex*PGPR	23.536	2	11.768	831.443***
	Complex*NaCl*Level	6.720	4	1.680	118.698***
	Complex*NaCl*PGPR	40.578	4	10.144	716.735***
	Complex*Level*PGPR	4.722	4	1.180	83.404***
	NaCl*Level*PGPR	13.021	8	1.628	114.992***
	Complex*NaCl*Level*PGPR	6.495	8	0.812	57.362***

***Significant at $p < 0.001$

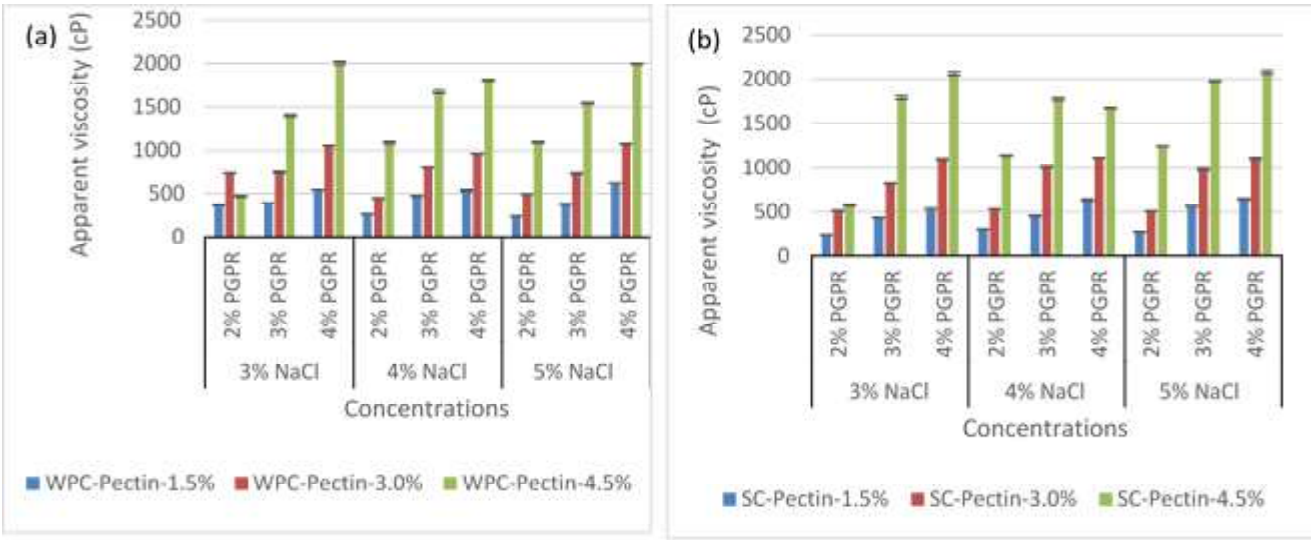

Fig 1. Effect of NaCl, PGPR and dairy protein-polysaccharide complexes (a): WPC-P, (b): SC-P on apparent viscosity of double emulsion; n=3

Apparent viscosity of the primary emulsion (W_1/O) increased with increasing PGPR concentration (Choudhary et al. 2018). Hence, the increased apparent viscosity of the final double emulsion ($W_1/O/W_2$) may be due to increased resistance to shearing during secondary emulsification. Our results were in agreement with studies reported by Su et al. (2006) and Bahtz et al. (2016), who found that the viscosity of the W/O emulsion increased with increasing concentrations of PGPR. This may have led to a subsequent reduction in the rate of coalescence and Ostwald ripening of the water droplets by decreasing the droplet diameter. On the other hand, though with initial increase in NaCl concentration, the apparent viscosity increased, it reduced at higher NaCl concentration. This may be because NaCl plays an important role in the formation of double emulsion by matching the osmotic and laplace pressure between the two aqueous phases (W_1 and W_2) of the double emulsion. Presence of excess NaCl concentration in inner aqueous phase causes a migration of the water droplets from outer aqueous phase (W_2) to inner aqueous phase (W_1), leading to subsequent swelling and sometimes bursting of the W_1/O droplets in the presence of concentration gradient between the two aqueous phases (Lutz and Garti, 2006; Rosano et al. 1998).

Zeta potential

The effect of the variables on ζ-potential of the double emulsion is shown in Fig. 2 (a,b). The double emulsion stabilized with the WPC-P complex exhibited higher ζ-potential than their SC-P stabilized counterparts. The mean ζ-potential values increased with increase in concentration of SC-P complex (1.5-4.5%), whereas it was found decreased with increase in concentration of WPC-P complex. The ζ-potential values of both complexes (WPC-P and SC-P) stabilized double emulsion increased with increasing concentration of PGPR while it was found to decrease up to 4% NaCl before increasing at 5% NaCl concentration. All three variables and their interactions showed a very highly significant ($p< 0.001$) effect on ζ-potential values of the double emulsion (Table 2).

According to Mirhosseini et al. (2008), an emulsion is considered as stable if the absolute zeta potential value is more than 25. Hence, ζ-potential is considered a key indicator for the stability of double emulsion (W/O/W) because the stability is affected by surface charge (ζ-potential) of the emulsion droplets. ζ-potential values below 25 indicated flocculation, while those above 25 mV indicated deflocculating emulsions (Mirhosseini et al. 2009). The negative surface charge of the double emulsion increased with increase in concentration of the complexes in the external aqueous phase (W_2) of the double emulsions ($W_1/O/W_2$). This effect can be attributed to the increase in electrostatic repulsion between the protein-polysaccharide complex molecules associated with their increased electrical charge, which prevented the aggregation and flocculation of the droplets. Our results are in good agreements with Guzey and McClements (2007), who reported that as the pectin concentration (0-0.05%, wt%) increased, the magnitude of zeta potential changed from positive to negative because the anionic pectin molecules adsorbed to the surface of cationic protein-coated droplets until the saturation of pectin concentration occurred. Further adsorption of the pectin may be prevented because the cationic binding sites get saturated with anionic groups of the pectin particles or due to the electrostatic repulsion between adsorbed and non-adsorbed pectin particles.

Prichapan and Klinkesorn (2014) reported that zeta potential was higher in $W_1/O/W_2$ emulsions prepared with SC than those with WPC, and therefore, it had more electrostatic repulsive force between droplets, which prevents droplet flocculation and coalescence. In presence of high salt concentration, the magnitude of ζ-potential decreased may be due to the screening effect of electrically charged ions. This meant that the counter-

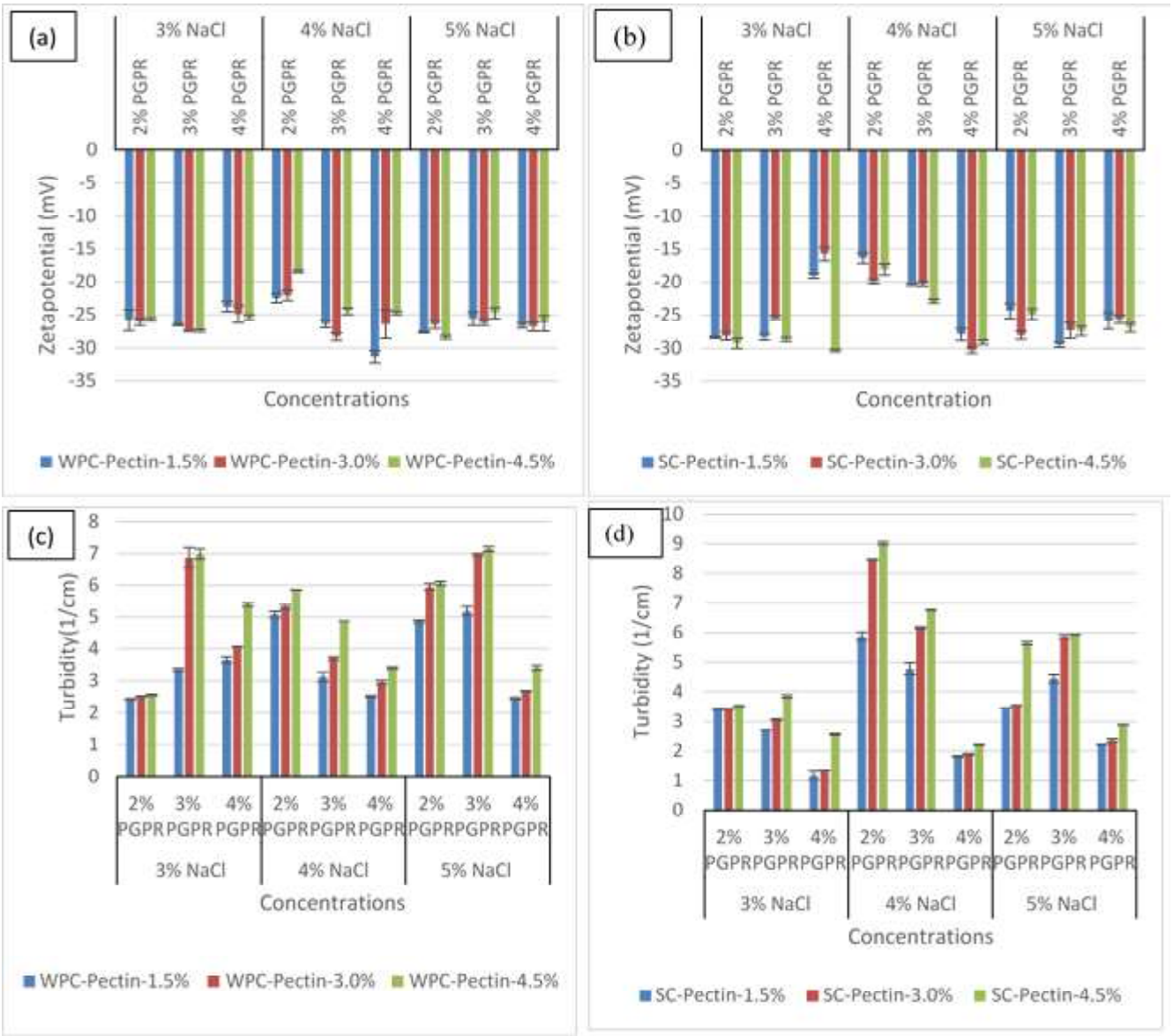

Fig 2. Effect of NaCl, PGPR and dairy protein-polysaccharide complexes (a): WPC-P (b): SC-P on zeta potential and (c): WPC-P (b): SC-P on turbidity of double emulsion; n=3

ions (Na^+) in inner aqueous phase accumulated loosely around the negatively charged $–COO^-$ groups on the protein-polysaccharide complex surface, due to the electrostatic interaction between the emulsion droplets and ion-binding effect. Low ionic (salt) concentration facilitated a strong electrostatic repulsion between the emulsion droplets, which prevents ions coming into close contact. However, once a critical ion (salt) concentration was reached, above this concentration electrostatic repulsion was no longer strong sufficient to overcome the attractive forces such as van der Wall and hydrophobic forces acting between the emulsion droplets, thus the emulsion droplets aggregate (McClements 2004). As a result of salt addition, the negative surface charge (ζ-potential) of the double emulsions shifted towards zero (slightly positive). This results supports the finding of Liu et al. (2012) and Onsaard et al. (2014) that the magnitude of the electrical charge (ζ-potential) of emulsions prepared with SC-carboxymethylcellulose and WPC-maltodextrin/carrageenan was negative at all salt (NaCl) concentrations studied (0-500 mM), but the magnitude of surface charge decreased as well as the salt concentration increased due to reduction in the electrostatic repulsion between oil-water emulsion droplets. Lutz et al. (2009b) also found a lower ζ-potential value (-37±3 mV) of double emulsions stabilized with most negatively charged WPI/U63 as compared to other pectin type WPI/C63 (-28±3 mV). This negative surface charge prevented flocculation and coalescence by facilitating electrostatic repulsion between the emulsion droplets.

Turbidity

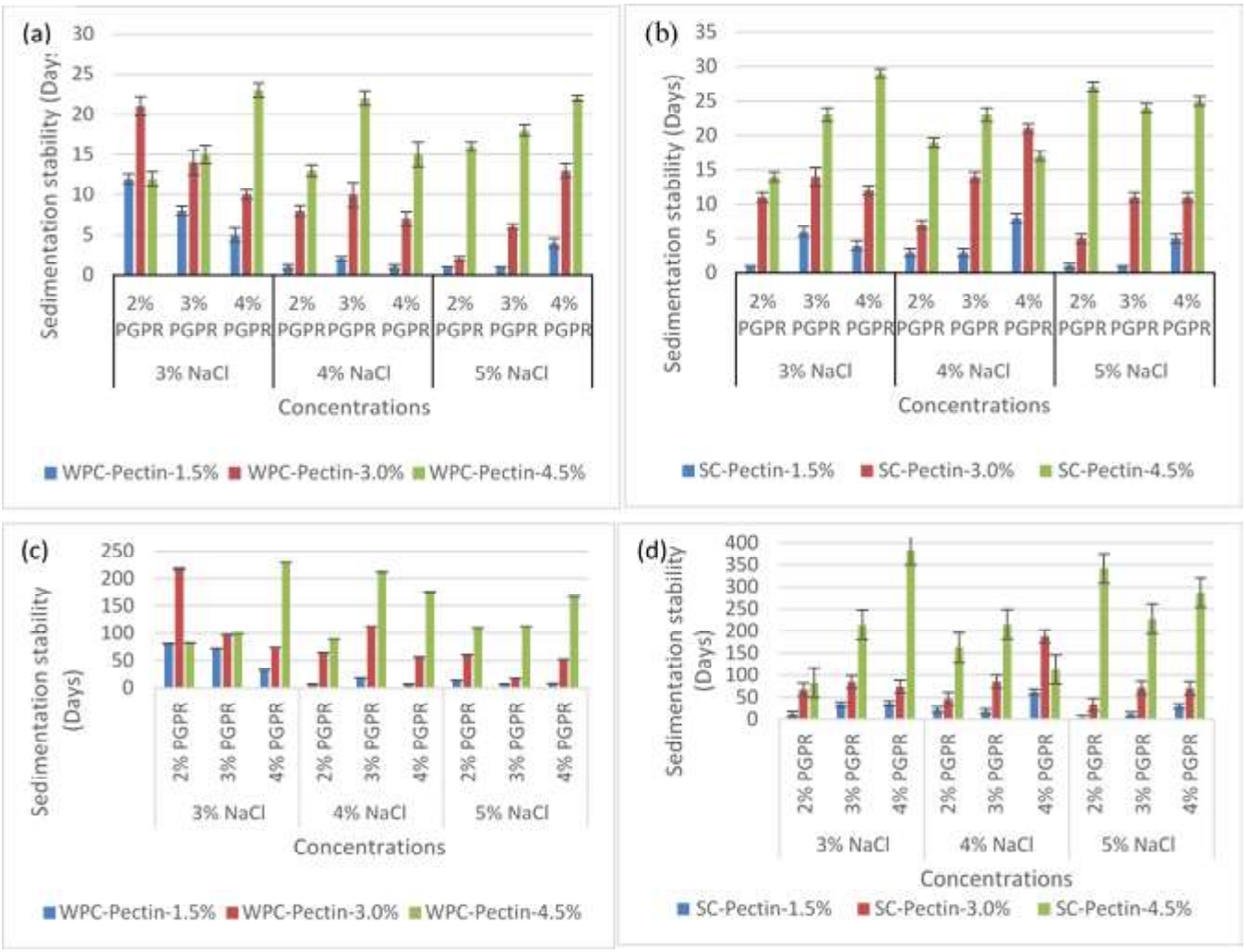

Fig 3. Effect of salt, PGPR and dairy protein-polysaccharide complexes (a): WPC-P (b): SC-P (37°C) (c): WPC-P (d): SC-P (7°C) on sedimentation stability of double emulsion; n=3

The effect of the variables on the turbidity of the double emulsion is shown in Fig. 2 (c,d). The average turbidity values were found lower in SC-P stabilized double emulsion than WPC-P stabilized samples and it increased with increasing concentration of both complexes (1.5-4.5%). The range of turbidity values with the minimum and maximum concentration of the complex were as follow: DE_{SC-P} (2.95±0.22 cm^{-1} to 5.04±0.43 cm^{-1}) < DE_{WPC-P} (3.63±0.21 cm^{-1} to 4.94±0.34 cm^{-1}). The mean turbidity values of both complexes (SC-P and WPC-P) stabilized double emulsion increased up to 3% PGPR, after that it decreased at 4% PGPR concentration. The range of turbidity values with the minimum and maximum concentration of NaCl are as follow: DE_{SC-P} (3.12±0.30 cm^{-1} to 4.04±0.27 cm^{-1}) < DE_{WPC-P} (3.86±0.28 cm^{-1} to 4.97±0.33 cm^{-1}). There was very high significant ($p< 0.001$) difference in the turbidity of double emulsions stabilized with each of the three variables i.e., NaCl, PGPR and complexes (WPC-P and SC-P). Their interaction also showed a very high significant ($p< 0.001$) effect on turbidity of the double emulsion (Table 2).

Several factors were reported to influence the emulsifying properties of protein-polysaccharide complexes such as protein-to-polysaccharide molar mass ratio, their total concentration, polymer charge density, pH, ionic strength, temperature and method of mixing (Benichou et al. 2002; Schmitt and Turgeon 2011). Ettelaie et al. (2005) reported that electrostatic protein-polysaccharide complexes onto the surface of protein-coated oil droplets led to increased emulsion stability by a thick secondary layer of charged polysaccharide. This could be due to the electrostatic and steric interaction between the protein and charged polysaccharide molecules. It has been reflected that the chemically modified proteins via electrostatic and steric complexation with polysaccharide could enhance emulsifying properties, especially below their isoelectric pH and at low ionic (salt) concentrations by maintaining molecular integrity and altered solubility (Surh et al. 2006; Lutz et al. 2009b). Emulsion activity (EA) and emulsion stability (ES) of double emulsion can be enhanced by covering the emulsion droplets by surfactants.

The biopolymers form a bulky and flexible film that may be strongly anchored on the O/W interface (Benichou et al. 2007; Lutz and Aserin 2007). Surh et al. (2006) associated the high viscosity of SC-emulsion with the addition of pectin at pH 3 and 4, due to high degree of droplet flocculation caused by charge neutralization and bridging flocculation of oppositely charged SC-(+ve)-stabilized emulsion droplets and pectin (-ve) molecules. Double emulsions ($W_1/O/W_2$) exhibited higher optical turbidity with increasing concentration of the dairy protein-polysaccharide complexes, probably at higher concentration of the complexes led to high viscosity of the emulsion by inducing strong electrostatic interaction. Lutz et al. (2009a,b) reported that WPI-modified pectin soluble complexes had net negative zeta-potential at pHd"6.0 that enable the repulsive interaction (electrostatic and steric) between the droplet interface, which results in a stable emulsion. Su et al. (2008) reported that the ionic environment reduced the emulsifying ability of the hydrophobic emulsifier (PGPR) from 4 % (w/w) PGPR in a buffered system to 2 % (w/w) PGPR in distilled water systems and with an encapsulation efficiency >90% of W/O/W emulsions prepared with SUPER GUM™.

Sedimentation stability

The double emulsion samples were kept at 37±1°C and 7±1°C and observed daily for phase separation. It was observed that the sedimentation stability (by visual separation) is a function of storage temperature. Fig. 3 depicts the effect of variables such as NaCl, PGPR and complexes (WPC-P and SC-P) on sedimentation stability. The double emulsions prepared with SC-P were stable for almost one month (29 days) and more than one year (383 days) while WPC-P stabilized emulsions were stable for 23 days and 229 days at 37°C and 7°C, respectively. It was found that as concentration of both complexes increased, sedimentation

Table 3 Analysis of variance of sedimentation stability of double emulsion stabilized with WPC/SC-P complex

Temperature	Components	Type III Sum of Squares	Degree of freedom (Df)	Mean Square	F value
37°C	NaCl	27.938	2	13.969	11.203***
	PGPR	405.975	2	202.988	162.792***
	Complex	769.191	1	769.191	616.876***
	Level of Complexes	7665.198	2	3832.599	3073.668***
	PGPR*NaCl	25.988	4	6.497	5.210***
	Level*PGPR	118.840	4	29.710	23.827***
	NaCl*Level	267.210	4	66.802	53.574***
	NaCl*Complex	54.531	2	27.265	21.866***
	Complex*Level	606.309	2	303.154	243.124***
	Complex*PGPR	35.901	2	17.951	14.396***
	Complex*NaCl*Level	334.025	4	83.506	66.970***
	Complex*NaCl*PGPR	109.321	4	27.330	21.918***
	Complex*Level*PGPR	76.543	4	19.136	15.347***
	NaCl*Level*PGPR	560.420	8	70.052	56.181***
	Complex*NaCl*Level*PGPR	99.679	8	12.460	9.993***
7°C	NaCl	12291.012	2	6145.506	10479.705***
	PGPR	25711.272	2	12855.636	21922.242***
	Complex	279000.469	1	27900.469	47577.642***
	Level of Complexes	685042.123	2	342521.062	584088.547***
	PGPR*NaCl	24835.877	4	6208.969	10587.926***
	Level*PGPR	34540.210	4	8635.052	14725.037***
	NaCl*Level	44290.802	4	11072.701	18881.868***
	NaCl*Complex	23422.864	2	11711.432	19971.074***
	Complex*Level	66340.198	2	33170.099	56563.747***
	Complex*PGPR	13283.864	2	6641.932	11326.242***
	Complex*NaCl*Level	53243.025	4	13310.756	22698.342***
	Complex*NaCl*PGPR	30957.802	4	7739.451	13197.800***
	Complex*Level*PGPR	24984.358	4	6246.090	10651.226***
	NaCl*Level*PGPR	141898.642	8	17737.330	30246.816***
	Complex*NaCl*Level*PGPR	33340.420	8	4167.552	7106.774***

stability increased. The sedimentation stability was found to increase with increase in PGPR (2-4%) concentration in both SC-P and WPC-P complex stabilized double emulsion, It could be seen from Fig. 3(a,c) that sedimentation stability was highest at 3% NaCl, followed by 5% and 4% NaCl at 37°C and it was found to decrease with increase in salt concentration (3-5%) at 7°C. Whereas Fig. 3(b,d) shows that it was highest at 4% NaCl, followed by 3% and 5% NaCl at 37°C and, it increased with increase in NaCl concentration (3-5%) at 7°C.

It is evident from Table 3 that the sedimentation stability (by visual observation) of double emulsion was very highly significantly affected by the variables such as NaCl, PGPR, WPC-P and SC-P complexes and their interaction.

Lutz et al. (2009a) observed no coalescence in double emulsions stabilized with soluble WPI/modified pectin complex for almost one month, because oil droplets were fully covered by external oil-water interfacial (O/W_2) film comprising of the WPI/modified pectin. The soluble complexes of WPI/pectin also reduced the interfacial tension on surface of the emulsion droplets. Further they reported that the level of creaming also depended on the NaCl concentration (1.0, 1.5 and 4.4%, w/w) in the inner aqueous phase. Salminen and Weiss (2014) studied the electrostatic adsorption of heat-treated biopolymer complexes [Whey protein isolate (WPI)-apple pectin at 85°C for 20 min] and stability of these complexes on emulsion interfaces. They found that the emulsions covered with the complexes showed good stability to salt (up to 200 mM) and heat (up to 90°C) by creating a thick polymer layer. Muschiolik et al. (2006) investigated the effect of addition of salt (NaCl) in inner aqueous phase of multiple emulsions prepared with 4% PGPR during storage. They found that presence of the salt in the inner aqueous phase (W_1) of multiple emulsions is essential to carry out coalescence free W/O emulsions prepared with PGPR, an addition of salt increased the encapsulation stability and oil droplet stability of multiple emulsions. Actually, salt is considered to be a co-stabilizer, a lipophobic that builds-up the osmotic pressure to counterbalance the Laplace pressure, and consequently stabilizes emulsions against diffusional degradation known as Ostwald ripening (Landfester 2006; Capek 2010; Colman et al. 2014). Bahtz et al. (2016) reported that PGPR, when used as an oil-soluble surfactant, was able to spontaneously form tiny water droplets in oil phase that cause structural changes in the oil layer. Further, they described that the overall water transport rate increases and water transport stagnates because of maximized structure formation at below and above a critical concentration of the oil-soluble surfactant, respectively.

Conclusions

An attempt was made to fabricate a stable water-in-oil-in-water ($W_1/O/W_2$) double emulsion encapsulating bitter gourd extract, using a two-step emulsification technique. The results of the present study indicated that several factors such as NaCl, PGPR and protein-polysaccharide complexes (WPC-P and SC-P) and their interaction exhibited a synergistic effect on the stability of the fabricated double emulsion. The deciding attributes (turbidity, zeta-potential, apparent viscosity and sedimentation stability) were very highly dependent on the all the variables such as NaCl, PGPR and complex and their levels. SC-P stabilized double emulsions were found more stable than WPC-P stabilized samples.

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RESEARCH ARTICLE

Suitability studies of different milk proteins for supplementation in functional extruded snack

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Abstract: Carbohydrate based snacks clubbed with frying have substantial dominance on Indian snack market, depriving nutrition. But awareness for alternative healthier snacks has already attracted market attention. In the present study, an effort was ventured by utilizing the combined benefits of high carotene sweet potato flour, barley flour, rice flour and milk protein (Whey Protein Concentrate (WPC)-70/ Skim Milk Powder (SMP)/ rennet casein) to design a low fat-high protein, crisp snack by extrusion cooking. The conditions employed for snack processing were the temperature of heater 1 (110°C) and 2 (40°C), screw speed (370 rpm) and die diameter (4 mm). The effect of different proteins and their levels on snack quality were evaluated by physical and sensory responses like hardness (N), L*, a* and b* color values, expansion index (EI) and sensory scores (color and appearance, flavor, texture and overall acceptability). The results revealed that addition of SMP at different rate did not showed significant ($p>0.05$) effect on expansion index, color & appearance and flavor score of the snack. Whereas, incorporating rennet casein as a source of quality protein, EI increased significantly ($p<0.05$) at 15% and there was a significant effect on the sensory parameters with increasing rennet level. However, increasing WPC-70 addition, the EI and sensory scores declined, and the hardness of the snack increased. Highest EI (3.18) and sensory scores were observed at 15% level of casein. From quantified parameters, casein was deduced to be most acceptable milk protein source for the intended snack.

Keywords: Composite flour, Extruded snacks, Functional food, Milk proteins, Physical properties

Introduction

Extrusion is a HTST technology that combines several unit operations comprising mixing, cooking, kneading, shearing, shaping, and forming (Steel et al. 2012). This process gelatinizes starch, denatures proteins, modifies lipids, and inactivates enzymes, microbes, and antinutritional factors (Singh, Gamlath and Wakeling 2007).The worldwide demand of extruded snack foods is high as they are available in variety of shapes, textures, colours and flavours. However, extruded snacks are typically high in calories and fat with low content of protein, fibre, and perceived as unhealthy or junk food to many consumers (Korkerd et al. 2016; Basto et al. 2016). A possible solution to this problem is incorporation of alternative ingredients like fruits and vegetables, banana powder, legume flour, oats and whole grains etc. in extruded product preparations to enhance their nutritional quality (Sukumar and Athmaselvi, 2019; Oliveira et al. 2018). However, many researchers have observed that addition of dietary fibre often leads to lower expansion volumes, higher density, harder texture, less crispness, and thus reducing the acceptability of the extruded snack (Veronica et al. 2006; Brennan et al. 2008; Robin et al. 2011; Chanvrier et al. 2013; Chassagne-Berces et al. 2011).

Milk proteins are recognized for their nutritive and health benefits (Zimecki and Kruzel 2007). These proteins have many biological activities: cancer prevention, tumour cell vulnerability increase, antimicrobial activities and immunomodulation (Madureira et al. 2007). Milk proteins have good digestibility (Hambraeus, 1992), as well as Protein Efficiency Ratio (PER), whey protein and caseins have a PER of 3.2 and 2.6, respectively (Walzem et al. 2002). Lately, milk constituents have been acclaimed as functional foods,

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as they impart numerous therapeutic benefits which improve health. Use of dairy proteins in cereal based products, improves the nutrient density of extruded snacks by increasing protein and mineral content (Brncic et al. 2011; Pordesimo et al. 2009). Attempts have been made to use whey protein concentrates or dried whey for extrusion purpose (Onwulata and Konstance, 2006; Onwulata et al. 2010; Fernandes et al. 2016; El-Ghany, et al. 2013; Chaudhari et al. 2020). The type and level of milk protein incorporated into an extrudate affects the water holding capacity, nitrogen solubility index and sorption characteristics of extruded products (Allen et al. 2007). This study was focused on combining different milk proteins (Skim milk powder/ whey protein concentrate-70/ rennet casein) with composite flour (sweet potato, barley and rice) to enhance the nutritional status of extruded snack and evaluate the effect of level and type of these milk proteins on physical and sensorial properties, that would ultimately have an effect on acceptability of the product by the consumer in market.

Materials and Methods

Raw Materials

Carotene rich variety of sweet potato, *Indramadhur* was purchased from Regional Centre of Central Tuber Crop Research Institute (CTCRI), Bhubaneswar, Odisha. Barley (DWRB-73 variety) was procured from the Directorate of Wheat Research (DWR), Karnal, Haryana. Paddy (PR-44) was procured from Indian Agricultural Research Institute (IARI- Regional Centre), Karnal, Haryana. Milk Protein Sources (WPC-70, Rennet Casein and SMP) were procured from M/s Modern Dairies Ltd., Karnal, Haryana. Sweet potatoes were washed, sliced, dipped in 0.1% KMS solution for 5 min and then dried in tray drier at 60°C for 4-5 hr. The dried slices were then ground to fine powder. Barley and paddy were de-husked, cleaned and then subjected to milling.

Proximate analysis of the raw material

Standard procedures as described in AOAC (2000) were used for proximate analysis of sweet potato, barley and rice flour and skim milk protein, casein, and WPC-70 (Moisture, protein, crude fat and ash content).

Preparation of composite extruded snack

Based on preliminary trials, the composite flour ratio (sweet potato, barley and rice flour) were fixed at 20, 25 and 30 parts, respectively. The three types of milk protein (WPC-70, SMP and rennet casein) used for the study were at proportion of 5, 10 and 15 parts. The methodology adopted was as described by Yadav et al. 2016. The ingredients were blended in a Hobart mixer at 60 rpm, moisture was adjusted to 12% and the blend was passed through 2 mm size sieve and again blended for 10 min. to get uniform mixing. The premix was packed in 1 kg PE bag and preconditioned overnight in a refrigerator before extruding using twin-screw extruder. The speeds of feeder screw and extruder screw were kept constant at 40 and 370 rpm, respectively. The temperature of the two heating zones were 40°C and 120°C, respectively. As the material was extruded through 4mm die and was cut into pieces of desired length by a rotating-blade cutter at 10 rpm. Extrudates were then packed in metalized PE pouches and stored at 37°C until further analysis.

Sensory evaluation

Sensory evaluation was conducted by 10 trained panelists from NDRI, Karnal. Product evaluation was based on its color & appearance, flavor, texture, and overall acceptability on 9-point hedonic scale (1 = dislike extremely to 9 = like extremely) (Deshpande and Poshadri, 2011).

Hardness (Texture Analysis)

Hardness is the peak force during compression of the product. It was determined using TA-XT2i (Stable Micro Systems, UK) Texture Analyzer fitted with a 25 kg load-cell and Warner-Bratzler Blade. The pre-test and post-test speed were 2.0 mm/sec, while the test speed was 1.0 mm/sec.

Expansion ratio

The ratio of diameter of extrudate and the diameter of die was used to express the radial expansion of extrudate (Fan et al., 1996). The extrudate expansion ratio was calculated as follows:

$$\text{Expansion ratio} = \frac{\text{Extrudate diameter (mm)}}{\text{Die diameter (mm)}}$$

Hunter color analysis

The color of the product was measured using a Hunter Lab Colour flex colourimeter (Hunter Associated Laboratory, Inc., USA) using the Universal software version 4.10. Measurement was made by taking finely ground sample in the glass sample holder up to 2 cm height followed by tapping for ten times on bench-top. Data were received through the software in terms of L* (lightness), ranging from zero (black) to 100 (White), a* (Redness), +60 (red) to -60 (green) and b* (yellowness), ranging from +60 (yellow) to -60 (blue) values of the international (CIE) color system.

Statistical analysis

The data obtained from the given experiments for milk protein sources incorporation in composite extruded snack were subjected to analysis using two-way ANOVA using SPSS Statistical tool (version 20, IBM Corp., New York, NY, USA) as described by Snecdecor and Cocharan (1994). The data were presented as Means ± Standard Error (SE).

Results and Discussion

Proximate analysis

The results obtained for proximate analysis of raw material are presented in Table 1. It is evident, that rennet casein had highest crude protein content i.e. 80.16 %, followed by WPC-70 (69.70 %) whereas, sweet potato flour (SPF) had the least amount of proteins (1.05 %). The fat content in rice flour was least with the value being 0.45 % and highest fat content was observed in WPC-70 i.e. 4.66 %. Casein and SMP had high percentage of ash content (7.94 and 7.89 %, respectively) whereas barley and rice flours had lower levels (1.55 and 0.45%). The carbohydrate content of the flours was calculated by subtracting percentage of all other components from 100. Similar values of proximate analysis of milk proteins was reported by Ponbhagavathi et al. (2018)

Hunter color values of raw materials

The color of the raw material has a prominent effect on the appearance of the processed product, thereby effecting its consumer acceptability. The L* value was found to be maximum with Skim milk powder (92.03), thus indicating that SMP was the brightest and whiter among all the ingredients (Table 2). The lowest L* value was observed for SPF (72.61) but a* and b* values were highest for SPF indicating that it was darker compared to other raw materials.

Effect of incorporation of milk proteins on expansion index and hardness

A higher value of expansion index in extruded snacks represent a product with greater crispiness and less dense structure. The composition of the raw ingredients has a significant effect on the expansion. During extrusion the high temperature and shear, gelatinize the starch forming a matrix with entrapped water molecules, due to sudden pressure drop at the exit, expansion of the extrudate occurs as the water molecules expand forming bubbles and creating a porous structure (Guy and Horne, 1988). When raw material have higher amounts of protein and/ or fibre, the starch level drops, resulting in lesser expansion and increased density. The values of expansion index and hardness are represented in Table 3. The values ranged from a lowest of 1.68 (15% WPC 70) to 3.18 (15% rennet casein). SMP did not have have any significant difference with increase in level, while in case of WPC, the E.I. decreased tremendously with increase in level from 5 to 15%. Ding et al. (2005) reported values between 2.02 and 3.87 for rice-based extrudates. It is evident from the table that increasing level of SMP did not have any significant effect on E.I. of extrudates, whereas in case of rennet casein, E.I. increased significantly (p d" 0.01) as the level increased from 5 to 15 %. Patel et al. (2016) also observed that the increasing level of rennet casein resulted in a significant increase in the expansion ratio ($p < 0.05$). This may be due to the large molecular structure of casein, hydrophobic properties and random coil conformation in comparison to whey protein concentrate and isolate (Onwulata and Konstance 2006). However, an opposite trend was observed in case of WPC-70, as the level of incorporation was increased, there was reduction in the expansion of the snack. Reduction in expansion with increasing whey protein concentration as a result of starch-protein interactions was reported by Allen et al. (2007). Another study conducted by Chinnaswamy and Hanna (1988) also concluded that there was decrease in the expanded volume of cereal flour-based snacks when level of lipid and protein is increased in the mixture.

Table 1 Proximate composition (g/ 100g) of raw materials used for extruded snack preparation

Constituent	Moisture	Crude protein	Fat	Carbohydrate*	Total ash
SPF[#]	5.94±0.36	1.05±0.45	0.69±0.05	88.93	3.38±0.03
Barley Flour	5.63±0.18	14.97±0.00	1.68±0.11	76.17	1.55±0.00
Rice Flour	11.22±0.22	8.40±0.45	0.45±0.19	79.48	0.45±0.042
Casein	8.90±0.04	80.16±0.44	0.96±0.04	—	7.94±0.021
WPC-70%	3.76±0.27	69.70±0.46	4.66±0.05	—	3.89±0.197
SMP	3.8±0.48	34.05±0.44	1.2±0.31	53.06	7.89±0.003

*By difference; [#] Sweet potato flour; Values are mean ± standard error (n= 3)

Table 2 Hunter color parameters (L, a, b values) of raw materials

Constituent	Hunter color parameters		
	L* value	a* value	b* value
SPF	72.61 ± 0.32	4.31 ± 0.09	20.65 ± 0.38
Barley flour	81.52 ± 0.14	1.43 ± 0.27	9.15 ± 0.04
Rice flour	86.71 ± 0.23	-0.01 ± 0.02	6.51 ± 0.02
Rennet Casein	83.03 ± 0.11	-0.64 ± 0.05	10.98 ± 0.02
WPC-70 %	84.55 ± 0.16	0.92 ± 0.13	20.68 ± 0.21
SMP	92.03 ± 0.01	-3.02 ± 0.04	16.71 ± 0.02

Values are mean ± SE (n=5)

Hardness is the peak force required for a probe to penetrate or break the extrudate (Ponbhagavathi et al. 2018). Expansion index and the cell structure of the extruded snack have an impact on the hardness. The values ranged from 21.51N to 54.13 N, the highest value was observed for 15% WPC 70. When extrudate was made with incorporation of skim milk powder, the hardness of the product reduced from 25.64 N to 21.72 N. There was no significant change ($p > 0.05$) in the hardness values when extrudate was made with incorporation of rennet casein. It was reported by Voort and Stanley (1984), that there was no significant effect on hardness value of wheat flour extrudates by incorporation of rennet casein (10 to 30 %). Another study concluded that rennet casein, tended to decrease the hardness at lower levels, but the effect was not significant at higher levels (Patel et al. 2016). When WPC 70 was added to the ingredient mix as the milk protein source, the hardness of the product increased significantly ($p <0.01$) from 28.69 N (5%) to 54.13 N (15%). It is evident that hardness level increased with increase in level of WPC 70. Similar results were obtained by Yadav et al. (2013), in pearl millet-WPC extruded snacks. This may be due to fact that the protein competes with the starch for the water during extrusion, reducing the rate of gelatinization and thereby

Table 3 Physical properties of composite flour based extruded snack affected by different milk proteins

Physical Properties	Level (%)	Milk Protein Type[#]		
		SMP	Rennet Casein	WPC-70
Hardness	5	$25.64^{bB} \pm 0.55$	$22.27^{aA} \pm 0.73$	$28.69^{aC} \pm 1.53$
	10	$27.35^{cB} \pm 0.09$	$21.51^{aA} \pm 1.08$	$35.22^{bC} \pm 2.61$
	15	$21.72^{aA} \pm 0.12$	$22.26^{aA} \pm 0.13$	$54.13^{cB} \pm 1.42$
Expansion Index	5	$2.403^{aA} \pm 0.21$	$2.91^{aB} \pm 0.04$	$2.91^{cB} \pm 0.19$
	10	$2.49^{aA} \pm 0.12$	$2.87^{aB} \pm 0.02$	$2.32^{bA} \pm 0.13$
	15	$2.39^{aB} \pm 0.10$	$3.18^{bC} \pm 0.18$	$1.68^{aA} \pm 0.02$
L value	5	$62.25^{aA} \pm 1.58$	$65.46^{bB} \pm 0.30$	$63.79^{aAB} \pm 0.02$
	10	$64.55^{bB} \pm 0.13$	$64.05^{aA} \pm 0.14$	$65.62^{bC} \pm 0.29$
	15	$64.98^{bB} \pm 0.09$	$64.45^{aA} \pm 0.12$	$68.79^{cC} \pm 0.23$
a value	5	$8.18^{aB} \pm 0.56$	$5.59^{aA} \pm 0.67$	$7.60^{cB} \pm 0.31$
	10	$7.40^{aB} \pm 0.31$	$7.71^{bB} \pm 0.57$	$5.69^{bA} \pm 0.73$
	15	$7.54^{aB} \pm 0.22$	$7.52^{bB} \pm 0.50$	$4.71^{aA} \pm 0.30$
b value	5	$25.02^{aA} \pm 0.33$	$25.25^{aA} \pm 0.51$	$24.93^{cA} \pm 0.54$
	10	$24.79^{aB} \pm 0.14$	$25.05^{aB} \pm 0.56$	$23.37^{bA} \pm 0.44$
	15	$25.63^{bB} \pm 0.18$	$25.501^{aB} \pm 0.63$	$20.34^{aA} \pm 0.25$

[#]Milk Protein Type: SMP- Skim milk powder, WPC-70- whey protein concentrate

[abc; ABC] Means ± S.E. (n=15) with same superscripts in a row (A, B, C) or in a column (a, b, c) do not differ signiûcantly (P d" 0.01).

Table 4 Sensory scores of composite flours based extruded snack affected by different milk proteins

Sensory Attribute*	Level (%)	Milk Protein Type[#]		
		SMP	Rennet Casein	WPC-70
Color & appearance	5	$5.93^{aA} \pm 0.58$	$6.87^{bB} \pm 0.08$	$6.49^{cAB} \pm 0.06$
	10	$6.69^{aB} \pm 0.27$	$6.43^{aB} \pm 0.04$	$5.84^{bA} \pm 0.10$
	15	$6.80^{aB} \pm 0.44$	$7.50^{cC} \pm 0.20$	$4.90^{aA} \pm 0.13$
Flavor	5	$5.98^{aA} \pm 0.51$	$6.83^{aB} \pm 0.14$	$6.23^{bAB} \pm 0.43$
	10	$6.44^{aA} \pm 0.17$	$6.26^{aA} \pm 0.41$	$5.78^{bA} \pm 0.49$
	15	$6.73^{aB} \pm 0.36$	$7.67^{bC} \pm 0.08$	$4.36^{aA} \pm 0.32$
Texture	5	$5.65^{aA} \pm 0.78$	$7.03^{bB} \pm 0.19$	$6.29^{cAB} \pm 0.53$
	10	$6.77^{bB} \pm 0.19$	$6.77^{aB} \pm 0.11$	$5.47^{bA} \pm 0.18$
	15	$6.93^{bB} \pm 0.04$	$7.54^{cC} \pm 0.09$	$4.05^{aA} \pm 0.41$
Overall Acceptability	5	$5.88^{aA} \pm 0.77$	$6.83^{bA} \pm 0.06$	$6.17^{cA} \pm 0.40$
	10	$6.64^{abB} \pm 0.06$	$6.53^{aB} \pm 0.13$	$5.49^{bA} \pm 0.18$
	15	$6.86^{bcB} \pm 0.12$	$7.32^{cC} \pm 0.10$	$4.12^{aA} \pm 0.16$

[*]On a 9-point hedonic rating scale: 9-like extremely; 1-dislike extremely.

[#]Milk Protein Type: SMP- Skim milk powder, WPC-70- whey protein concentrate

[abc; ABC] Means ± S.E. (n=10) with same superscripts in a row (A, B, C) or in a column (a, b, c) do not differ signiûcantly (P d" 0.01).

decreasing expansion and increasing hardness of the product. In addition, the higher amounts of protein may also result in variation in temperature and pressure inside the extruder, affecting the degree of expansion and textural properties (Anton et al. 2009; Sumargo et al. 2016). Anton and Luciano (2007) also stated that the extrudates hardness depends on raw material composition, as well as feed moisture and extrusion conditions. Protein rich extrudates produce less expandable products and more rigid network resulting in higher resistance to shear and lower expansion (Li et al., 2005).

Effect of incorporation of milk proteins on colour parameters

Food acceptability is affected by color of the product. It acts as an indicator of quality, flavor expectation and commercial value (Fradique et al., 2010). Color of an extrudate is an indicator of thermal history and account of non-enzymatic browning resulting from maillard browning during extrusion process (Yadav et al. 2013). The L*, a* and b* value of extruded snack as effected by type and level of milk protein is presented in table 3. The L* values for extrudates ranged from 62.25 to 68.79. The L* value was highest in case of WPC 70, indicting a lighter product in comparison to SMP and rennet casein. Positive b* values indicate the yellowness of a sample and negative values are indicative of a blue color. As the extruded snack had sweet potato flour rich in soluble sugars, the b* value was on higher side (20.34 for WPC to 25.63 for SMP). Non-enzymatic browning reactions like caramelisation and the Maillard reactions forms compounds that have higher a* value (Ilo and Berghofer 1999). Melanoidins formed in the Maillard reaction impart a darker colour to extrudates. Process conditions during extrusion, high lactose content and protein content in whey powder also intensify the dark color of these compounds (Tamanna and Mahmood, 2015).

Effect of incorporation of milk proteins on sensorial properties

The consumer acceptance of any product depend on the sensorial properties of the product. The sensory properties like colour, flavour, texture, and overall acceptability were significantly ($p<0.01$) affected by the level and type of milk protein incorporated (table 4). The lowest score (4.90) for colour and appearance was observed for 15% WPC 70 while highest (7.50) was observed for rennet casein 15 %. The scores improved with increase in level of SMP and rennet casein from 5 to 15 %, whereas the values decreased in case of increment of WPC 70. More or less similar trend was also noticed for flavour, texture and O.A. scores of the extruded snack. On comparison of different protein sources, it can be observed that rennet casein at 15% level, had significantly higher scores ($p<0.01$) in all sensory parameters compared to SMP and WPC 70. Higher acceptability in case of rennet casein incorporation maybe attributed to higher expansion and crisp texture of the extruded snack.

Conclusions

The study indicated that types and levels of milk proteins had a significant ($p<0.01$) effect on the hardness, expansion index and color values of the extruded snack. The sensory scores were significantly affected by the type of milk protein used to make high protein extruded snack. Rennet casein had better acceptability as depicted by the trained sensory panelist compared to product prepared with incorporation of WPC. The snack prepared using 5% level of WPC was acceptable but at higher levels (10% and 15%), the overall quality of the snack declined extremely. Increasing level of SMP didn't show significant effect on the sensory and physical parameters. Significant improvement in the expansion index and sensory scores of extruded snack with rennet casein was observed at all levels. Thus, a highly acceptable, high protein product with desirable physical, sensory characteristics could be prepared with rennet casein as a source of quality protein.

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RESEARCH ARTICLE

Optimization of process parameters for pickle *masala* flavored probiotic Greek yoghurt

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Abstract: In this study, Greek yoghurt was developed using standardized homogenized milk and fermented with suitable indigenous starter culture with optimized process parameters and pickle masala as novel flavor. The product was prepared by fermenting with four culture. Suitability of starters was evaluated based on starter culture counts, pH, titratable acidity and sensory parameters of the Greek yoghurt. The C4 starter containing *Streptococcus thermophilus* MTCC 5460, *Lactobacillus bulgaricus* NCIM 2358 and *Lactobacillus helveticus* MTCC 5463 (probiotic strain) was found to be most suitable as it received significantly ($P<0.05$) highest score for lactobacilli count (8.86 log CFU/ml), TS (23.41 %) and overall acceptability (8.88) compared to remaining. Among the straining period *viz,* 15 min, 30 min and 55 min, product prepared by straining for a period of 30 min was optimized based on pH (4.67), titratable acidity (% 0.89 LA) and overall acceptability scores (8.71). No significant effect of various straining period was observed on starter counts of the product. Pickle masala was used as novel flavor and it was added at the rate of 1 %, 1.5 % and 2 % of final product. The increased rate of pickle masala in Greek yoghurt affected adversely on starter count as well as sensory profile. So, 1% rate of addition of pickle masala in Greek yoghurt as flavor was considered more suitable having lactobacilli count (9.05 log CFU/ml), streptococci count (10.37 log CFU/ml), pH (4.81), titratable acidity (0.90 % LA) and overall acceptability score (8.80) compared to other. The composition of optimized Greek yoghurt was TS (23.02 %), fat (7.92 %), protein (6.17 %), lactose (4.91 %), calcium (171 mg/ 100g) and ash (1.29 %).

Keywords: Greek yoghurt, Starter culture, Pickle masala, LAB

Introduction

It is generally observed that the fermented milks like yogurt were discovered accidentally when our forefathers used to store milk in sheep skin bags to preserve it. The yoghurt making is evolved over centuries and now a days varieties with a range of flavors, forms and textures are commercially available (Weerathilake et al. 2014). One such variety is Greek yoghurt and also known differently according to their origin, such as Labneh in Eastern Mediterranean, Torba in Turkey, Stragisto in Greece, Ymer in Denmark, Skyr in Iceland and Chakka in India (Aryana and Olson, 2017; Uduwerella, et al. 2017; Tamime et al. 2014). It is prepared from the yoghurt by removing part of whey by straining for some period of time and most popular in Levantine, Eastern Mediterranean, Middle Eastern, Central Asian and South Asian people. Traditionally, nomads in the Middle East used the containers made from animal skin for the production of yoghurt and the yoghurt was left in these skins until it was consumed. While the yoghurt was hanging in the animal skin some of the liquid phase would have been absorbed into the skin, while some of the whey that had seeped through the skin would have been lost by evaporation. In this way concentration of the product took place and the new product was referred to as concentrated/ strained yoghurt. This product has better keeping quality than normal yoghurt, mainly as a result of the higher concentration of lactic acid (Tammie and Robinson, 2007) and low moisture content.

Greek yogurt is having creamier texture (Uduwerella, 2018) and thicker consistency with slightly sour taste. It is rich in protein content (Tamime and Robinson, 2007), lower carbohydrates (as decomposed by the starter culture), improved bioavailability of

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calcium, source of vitamin B 12 and health promoting microorganisms (Panahi et al. 2016). Greek yoghurt is popularly consumed as starter culture associated with it may also have the capacity to survive digestion, reach the gastrointestinal tract and ultimately provide several health benefits (Marco et al. 2017). Due to these reasons the demand of Greek yoghurt is increasing day by day and health conscious people have started to include it in their regular diet. Bell et al. (2018) suggested fermented foods like Greek yoghurt should be introduced to children early in life and incorporated into their everyday meal plans. This helps in boosting the immunity, improved digestion and increase the bioavailability of micronutrients.

Lactic acid bacteria (LAB) are known for their widespread occurrence and diverse commercial use. Several species of LAB are industrially formulated to produce fermented food as starter cultures and enhance human health through probiotics. Probiotics alter the intestinal microflora balance, inhibit the growth of pathogen (Wulandari et al. 2020), modulate immune response and can act in various other ways as health promoters (Trush et al. 2020). Probiotics strains of lactobacillus are usually given generally regarded as safe (GRAS) status, since most are isolated either from traditional dairy products or the gastrointestinal tract of healthy individuals (Salminnen et al. 1998). The technological application of probiotic organisms in fermented dairy products aims to combine the potential health benefits of the bacteria with their ability to grow in milk, resulting in a nutritionally healthy and desirable product for the consumers. Therefore, apart from health claims, probiotic strains intended for use in fermented dairy products should be selected on the basis of their overall effect in the products as many probiotic bacteria used today in yoghurt grow poorly in milk when compared with some common starter cultures.

Wide variety of Greek yoghurt with different flavours are available in Indian markets and sold under the brand names like Amul, Danone, Epigamia, Nestle, Chobani, Barambah etc. None of the commercially available Greek yoghurt are manufactured using Indian origin starter culture. Consumers always search for the novelty and uniqueness in the product. Taking these points in mind, the study was conducted with the objective to develop Greek yoghurt fermented by indigenous starter having probiotic potential, with underexplored novel flavor.

Materials and Methods

Collection of LAB and chemicals

The four combination of LAB were taken from the Culture Collection of Dairy Microbiology Department, Sheth M. C. College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India C1- *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus helveticus* MTCC 5463; C2- *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus rhamnosus* MTCC 5946; C3- *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus bulgaricus* NCIM 2358 (yoghurt culture) and C4- *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus bulgaricus* NCIM 2358 + *Lactobacillus helveticus* MTCC 5463. Sterilized reconstituted skim milk with 12% TS was used for the propagation of lactic culture and further stored at 5 ± 2 °C. All the chemicals and reagents utilized in the study were purchased either from Hi-media (Mumbai), Merck (Germany) or Loba.

Procurement of milk and pickle masala

The standardized homogenized milk (Amul T special milk) and pickle masala (Vasant achar masala and taste maker) were purchased from the local market of Anand city of Gujarat state (India).

Preparation of Greek yoghurt

The flow chart to prepare Greek yoghurt is shown in Figure 1.

Determination of Starter culture count

Lactobacilli count of Greek yoghurt was determined as per the method described by Kathiriya et al. (2016). Eleven grams of sample was taken out from the plastic container and added to 99 ml phosphate buffer flask. This made 1:10 dilution. Similarly, required numbers of serial dilutions were prepared. One ml diluted sample from appropriate tubes was transferred to labeled petri plates (performed in triplicates) then 15-20 ml of molten and cooled (45°C) MRS agar was poured to respective petri plates. The content was mixed thoroughly by tilting and rotating the plates and allowed it to solidify and then additional layer (5-7 ml) of the same agar was poured over the solidified medium. Again allowed it to solidify, then incubated anaerobically at 37°C for 48 h in inverted position. Typical colonies were calculated and the counts were expressed as log CFU/ml. For Streptococci count, the procedure is same as the lactobacilli count. But M17 growth media was used instead of MRS agar for the enumeration of streptococci (Kathiriya et al. 2016).

Measurement of pH

The pH of the Greek yoghurt was measured as per the procedure described in Indian Standard (1961) with a calibrated pH meter (OAKTON pH700, India). Well mixed 10 ml Greek yoghurt sample was put into a beaker and then pH was measured by immersing the pH meter probe into the fermented milk sample at 25°C. Standard buffer solution of pH 4, 7 and 9 was used to calibrate the pH meter before measuring the sample.

Measurement of titratable acidity

The titratable acidity (% Lactic acid) of Greek yoghurt was estimated by the procedure described in IS:1479 (Part I, 1960). Ten gram of sample was taken into a porcelain dish and an equal

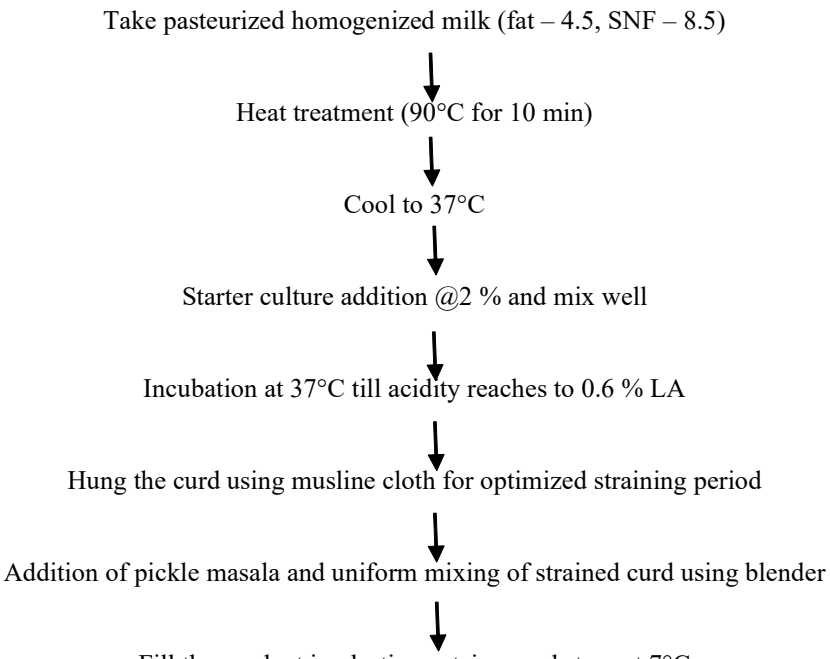

Fig. 1 Flow chart for the preparation of Greek yoghurt

volume of distilled water was added to it. Then 1 ml of phenolphthalein indicator was added and it was titrated against 0.1[N] NaOH till the appearance of light pink color, which persisted for 30 seconds in the solution. Titratable acidity was calculated as follow

$$\text{Acidity (\% Lactic acid)} = \frac{9 \times V \times N}{X}$$

Where, V = Volume of 0.1[N] NaOH required for the titration; N = Normality of NaOH solution and X = quantity of product taken for titration

Determination of total solids (TS)

The TS of product was determined by oven drying method (AOAC, 2012). Heat the clean dry empty dish in oven maintained at 100º ± 2ºC for one hour, cool in a desiccator and weigh. Weigh about 5 g product. Add 1-2 drops of phenolphthalein solution to the sample in the dish and neutralise with 0.1 N sodium hydroxide solution to a faint pink colour. Note the volume of 0.1N sodium hydroxide required to neutralize the sample. Place the dish on a boiling water bath until the water is removed from the sample. Wipe the under-surface of the dish and place in the oven maintained at 100 ± 2ºC, for 3 h. Remove the dish and cool in a desiccator and weigh. Continue heating and re-weighing at hourly intervals until successive weighings do not vary by more than 0.5 mg. Deduct half weight of the 0.1N sodium hydroxide added to neutralize the sample from the residue after drying and calculate total solids using following formula (FSSAI Lab manual 1, 2012).

$$a = \frac{N \times TV \times 40}{1000 \times 2}$$

$$\text{Total solids (\% w/w)} = \frac{(W2 - a)}{W1} \times 100$$

Where, N = Normality of NaOH; TV = Titre value; W2 = Weight in g of residue left after drying; W1 = Weight in g of the prepared sample taken and a = Half of the volume of 0.1 N Sodium hydroxide added

Sensory analysis of the product

The Greek yoghurt was subjected to the sensory evaluation by trained panel of nine judges on nine point hedonic scale (Stone and Sidel, 2004) with sensory perameters viz. flavor, body and texture, color and appearance and overall acceptability. The product was served at 10°C.

Compositional analysis of the product (Fat, Protein, Lactose, Calcium and Ash)

Fat percentage of Greek yoghurt was determined by Gerber method as per adopting the procedure FSSAI Lab manual 1 (2012). The protein content of Greek yoghurt was determined by kjeldahl method described in AOAC (1990). The lactose content of Greek yoghurt was determined by Lane Eynon method as per FSSAI

Lab manual 1 (2012). The calcium content of Greek yoghurt was determined by following the method mentioned in the BIS handbook (BIS: Part XI, 1981). Total ash was determined according to method described by Ranganna (1986).

Statistical analysis

All the data were subjected to statistical analysis using Completely Randomized Design (CRD) as per the methods described in Steel and Torrie (1980). The significance was tested at 5 % level using mean value, co-efficient of variance (% CV) and critical difference (CD). The values for starter counts were log transformed before analysis. The results were presented as mean ± SD of the replicates. All the observations were taken in triplicates.

Results and Discussion

In order to minimize the fat losses during straining, homogenized milk was taken for the preparation of Greek yoghurt in our experiment. Desai et al. (1985) reported that fat losses in the whey were reduced during the manufacture of strained yoghurt by homogenization of the milk before addition of starter cultures during fermentation stage.

Selection of suitable starter culture

Each starter culture was inoculated at the rate of 2 % in previously heat treated and cooled standardized homogenized milk to prepare Greek yoghurt. The prepared product was subjected to lactic count, pH, titratable acidity, TS and sensory analysis in order to select the best suited starter culture to prepare Greek yoghurt.

From the Table 1 it is observed that lactobacilli counts were ranged from 8.39 to 8.86 log CFU/ml in Greek yoghurt prepared with various starter cultures. Lactobacilli counts of Greek yoghurt prepared by C4 starter was higher (8.86 log CFU/ml) followed by C3 (8.82 log CFU/ml), C1 (8.77 log CFU/ml) and C2 (8.39 log CFU/ml). No significant change in lactobacilli count was observed between C4 and C3 starter containing Greek yoghurt. While lactobacilli counts of C1 and C2 starter containing Greek yoghurt differed significantly ($p<0.05$). In case of streptococci count of Greek yoghurt prepared by various starters, the counts were in range of 9.20 to 10.17 log CFU/ml. Highest streptococci count was in C2 treatment (10.17 log CFU/ml) followed by C1 (10.16 log CFU/ml), C3 (9.69 log CFU/ml) and C4 (9.20 log CFU/ml). pH of the Greek yoghurt prepared with different starter cultures was found in the range of 4.87 to 4.49. C2 starter showed highest reduction in pH (4.49) followed by C3 (4.60), C4 (4.65) and C1 (4.87). No significant difference observed in C2, C3 and C4 treatment. The acidity (% LA) produced by the different treatments was in the range of 0.87 % LA to 0.97 % LA. The Greek yoghurt containing starter culture C3 showed highest acidity production (0.97 % LA) followed by C4 (0.94 %), C1 (0.92 % LA) and C2 (0.87 % LA). No significant difference observed between C3 and C4 treatment. Total solids (TS) of Greek yoghurt prepared with different starters was found in the range of 22.27 to 23. 41 %. Among all the starters, the Greek yoghurt prepared with C4 showed the highest TS (23.41 %) followed by C3 (23.12 %), C1 (22.42 %) and C2 (22.27 %).

The sensory evaluation score of the Greek yoghurt prepared with different starters is shown in Fig. 2. Significant ($p<0.05$) difference was observed in body and texture as well as overall acceptability scores of product while no significant difference observed in flavor and color & appearance of product prepared using different starters. In case of body and texture, C4 treatment received highest score (8.63) followed by C2 (8.21), C3 (7.83) and C4 (7.71). C4 again received highest overall acceptability score (8.88) followed by C3 (8.67), C1 (8.19) and C2 (8.08).

The Greek yoghurt prepared with starter C4 received highest score for lactobacilli count, TS and overall acceptability compared to remaining. The pH and acidity (% LA) value of product containing C4 starter was at par with the product containing other starter received highest score for pH and acidity (% LA). So the C4 is considered most suitable among all starters to prepare Greek yoghurt.

Total plate counts of 7 log to 8 log CFU/ml have been reported for strained yoghurt (Rosenthal et al. 1980; Yamani and Abu-Jaber, 1994; Al-Kadamany et al. 2003). In another study, conducted

Table 1 Effect of different starter on lactic count and chemical parameters of product

LAB Combination	Lactobacilli (Log CFU/ml)	Streptococci (Log CFU/ml)	pH	Acidity (% LA)	TS (%)
C1	8.77 ± 0.25	10.16 ± 0.18	4.49 ± 0.24	0.92 ± 0.04	22.42 ± 0.14
C2	8.39 ± 0.27	10.17 ± 0.32	4.87 ± 0.13	0.87 ± 0.02	22.27 ± 0.49
C3	8.82 ± 0.06	9.69 ± 0.86	4.60 ± 0.14	0.97 ± 0.04	23.12 ± 0.81
C4	8.86 ± 0.23	9.20 ± 0.30	4.65 ± 0.13	0.94 ± 0.05	23.41 ± 0.64
SEm	0.11	0.24	0.08	0.02	0.29
CD (0.05)	0.33	0.75	0.26	0.06	0.89
CV %	2.49	4.98	3.58	3.94	2.53

Data are presented as mean ± SD (n=3)

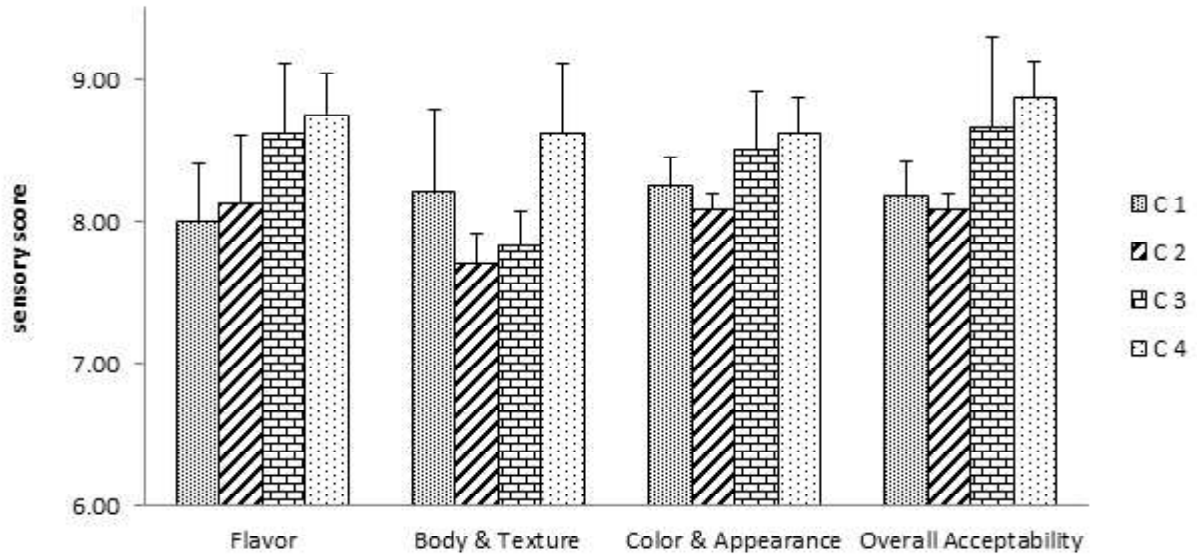

Fig. 2 Effect of different starter on sensory parameters of Greek yoghurt (C1: *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus helveticus* MTCC 5463; C2: *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus rhamnosus* MTCC 5946; C3: *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus bulgaricus* NCIM 2358 (yoghurt culture) and C4: *Streptococcus thermophilus* MTCC 5460 + *Lactobacillus bulgaricus* NCIM 2358 + *Lactobacillus helveticus* MTCC 5463); Error bars show standard deviation (n=3)

Table 2 Effect of straining period on lactic count and chemical parameters of product

Straining Period (Min)	Lactobacilli (Log CFU/ml)	Streptococci (Log CFU/ml)	pH	Acidity (% LA)	TS (%)
15	9.09 ± 0.32	10.97± 0.18	4.83 ± 0.13	0.75 ± 0.10	21.76 ± 0.67
30	9.32 ± 0.49	10.37 ± 0.79	4.67 ± 0.25	0.89 ± 0.08	23.99 ± 0.94
55	9.25 ± 0.45	10.47 ± 0.47	4.50 ± 0.08	1.08 ± 0.12	25.30 ± 0.86
SEm			0.07	0.04	0.37
CD (0.05)	NS	NS	0.23	0.14	1.14
CV %			3.55	11.03	3.51

Data are presented as mean ± SD (n=3) NS -Non significant

by Serhan et al. (2016) reported around 7 log CFU/ml starter culture counts in the concentrated yoghurt (labneh) made using goat milk. Ozer and Robinson (1999) reported that lactobacilli counts and streptococcus counts at the end of incubation period (pH 4.3) was 8 log CFU/ml and 9 log CFU/ml respectively. In this study also the starter count was in the range of 8 to 10 log CFU/ml. Aboudonia et al. (1992) and Amer et al. (1997) reported the use of different starter culture combinations for making strained yoghurt affects the overall quality of the product. We obtained the similar results when Greek yoghurt prepared with different starters. In one of the study acceptable strained yoghurt was prepared using *L. delbrueckii* subsp. *bulgaricus* in combination with *Enterococcus faecalis* instaed of using *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (El-Samragy et al. 1988). We also tried to prepare Greek yoghurt by utilizing starter other than *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and superior quality of Greek yoghurt was obtained.

Optimization of straining period

Greek yoghurt prepared using C4 starter was strained for different time periods viz. 15 min, 30 min and 55 min. The product strained at different time intervals was subjected to lactic count, pH, titratable acidity, TS and sensory analysis order to get most suitable straining period for Greek yoghurt preparation.

Table 2 shows that starter count i.e. lactobacilli and streptococci counts of product prepared by straining for different time period, has no significant difference. Whereas significant ($p<0.05$) pH drop was observed with increasing straining time from 15 min to 55 min. The pH of product strained at 15 min, 30 min and 55 min are 4.83, 4.67 and 4.50 respectively. While no significant drop in

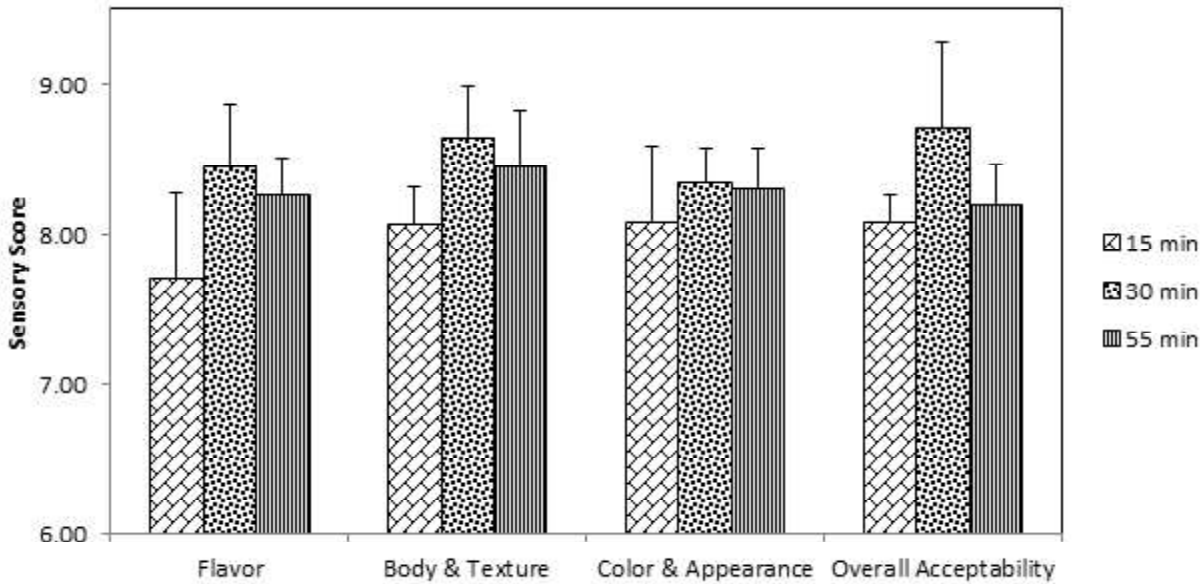

Fig. 3 Effect of straining for different period of time on sensory parameters of Greek yoghurt; Error bars show standard deviation (n=3)

pH was found in the product when straining period was increased from 15 min to 30 min. Also no significant drop in pH was found in the product when straining period was increased from 30 min and 55 min but the pH was reduced significantly ($p<0.05$) when product made by straining for 15 min and 55 min. Similar trend was observed in titratable acidity. The acidity was increased significantly ($p<0.05$) with increasing the straining period. Titratable acidity of the product strained for 15 min, 30 min and 55 min are 0.75 % LA, 0.89 % LA and 1.08 % LA respectively. While no significant increase in titratable acidity observed when straining period was increased from 15 min to 30 min. The titratable acidity was increased significantly ($p<0.05$) when straining period further increased to 55 min. TS of the product was also increased significantly ($p<0.05$) with increasing the straining period. TS of the product strained for 15 min, 30 min and 55 min are 21.76 %, 23.99 % and 25.30 % respectively.

The sensory evaluation score of the Greek yoghurt prepared by straining for different period of time is shown in Fig. 3. The color and appearance scores of Greek yoghurt prepared by straining for 15 min, 30 min and 55 min were non significant. Significant ($p<0.05$%) difference was observed in flavor, body and texture as well as overall acceptability scores. The scores for flavor was highest in Greek yoghurt prepared by 30 min straining period (8.46) followed by 55 min (8.26) and 15 min (7.70). The scores for body and texture was highest in Greek yoghurt prepared by 30 min straining period (8.64) followed by 55 min (8.45) and 15 min (8.07). The scores for overall acceptability was highest in Greek yoghurt prepared by 30 min straining period (8.71) followed by 55 min (8.20) and 15 min (8.08).

The straining period has no significant effect on starter count. The overall acceptability score of 30 min straining treatment was significantly ($p<0.05$) higher compared to remaining treatments. The overall acceptability score in sensory evaluation of product prepared by 15 min is the lowest among three treatments. This can be correlated with the comparatively less reduction in pH, lesser titratable acidity (% LA) and low TS (%) of the product prepared by 15 min than 30 min and 55 min. Straining the curd for 55 min has adversely affected the pH, titratable acidity and sensory parameters of Greek yoghurt. Hence it was concluded that Greek yoghurt prepared by straining for 30 min is more acceptable than the remaining tratments.

Vargas *et al.* (2008) reported that longer the draining of the yogurt in the cloth bags, the higher the total solids of the final product. Thus, moisture content is related to syneresis. Similar result was also observed in this study, the total solid content in the product was increased with increasing the straining period from 15 min to 55 min. Tamime (1977) reported that use of various strains of yoghurt starter culture affects the rate of whey drainage during straining. The sensory properties of Greek yoghurt made by traditional method (straining in muslin cloth bag) are excellent (Robinson, 20002) compared to other methods. Preparation of Greek yoghurt by straining in muslin cloth bag is still preferred in Middle East, as the investment in mechanized system of production are quite high (Lange, 2020).

Optimization of rate of addition of pickle masala

Greek yoghurt was prepared using C4 starter and strained for 30 min with different rate of addition of pickle masala viz. 1%, 1.5% and 2%. The product containing different proportion of pickle masala was subjected to starter count, pH, titratable acidity, TS and sensory analysis in order to select most appropriate rate of addition of pickle masala.

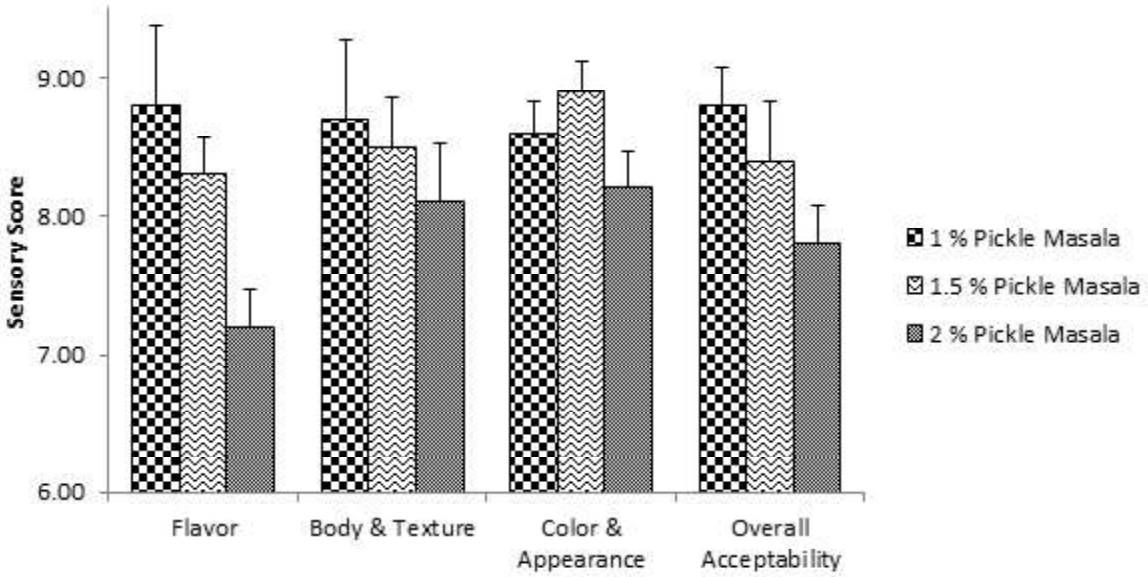

Fig. 4 Effect of different concentration of pickle *masala* on sensory parameters of Greek yoghurt; Error bars show standard deviation (n=3)

Table 3 Effect of varying rate of addition of pickle masala on lactic count and chemical parameters of product

Rate of pickle masala addition (%)	Lactobacilli (Log CFU/ml)	Streptococci (Log CFU/ml)	pH	Acidity (% LA)	TS (%)
1.0	9.05± 0.16	10.37± 0.18	4.81± 0.07	0.90± 0.06	23.02± 0.17
1.5	8.73± 0.16	10.01± 0.28	4.67± 0.05	0.98± 0.03	23.13± 0.31
2.0	8.48± 0.32	10.00± 0.23	4.62± 0.09	0.98± 0.07	23.54± 0.22
SEm	0.10	0.11	0.03	0.025	0.11
CD (0.05)	0.32	0.32	0.10	0.076	0.33
CV %	2.62	2.32	1.52	5.78	1.03

Data are presented as mean ± SD (n=3)

Table 3 shows that with increasing rate of addition of pickle masala from 1 % to 2% has significantly ($p<0.05$) reduced the lactobacilli and streptococci count. The lactobacillus count was in the range of 8.48 log CFU/ml to 9.05 log CFU/ml. streptococcus count was in the range of 10.00 log CFU/ml to 10.37 log CFU/ml. Significant ($p<0.05$) pH reduction was observed with increasing rate of pickle masala addition from 1 % to 2 %. The pH of product containing 1 %, 1.5 % and 3 % are 4.81, 4.67 and 4.62 respectively. Similar trend was observed in titratable acidity. The acidity was increased significantly ($p<0.05$) with increasing the rate of pickle masala addition. Titratable acidity of the product containing 1 %, 1.5 % and 3 % are 0.90 % LA, 0.98 % LA and 0.98 % LA respectively. TS of the product was also increased significantly ($p<0.05$) with increasing rate of pickle masala addition. TS of the product containing 1 %, 1.5 % and 3 % are 23.02 %, 23.13 % and 23.54 % respectively.

The sensory evaluation score of the Greek yoghurt prepared with different rate of addition of pickle masala is shown in Fig. 4. The body and texture scores of Greek yoghurt prepared by 1 %, 1.5 % and 2 % pickle masala were non- significant. Significant ($p<0.05\%$) difference was observed in flavor, color and appearance as well as overall acceptability scores. The scores for flavor was highest in Greek yoghurt prepared by 1 % pickle masala (8.80) followed by 1.5 % (8.30) and 2 % (7.20). The scores for body and texture was highest in Greek yoghurt prepared by 1.5 % pickle masala (8.60) followed by 1 % (8.90) and 2 % (8.20). The scores for overall acceptability was highest in Greek yoghurt prepared by 1 % pickle masala (8.80) followed by 1.5 % (8.40) and 2 % (7.80).

Addition of pickle masala affected adversely on starter count as well as sensory profile of the Greek yoghurt. So 1% rate of addition of pickle masala as flavoring agent was considered as more suitable compared to 1.5 % and 2 %.

The streptococci count and lactobacilli counts were around 8 log CFU/ml in fresh fruit flavored Greek yoghurt (Abou et al. 2013). Upadhyay et al. (1984) and Upadhyay et al. (1985) found a positive correlation between the chemical changes and microbial counts of sweetened strained yoghurt and a sensory evaluation of fresh samples. While we observed the inverse relation between chemical changes and starter counts of pickle flavored Greek yoghurt. The reason behind getting such result is not known

Table 4 Compositional analysis of optimized Greek yoghurt

Constituents	Concentration
Fat (%)	7.92 ± 0.22
Protein (%)	6.17 ± 0.17
Lactose (%)	4.91 ± 0.28
Calcium (mg/100g)	171.00 ± 0.82
Ash (%)	1.29 ± 0.15
Total Solids (%)	23.02 ± 0.17

Compositional analysis of optimized Greek yoghurt

The optimized Greek yoghurt was prepared from standardized homogenized milk (4.5% fat and 8.5% SNF) with C4 starter culture. Straining was carried out for 30 min and the pickle masala was added at the rate of 1% of strained curd weight. The product was subjected to TS (%), fat (%), protein (%), lactose (%), calcium (mg/100g) and ash (%) analysis. The composition of the product is shown in Table 4.

Composition of Greek yoghurt varies, containing usually 23 to 25 % TS (Abu-Jdayil et al. 2002) and 6 to 11 % fat (Nsabimana et al. 2005, Tamime and Robinson, 2007). According to the Codex Standard for Fermented Milk (Codex Alimentarius, 2003) strained yogurt should have minimum of 5.6 % protein. Tamime et al. (2014) analysed 109 commercial concentrated fermented milks with different geographical origins. The protein content in these commercial products varied from approximately 4.5 to 8%. In a similar study, Desai et al. (2013) collected 24 samples of Plain commercial Greek yogurts from across the United States. They reported that Protein and TS of all the samples ranged from 5.8 to 10.6 % and from 15 to 23.8 %, respectively. Ozer and Robinson (1999) reported that concentrated yoghurt prepared by traditional method contains 5.66 % lactose. Serhan et al. (2016) reported that ash content of concentrated yoghurt (labneh) prepared from cow milk was 1.16 %. While Calcium levels in 38 samples of strained yoghurts ranged between 43 to 921 mg/100 g (Abou Jaoude et al. 2010). Greek Yoghurt optimized in this study contains 23.02 % TS, 7.92 % fat, 6.17 % protein, 4.91 % lactose, 1.29 % ash and 171 mg/100g calcium. All the compositional parameters of Greek yoghurt prepared in this study are either in the range or nearer to the results reported by above mentioned authors.

Conclusion

The Greek yoghurt can be prepared at large scale using indigenous starter culture (*Streptococcus thermophilus* MTCC 5460, *Lactobacillus bulgaricus* NCIM 2358 and *Lactobacillus helveticus* MTCC 5463) with 30 min straining in muslin cloth bag and containing 1% pickle masala as novel flavor. The viable cells of probiotic strain *Lactobacillus helveticus* MTCC 5463 were more than 8 log CFU/ml in the optimized product. To make the manufacturing of Greek yoghurt more feasible at large scale production, instead of straining it in muslin cloth trials can be taken by centrifugation, ultrafiltration or other less time consuming methods.

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RESEARCH ARTICLE

Antibacterial sensitivity of *Escherichia coli* isolated from milk and milk products in Jabalpur, MP, India

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Abstract: Milk and milk products are an essential element of the diet. Apart from its nutritional value, it can also serve as a carrier for multidrug-resistant bacteria attributed to many infectious diseases. The present study was conducted to detect antibiotic-resistant bacteria from milk and milk products (Raw milk, pasteurized milk, and cottage cheese) marketed in Jabalpur city of Madhya Pradesh, India. A total of 640 samples of pasteurized milk, raw milk, and cottage cheese were collected in a sterile container from different dairies and shops of Jabalpur city. Immediately after collection, samples were brought to the microbiology lab within 1 hour. The *Escherichea coli* were isolated from milk and cottage cheese samples, based on cultural and molecular tests. These isolates were further subjected to antibacterial susceptibility against commonly used antibiotics by the disk diffusion methods. Out of 640 samples examined, 118 (18.44%) were positive for *Escherichea coli.* The highest isolation of *Escherichea coli* was from cottage cheese (32%), followed by raw milk (22.5%) and pasteurized milk (0%). Antibiotic susceptibility profile showed that *Escherichia coli* were resistant for nitrofurantoin (61.8%), nalidixic acid (37.2%) and cefotaxime (30.50%). The analysis showed that 89.8 % of isolates showed multidrug resistance comprising 2-3 antimicrobials. The presence of *Escherichea coli* with multiple antibiotic resistances poses a significant threat to public health and food safety. These findings stress the need for better sanitation practices in the production and consumption of milk and milk products and strict monitoring of uses and misuses of antibiotics in humans and food animals.

Keywords: Antibiotic resistance, Antibiotic-resistant bacteria, Milk and milk products

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Introduction

Milk and milk products are an indispensable part of the Indian diet. When milk is secreted from mammary glands to alveoli of the udder, it is generally free from microbes (Tolle, 1980). However, later on, different sources might contribute to the contamination of milk with a wide variety of microbial populations (Mennane et al. 2007). Unhygienic practices in pre milking preparation of udder, substandard hygiene of milk handlers, and poor sanitation practices related to milking and storage equipments are the responsible factors for contamination of raw milk at different critical points (Gardew et al. 2012). Milk and milk products are rich in various nutrients such as; proteins, fats, carbohydrates, minerals, and vitamins. These nutritional contents work as a perfect medium for the growth of microbes. Microbial quality of the milk and milk products also depends upon production procedures and post-production processing, handling, packaging, and storage of products (Kumar et al. 2014). In India, several studies reported contamination of dairy products with various pathogenic microorganisms that could cause disease in humans (Desale et al. 2009; Godbole et al. 2013). According to Elmnoir et al. (2018), up to 5% of foodborne infections are attributed to the consumption of milk and dairy products in developing countries like India. Therefore, milk and its products can be an efficient vehicle for transmission of diseases causing agents to human beings (Garedew et al. 2012).

Escherichia coli bacteria are frequently used as an indicator of fecal contamination of milk and dairy products and may impose the presence of disease-causing serotypes for humans. Various strains of *E. coli* have been associated with several life-threatening food-borne outbreaks worldwide (Elmnoir et al. 2018).

Antibacterial resistance can further increase the mortality rate as various resistant strains of *Escherichea coli* have been reported globally (Bell et al. 2002). Being referred to as 'AMR Capital'

condition for India is challenging as other infectious diseases such as Malaria, Tuberculosis, and Cholera are still prevalent in the communities (Chaudhry et al. 2017). Due to a lack of awareness about communicable diseases and poor access to health sectors, the Indian population, usually self medicate with antimicrobial agents, having no knowledge of doses and duration of treatment (Morgan et al. 2011). Various other factors, such as poverty, illiteracy, malnutrition, and overcrowding, further increase the problem (Swaminathan et al. 2016).

In milk production, India acquires the first position in the world (Awan et al. 2014). It is estimated that India's milk production will reach up to 180-200 million by 2021-2022, with a growth rate of 5% per annum (Parekh, 2011). However, with the amplifying production of milk, the demands of milk have also been increased over the years due to exponential population growth rate, increased urbanization, and scattered colonization (Awan et al. 2014). Still, there exists a gap in supply and demand. Because of this gap and poorly organized non-regulatory marketing system, the quality of milk is being compromised. Therefore, this study aimed to investigates the prevalence of *Escherichea coli* as a principal foodborne agent in milk and dairy products collected from markets in Jabalpur town of India. Antibiotic resistance of this pathogen was also studied.

Material and Methods

Study area

This study was conducted in the Jabalpur city of Madhya Pradesh, India. This city is purposively selected because it is one of three most populous city of Madhya Pradesh (Jabalpur ranks third after Indore and Bhopal with a population of 1,268,848 in 2011 survey) making it more prone to faulty practices in milk distribution to fulfill the demand of such a large population

Experimental design

A cross-sectional study was conducted from October 2015 to March 2016 to determine the incidence and antibacterial resistance of *Escherichea coli* in milk and milk products (Raw milk, pasteurized milk, and cottage cheese) samples. In the present study, 640 samples (Raw milk = 240, pasteurized milk = 200 and cottage cheese = 200) were collected. Dairy outlets, shops, and supermarkets that had a high level of consumers were included in this study.

Collection and analysis of samples for laboratory analysis

Samples were collected according to the instructions introduced by the International Dairy Federation. Until the analysis was performed, the samples had kept at 4°C.

Analysis of samples

Isolation and Identification of *Escherichea coli*

Enrichment of the sample was done by using EC-broth at 37^0 C for 24 hours, after that, Mac Conkey agar media were streaked by enriched sample and incubated at 37^0 C for 24 hours. The single pink colony was then picked up and gram stained for morphological identification and further transferred on Eosin Methylene Blue agar to get typical metallic sheen colonies of *Escherichea coli*. These metallic sheen colonies were transferred to nutrient agar to conduct further confirmatory biochemical tests (IMViC).

Testing for antibacterial susceptibility

The disc diffusion method was used to determine the antibacterial susceptibility. Mueller-Hinton agar media was used for this purpose as per the criteria defined by the National Committee for Clinical Laboratory Standards (Kiehlbauch et al. 2000). The isolated *Escherichia coli* were tested for sensitivity to the most commonly used antibiotics including, Ciprofloxacin (CIP) (05 mcg), Ceftazidime (CAZ) (30 mcg), Cefotaxime (CTX) (30 mcg), Netillin (NET) (30 mcg), Ofloxacin (OF) (01 mcg), Norfloxacin (NX) (50 mcg), Nalidixic acid (NA) (30 mcg), Nitrofurantoin (NIT) (300 mcg), Gentamicin (GEN) (30 mcg). Antibiotics discs were procured from HiMedia Laboratories Pvt Ltd, Mumbai, and Maharashtra.

Results and Discussion

The results of analysis for prevalence and antibacterial sensitivity of *Escherichia coli* are presented in Tables 1, 2, and 3. The present research revealed that *Escherichea coli* were isolated from 18.44% of milk and milk products (raw milk, pasteurized milk, and cottage cheese). Meanwhile, this study also confirmed that *Escherichea coli* were not present in pasteurized milk. The presence of *Escherichea coli* in pasteurized milk does not necessarily show that organism can survive the pasteurization temperature. Poor hygienic handling after the milk is pasteurized might contributes to milk contamination. Our findings simulate with the results obtained by Bedasa et al. (2018), where they observed the absence of *Escherichea coli* in pasteurized milk.

In the current research, 22.5% of raw milk samples were found to harbor *Escherichea coli,* which is somewhat in agreement with the report of 21.66% by Bonyadian et al. (2014). However, prevalence in current study is much lower when compared to 44.5%, 100%, 51.66%, and 83% prevalence reported by Tadeesi et al. (2018) from Ethiopia, Swai and Schoonman (2011) from Tanzania, Soomro et al. (2002) from Tandozam Pakistan (51.66%) and Kilango et al. (2012) from Deres Sallam, Tanzania respectively and far higher when compared to 14.65% prevalence reported by Younis et al. (2018). These variations might be due to differences

in animal management, milking system, and milk handling and storage practices in different countries.

Further, in the present study, 32% isolation rate of *Escherichea coli* was recorded from cottage cheese (Cottage cheese) samples. This prevalence is slightly higher than the report of Ombark et al. (2018) (29.7%) and De Campos et al. (2018) (19.8%). Though, when Indian cottage cheese (Cottage cheese) is manufactured under strict conditions, it may not contain any pathogens, but unsanitary practices during handling, storage, and packaging, after the product is prepared, might contribute to the growth of these organisms (Rao et al. 1992**;** Kumar et al. 2014). Several studies reported a high prevalence of *Escherichea coli* (Kumar et al. 2010; Ahmadi and Panda, 2015; Selvamalar et al. 2018) because other than the contamination during handing, *Escherichea coli* was also found to survive the manufacturing of cottage cheese. Unpasteurized and improperly pasteurized milk could be a vital source for the transmission of this pathogen (Wahi et al. 2006)

Most of the foodborne illness is associated with foods of animal origin. Now a day, the drug-resistant pathogen in milk and milk products is becoming an increasing public health problem worldwide due to the excessive use of antibiotics in animal feed (Pérez-Rodríguez et al. 2019). Resistance towards drugs also emerges from the extensive use of antibacterial in humans and animals and consequent transfer of resistance genes among animals, human beings, animal products, and surroundings (Tadeesi et al. 2018).

In India, there have been reports on drug resistance of *Escherichea coli* isolates from milk and milk products (Selvamalar et al. 2018; Singh et al. 2018). Other researchers have previously reported the link between the use of antibacterial drugs in animal farming and the incidence of antibacterial resistant organisms in the food products obtained from these animals (Aaresbeep, 2000; Asai et al. 2005; Van den Boogard et al. 2001).

The high antibacterial resistance observed in this research might be due to the extensive use of antibiotics in animals to treat different diseases. In the current study, maximum numbers of isolates were resistant towards nitrofurantoin (61.8%), nalidixic acid (37.2%) and cefotaxime (30.50%). Meanwhile, this study also revealed that all the *Escherichea coli* isolates were sensitive to ofloxacin. Similarly, Esquivel et al. (2008) and Bhatt & Lakhy (2008) also reported the sensitivity of *Escherichea coli* towards ofloxacin. Reported resistance of nitrofurantoin and nalidixic acid in this study was similar to the findings of Uddin et al. (2011). However, various researchers reported that *Escherichea coli* is

Table 1 Prevalence of *Escherichea coli* in different sources (n=640)

Products	Prevalence of *Escherichea coli*		
	No. of samples	No. of samples Positive	Percentage of sample positive
Pasteurized Milk	200	0	0%
Raw Milk	240	54	22.5%
Cottage Cheese	200	64	31.6%
Total	640	118	18.43%

Table 2 Antibiotic sensitivity of *Escherichea coli* isolated from Milk and Milk Products (n=118)

S.No.	Name of Antibacterial Agent	No. of isolates screened	Pattern of Antibiogram		
			Resistant	Intermediate	Sensitive
1	Norfloxacin(10mcg)	118	04 (3.38%)	06 (5.08%)	108 (91.5%)
2	Ofloxacin(5 mcg)	118	0(0%)	0(0%)	118(100%)
3	Ceftazidime(30mcg)	118	15 (12.71%)	09 (7.63 %)	94 (79.66%)
4	Ciprofloxacin(5mcg)	118	09 (7.63%)	19 (16.10%)	90 (76.27%)
5	Cefotaxime(30 mcg)	118	36 (30.51%)	35 (29.66%)	47 (39.83%)
6	Nalidixic acid(30 mcg)	118	44 (37.29%)	04 (3.39%)	70 (59.32%)
7	Nitrofurantoin(300mcg)	118	73 (61.86%)	22 (18.64%)	23(19.49%)
8	Gentamicin(30 mcg)	118	14(11.86%)	0 (0%)	104(88.13%)

Table 3 Multiple drug resistance in *E coli.* isolates

Number of Antimicrobials	Number of resistant Isolates	Percentage of resistant Isolates
One drug	12	10.16
Two drug	71	60.17
Three drug	35	29.66
Multi drug resistant isolates	106	89.83

highly susceptible to nitrofurantoin (Hafsa et al. 2013; Ntuli et al. 2016; and Abike et al. 2015), which is contrary to the results of our research. But in Egypt, Elmonir et al. (2018) reported that *Escherichea coli* isolates were resistant to nitrofurantoin, which is in line with the findings of the present study.

Furthermore, the current study also revealed that *Escherichea coli* showed resistance to gentamicin, ciprofloxacin, norfloxacin, ceftazidime. However, the percentage of resistance varied with the antibiotics. These variations could be a manifestation of the use and misuse of these antibiotics in the population. These findings are not shocking because, in India, the general population has easy access to various antibiotics at any drug store without any prescription from a medical practitioner.

Multidrug resistance analysis showed that 89.8% of tested isolates were resistant to two to three antibiotics. These results are in line with the finding of Mude et al. (2017), who showed 92% of multidrug-resistance. Moreover, various researchers (Bekele et al. 2014; Iweriebor et al. 2015; Atnafir et al. 2017) from the different countries recorded multidrug resistance pattern. According to Aarestrup (1999) and Levin et al. (1997), multiple resistances are capable of regional dissemination and can develop as a result of antibacterial selection pressure in either live stocks or humans. Several pieces of evidence suggest that transmission of a resistant pathogen can occur in humans through food too (Oosterom, 1991; Khachatourians, 1998).

Conclusions

Milk and milk products collected from Jabalpur city were contaminated with *Escherichea coli,* and these bacteria showed resistance to various antibiotics. Contamination may originate from infected animals or unsanitary practices during processing, handling, and distribution of these products. Importantly, the incidence of *Escherichea coli* and its multiple antibiotics resistant profile reveals a risk for public health and food safety (Ulukanli et al. 2006). Therefore, good hygiene and sanitation practices should be mandated in all the farms and dairy outlets. Furthermore, there is a need for stricter laws to limit the sale of antibiotics to the population with a valid prescription from qualified medical professionals only.

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RESEARCH ARTICLE

Single nucleotide polymorphism at cluster of differentiation 14 (CD14) gene and its association with fertility traits in crossbred cattle of Kerala

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Abstract: Cluster of differentiation 14 (CD14) gene is an important molecule for innate unity and found to be significantly associated with fertility traits in cattle. The objectives of present study were to detect the single nucleotide polymorphisms (SNPs) in exon 1 region of CD14 gene and to evaluate the association of genetic variants with fertility traits viz. Service period and Age at First Calving in crossbred cattle of Kerala. The study was conducted on two hundred and sixteen crossbred cattle maintained at various farms of Kerala Veterinary and Animal Sciences University. Genomic DNA was isolated and polymorphisms of gene were detected by Single Strand Confirmation Polymorphism. Two SNPs, c.445T>G and c.432C>T were detected in exon 1 coding region of CD14 gene. The frequencies of CC and CT genotypes were 0.82 and 0.18 in the population. In addition, a significant association between SNPs and service period was observed in crossbred cattle population under study. Crossbred cattle with CT diplo types showed lower values for service period than those with CC diplo types. The association of CD14 gene with these traits emphasizes the importance of bovine CD14 as a candidate gene for marker assisted selection for fertility traits in crossbred cattle

Keywords: Association, CD14, Crossbred cattle, Fertility, Polymorphism,

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Introduction

India has the world's second largest bovine population with 192.49 million cattle, which is about 12.9% of the world's total cattle population. Crossbred cattle contribute around 24.45% to total milk and 54.89% to total cow milk production in the country (Anonymous, 2019). Kerala, one of the southern states of India, has a total cattle population of 1.33 million of which 94.20 % is crossbred cattle and only 5.80 % is indigenous (BAHS, 2019). Crossbred cattle of Kerala having exotic inheritance 50- 65 % arise due to crossing of low yielding *Bos indicus* with Holstein Friesian.

Fertility is economically important as it brings cattle into lactation, reduces reproductive disorders and maximizes the profitability by in time calf crop. Since milk production and fertility traits are negatively correlated, selection for enhanced milk performance may cause decline of cow fertility. Multitude of studies in dairy animals of developed countries have shown that selection for higher milk yield alone is associated with reduced health and fertility (Lucy, 2001; Van Raden et al. 2004; Cole and Van Raden, 2010; De Vries, 2019). Fertility can be measured by calving interval, calving rate, service per conception and age at first calving. Conventional selection relies upon phenotypic information only and causing slow genetic gain in the population. To augment the selection response for milk yield in dairy cattle, traditional selection methods could be complemented with gene assisted or marker assisted selection (MAS) using genomic variation of the traits (Wakchaure et al. 2015).

Cluster of differentiation 14 (CD14) gene is an important molecule for innate immunity that can act against a wide range of pathogens. CD molecule ranges from 1 to 166 with differential structure and functions of these CD14 is the most important molecule functions both as a cell membrane receptor and a soluble receptor for bacterial lipopolysaccharide (Goldsby et al. 2000; Pal et al. 2011). CD14 is found to be related to fertility traits in Holstein cattle by Cochran et al. (2013) and Ortega et al. (2017). Immune function is an important determinant of reproductive function. It might be due to involvement of immune function in the establishment of pregnancy (Hansen, 2011). Banos et al. (2013) identified significant phenotypic correlations of immune traits

with reproductive performance traits. They found positive correlation of Cluster of differentiation (CD) cells within the peripheral blood mononuclear cell population and calving interval. Cochran et al. (2013) identified 10 genes containing SNP related to reproductive traits that were involved in immune function.

However there is no report regarding genetic variation of CD14 in crossbred cattle of Kerala state, India. Hence present study was carried out with the objective to identify genetic polymorphism in exon 1 of CD14 and to explore association of this region with fertility traits of crossbred cattle.

Material and Methods

Collection of blood and DNA isolation

Approximately, 5 ml of venous blood was collected from 216 crossbred cattle maintained at the University Livestock Farm, Mannuthy; Cattle Breeding Farm, Thumburmuzhy and different field centres of ICAR- Filed progeny testing scheme, Mannuthy. Samples were stored at -20 °C until isolation of DNA. Genomic DNA was isolated from the frozen blood samples using phenol-chloroform extraction method (Sambrook and Russel, 2001) and samples were checked for its quality, purity and concentration.

Polymerase chain reaction

Primers (forward 5'AGTGTGCTTGGGCAATGTTC 3', reverse 5'CGGGTACTCTCGTCTCAAGG 3') were designed using primers3 software from the published information available in Genbank (accession no: NC_037334.1) for the amplification of exon 1 region of CD14 and were custom synthesised (Sigma-Aldrich). PCR reactions were carried out in 25 µL volume using 50 ηg of genomic DNA, 5 mL of 10X Buffer,1 mL of 10mMdNTP,10 pM each of forward and reverse primers, and 1 mL of JumpStart AccuTaq LA DNA Polymerase (2.5U/mL) with proofreading 5 Activity (Sigma–Aldrich) in a thermal cycler (Bio-Rad – My Cycler). The optimum PCR conditions was 95ºC for 3 min, followed by 35 cycles (30 sec at 94ºC, 45 sec at 61ºC and 45 sec at 72ºC), followed by 1 cycle at 72ºC for 5 min, stopped at 4ºC. Amplified PCR products were loaded into the wells of 1.5% agarose gel with a standard 50 bp DNA ladder (GenerRuler, MBI Fermentas, Germany) as a marker to check the size of the fragment. Electrophoresis was carried out at the rate 6 Volts/cm in 1X TBE buffer. Gels were stained with ethidium bromide and visualised under UV light and documented in a gel documentation system (Bio-Rad, USA).

Genotyping

Genotyping of the samples was done by Single Stand Confirmation Polymorphism (SSCP). The amplified fragments were mixed with SSCP loading buffer in the ratio of 1:3 (10 µL sample with 30µL dye), denatured at 95ºC for 10 min and immediately snap chilled in ice. The products were run in 12% poly-acrylamide gel at 4ºC for 2 h 30 min at 200 V. The composition of poly-acrylamide gel was 30% acrylamide/bis-acrylamide (29:1)-12 mL, 10% Ammonium per sulphate-150µL, TEMED-30 µL, 1X TBE-3 mL and nuclease free water-14.82mL. Gels were stained with silver nitrate as per the procedure described by Byun et al. (2009) and SSCP fragments were visualised directly. Diplo types were detected directly by observing SSCP pattern of samples in the gels. The haplotype and diplo type frequencies were estimated by direct counting method (Falconar and Mackay, 1998). Representative samples from different diplo types were sequenced to find out the nucleotide differences between different haplotype by automated sequencer (ABI prism) using Sanger's dideoxy chain termination method.

Statistical analysis

Major fertility traits considered in the study were age at first calving (AFC) and service period (SP). Association analysis of genotypes with fertility traits and major non-genetic and genetic factors viz. season and period of calving, centre and sire, were analyzed using fixed General Linear Model (GLM) of SPSS V.21.

Results and Discussion

Fertility is a complex process, influenced by genetic, nutritional, environmental factors and genotype-environmental interactions. Fertility traits such as age at first calving and service period have important impact on the profitability of dairy industry. Therefore, selective breeding for optimal fertility traits is vital for improving genetic gain.

PCR-SSCP was performed to detect the SNPs in exon 1 of CD14. In the present investigation, 206 bp fragment were amplified and PCR-SSCP of this fragment, corresponding to the exon 1 region exhibited pattern with two bands were identified as CC and four bands as CT diplo type (Fig. 1). The sequencing analysis of different haplotypes reveales presence of two SNPs; one T to G transversion at 103th position and other C to T transition at 116th position of the 206 bp fragment (Fig 2). In comparison with available GenBank sequence (accession no: NC_037334.1), T to G transversion was located in nucleotide sequences at positions 445 in exon 1 of CD14 and at 149th position of ORF. Further analysis revealed c.445T>G, was a non-synonymous mutation and it resulted in an amino acid substitution due to a codon change of TCA to GCA. SNP (C'!T transition) at 432 position of the exon 1 (144th position of ORF) was a synonymous SNP with codon change of CGC to CGT. Ibeagha-Awemu et al. (2008) detected five SNPs in 5' untranslated region (UTR) (g.C1291T), two in the coding regions (g.A1908G and g.A2318G) and two in the 3' UTR (g.A2601G and g.G2621T) of CD14 in Canadian Holsteins and Jersey cows. Pal et al. (2011) observed 27 SNPs in CD14of crossbred cattle in which 18 SNPs are non-synonymous mutations. They also suggested that -synonymous substitutions exceeding synonymous substitutions indicate the evolution of

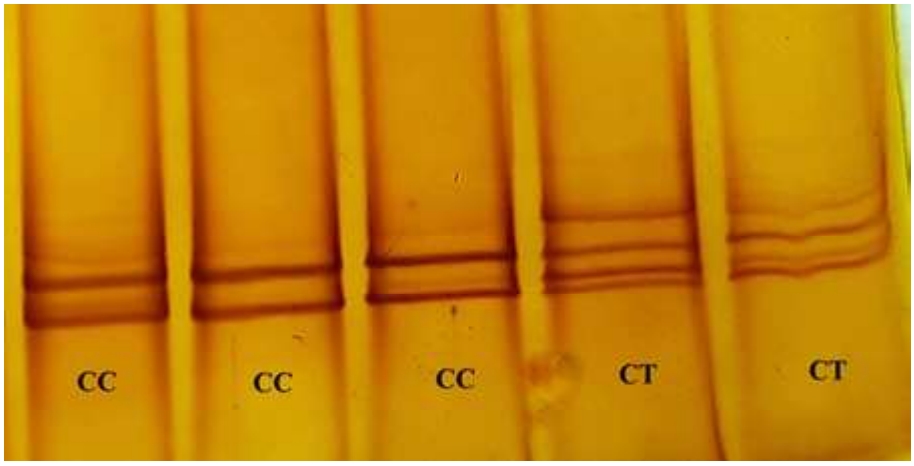

Fig 1. SSCP pattern of 206 bp fragment of CD14 gene (CC and CT genotype)

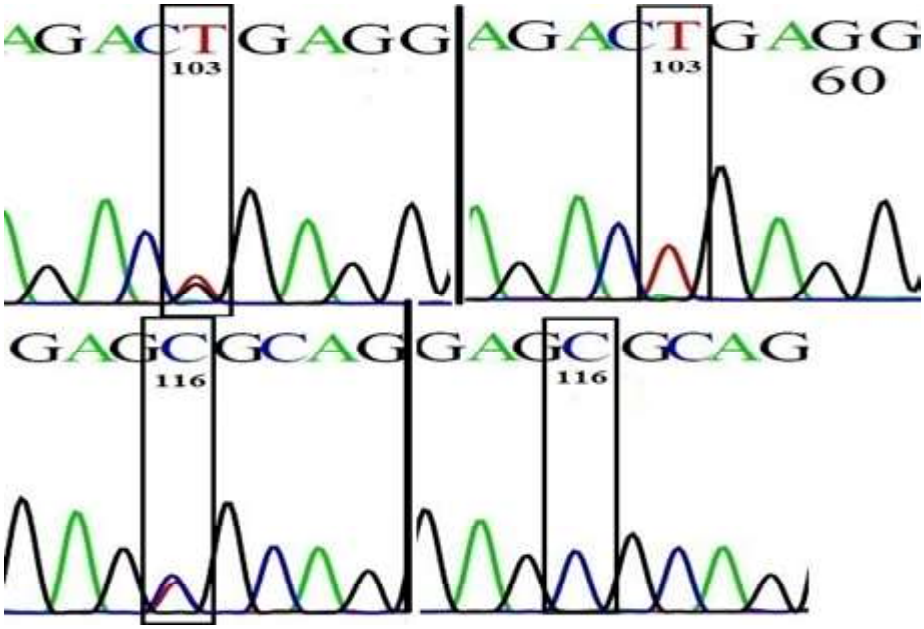

Fig 2. Sequence map for two Single Nucleotide Polymorphisms in the exon 1 of CD14 gene

this protein through positive selection among domestic animals. Selvan et al. (2014) screened Karan Fries (KF) cattle of India and reported six nucleotide changes in KF cows at positions T1117D, T1239G, T1291C, G1359C, G1361A and G1811A in a 832bp region (part of promoter, 5'UTR, exon 1, intron 1 and part of exon 2) of bovine CD14. However, Kumar et al. (2014) also reported these SNPs except T1117D in Sahiwal (*Bos indicus*) cows.

The diplotypic and allelic frequencies based on PCR-SSCP pattern are presented in the Table 1. CC diplo type frequency and the frequency of 'C' haplotypes were found to be predominant in studied population. In the present study homozygote, TT diplo type was not observed in the screened population and the 'C' haplotype was almost fixed in the population. Chi-square analysis showed non-significant differences between breeds with respect to CD14 locus. It revealed that the screened population is under Hardy Weinberg equilibrium ($\chi 2=2.13<3.841$).

In the association analysis of CD14 genotypes with fertility traits, it was revealed that SP was significantly influenced by genotype ($p < 0.05$). Significantly lower SP was observed for cattle with diplo type CT (98.85 days), compared to the heterozygous diplo type CC (131.393 days). AFC found to be significantly affected by centre and season ($p < 0.05$). Different genotypes of CD14 gene were not significantly associated with AFC. Ortega et al. (2017) evaluated 68 SNP in candidate genes associated with genetic merit for fertility traits such as predicted transmitting ability (PTA) for Daughter Pregnancy Rate and reported that SNP (rs109621328) in CD14 significantly associated with services per conception and days open. Cochran et al. (2013) genotyped 434 candidate SNPs in 550 Holstein breed and found that SNP (rs109621328) in CD14 is significantly associated with daughter pregnancy rate and heifer conception rate.

Table 1 Diplotype and haplotype frequencies of exon 1 (206 bp fragment) of CD14 gene based on SSCP pattern

Parameter	Total (216)
Diplotype Frequency	
CC	0.82 (177)
CT	0.18 (39)
Haplotype Frequency	
C	0.90 (393)
T	0.09 (39)
$\chi 2$	2.13

Conclusions

PCR-SSCP analysis of exon 1 of CD14 established presence of a non-synonymous mutation (c.445T>G) and a synonymous mutation (c.432C>T) in crossbred cattle population of Kerala. In the present study two diplo types of CD14 in the screened animals were observed. Diplo types and fertility traits especially service period in the tested population of crossbred cattle showed significant association. Although CC diplo type was predominant in the population, CT was significantly associated with lower service period. Therefore, genetic variants of CD14 appear to be potential candidates for the selection of fertility trait improvement. However, SNP identified in the current study may be characterized by functional genomics studies, as well as in a large population for further validation.

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RESEARCH ARTICLE

Number of pregnancies and season of calving influence the production and reproduction traits in Nili-Ravi buffalo

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Abstract: Production and reproduction traits in Nili-Ravi buffaloes maintained at the ICAR-Central Institute for Research on Buffaloes, Sub-campus, Nabha, Punjab, were analysed to study the impact of Number of pregnancies (parity) and season of birth on performance. The least squares method was used to elucidate the effect of parity and season on production and reproduction traits. The overall least squares means of production traits, 305 days or less milk yield, total lactation milk yield, and lactation length were 2603.22±47.66 kg, 2691.82±56.20 kg, and 305.72±4.95 days, respectively, and reproduction traits age at service, age at calving, service period, calving interval and dry period were 1418.76±16.60 days, 1741.30±18.52 days, 163.52±7.33 days, 466.55±6.70 days and 157.11±4.18 days, respectively. The parity had shown significant effect on all studied production and reproduction traits. However, the season impacted only dry period in Nili-Ravi buffaloes.

Keywords: Nili-Ravi, Parity, Production traits, Reproduction traits, Season of calving.

Introduction

India is blessed with wealthy pool of buffalo genetic resources and world's superior buffalo breeds are native to India. Presently, there are 17 registered breeds of buffalo in India (ICAR-NBAGR, 2020) and among these identified buffalo breeds, the best breeds maintained for milk production are Murrah, Nili-Ravi, Mehsana, and Jaffarabadi. The Nili-Ravi is one of the best riverine breeds of buffalo having its breeding region in Amritsar and Tarn-Taran areas of Punjab. Animals have black colour of skin, walled eyes and characteristic white markings are seen on the forehead, face, muzzle, feet, and tail and are considered the typical features of Nili Ravi breed of buffalo. The pure '*Panch Kalyani*' (with all-white extremities) has a beautiful appearance, and the breed has a good production and reproduction potential, despite of this, the breed has a limited breeding region in the North of India. The probable reason could be the market and breeders' preference for Murrah breed over the Nili-Ravi breed. It is interesting to note that while a population of pure Nili breed has been decreasing, despite the fact that its milk production is not lower than that of the Murrah. The quality of milk production and reproduction traits in Nili-Ravi are thought to be similar to an average breed of Murrah buffalo and is regarded to be superior in reproduction than Murrah (Vij and Tantia, 2005).

Production and reproduction is the significant economic trait and governs the economics of the dairying. The production and reproduction traits may be influenced by several factors which can be either genetic or non-genetic in nature. Variations in the environment may have great influence than the genetic components of variances (Price et al. 1991). Different non-genetic factors like season of calving, parity, period of calving, nutrition, temperature, services per conception, milking frequency, udder diseases, reproductive problems, housing and management conditions of the farm may masks the expression of precise genetic potential in the animals (Cunha et al. 2008). There are limited studies and scanty literature available which can indicate or identify the most important factor(s) influencing the performance in Nili-Ravi buffalo. Thus, it becomes imperative to identify important factors affecting the performance, so that an accurate estimate of genetic merit could be made in the Nili-Ravi breed of buffalo. With this knowledge, present study has been aimed to find out the effect of number of pregnancies (parity) and season of calving on various production and reproduction performance in the Nili-Ravi buffalo.

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Materials and Methods

Sample size and performance traits

The present study has been conducted at the ICAR-Central Institute for Research on Buffaloes, (sub campus-Nabha farm), Patiala, Punjab situated at 212 meters above mean sea level (29.17 N & 75.72 E). About 214 Nili-Ravi buffaloes sired by 23 bulls and 90 dams across different parity and from the period of 2010 to 2015 were included in the study.

The performance traits have been classified as production and reproduction traits. Production traits included in the study were standard 305 days or less milk yield, total lactation milk yield, and lactation length and reproduction traits were age at service, age at calving, service period, calving interval, and dry period. All the lactations were clustered and finally 214 observations has taken for the study. The normal lactation was considered as a period of milk production by a cow for at least 100 days, the milk production in lactation has recorded a minimum of 500 kg and buffaloes without having the abortions, still birth, delayed calving and other reproductive disorders has been included in the study.

Statistical model and analysis

According to the number of calving the animals have been classified and coded into three groups, namely, first parity, second parity, and third and above parity. Depending on the type of feed and fodder availability in the farm and prevailing climatic conditions in the region as recorded in CIRB, Nabha farm, Patiala district of Punjab, the year was classified and coded into three seasons of calving, viz. summer (April to June), rainy (July to October), winter (November to March). The effect of non-genetic factors on production and reproduction traits were estimated by least-squares analysis of variance with the help of LSMLMW PC-2 VERSION software package (Harvey, 1990). The model was used with the assumptions that different components being fitted into the model are linear, independent, and additive. The Model for production and reproduction traits of all lactation is described as under

Model: $Y_{jkl} = \mu + S_{-j} + P_k + e_{jkl}$

Where,

Y_{jkl} = observation on l^{th} buffalo of k^{th} parity in j^{th} season of calving of the animal

μ = Overall mean

S_j = Fixed effect of j^{th} season of calving (j = 1-3)

P_k = Fixed effect of k^{th} animals parity (k = 1-3)

e_{jkl} = Random error NID ~ $(0, \sigma^2 e)$

The difference of means between any two subclasses of parity and season was tested for significance using Duncan's multiple range test (DMRT) as modified by Kramer (1957).

Results and Discussion

Standard 305 days or less milk yield (S305DMY)

The milk production of a buffalo is a result of the interplay between heredity and the environment. For improved accuracy of selection, it is important that the records reflect as precisely as possible the practical genetic potential for milk yield. The actual records themselves may be a poor indicator of breeding values since many environmental factors have marked effect on a Nili-Ravi buffalo's performance during any lactation. In order to check the actual milk production potential of the Nili-Ravi buffalo this trait has been analysed. The MSS value and analysis of variance for production and reproduction traits in Nili-Ravi buffalo are depicted in Table 1 and 2.

The overall least squares means of 305 days or less milk yield in Nili-Ravi buffalo was estimated as 2603.22±47.66 kg, (Table 3). Lower estimates of standard 305 days or less milk yield than the present study were reported by Hussain et al. (2006) as 2191.858±35.353 kg, Ahmad (2008) as 2030.13±14.58 kg, and Chaudhary (2015) as 1913.87±133.61 kg in Nili-Ravi buffaloes.

Table 1 Analysis of variance (M.S.S Values) of production traits in Nili-Ravi buffalo

Source of variation	305 days or less milk yield	Total lactation milk yield	Lactation length
Parity	2359470.62**	1237182.22*	23946.82**
Season	211826.11	160049.87	675.66

Table 2 Analysis of variance (M.S.S Values) of reproduction traits in Nili-Ravi buffalo

Source of variation	Age at service	Age at calving	Service period	Calving interval	Dry period
Parity	17997975.86**	15849399.38**	20041.15*	17137.50*	6639.11*
Season	9144.95	7743.13	4533.54	3328.41	5915.14*

** (p<0.01), and * (p<0.05)

This indicates that over a period of last 10-15 years the performance at Nili-Ravi herd had improved, indicating the superior milch potential of the breed.

Parity has significant effect on standard 305 days or less milk yield ($p<0.01$). Maximum S305DMY was observed in second parity (2725.72±75.71 kg) and minimum S305DMY was observed in first parity (2394.11±57.50).

Season of calving had shown non-significant effect on of standard 305 days or less lactation milk yield. The S305DMY in Nili-Ravi buffalo for summer, rainy, and winter season were estimated as 2554.52±105.31 kg, 2669.11±51.03 kg, and 2586.03±66.70 kg, respectively. Standard 305 days or less lactation milk yield was the highest in rainy season being a favourable season for production as well as reproduction, as ample supply of grasses and good quality fodder is available.

Total lactation milk yield

The overall least squares means of total lactation milk yield in Nili-Ravi buffalo was estimated as 2691.82±56.20 kg, (Table 3). Lower estimates of total lactation milk yield than the present study were obtained by many workers. The overall least square mean for total lactation milk yield in Nili-Ravi buffalo were reported by Khan and Chaudhry (2000) as 1984 kg, Manzoor et al. (2003) as 1774±25.4 kg, Afzal et al. (2007) as 1831.6±530.9 kg, Khan et al. (2007) as 1984.4±773.43 kg, Ahmad (2008) as 2462.92±195.93 kg, Bashir et al. (2015) as 1840±8.0 kg, Charlini and sinniah (2015) as 1187±543 kg, Chaudhary (2015) as 1941.44±148.19 kg, and which suggests the lower production than the present study.

The parity had shown a significant effect ($p<0.05$) on total lactation milk yield. Maximum milk yield was obtained in the second parity (2802.92±89.28 kg) and Minimum milk yield was obtained in the third and above parity (2726.08±96.50 kg). Significant influence of parity on total lactation milk yield was reported by Khan and Chaudhry (2000), Afzal et al. (2007), Khan et al. (2007), and Chaudhary (2015) in Nili-Ravi buffaloes.

Season of calving had non-significant effect on total lactation milk yield. The least-squares mean for total lactation milk yield in Nili-Ravi buffalo for summer, rainy, and winter season were estimated as 2650.25±124.18 kg, 2748.99±60.18 kg, and 2676.22±78.66 kg, respectively. Significant influence of season of calving on total lactation milk yield was reported by Khan and Chaudhry (2000), Manzoor et al. (2003), Khan et al. (2007), Afzal et al. (2007), Bashir et al. (2015). However, such effect could not be observed in the present study while non-significant effect on season calving reported by Chaudhary (2015) and Ghaffar et al. (1991) in Nili-Ravi buffaloes. Variable milk yield lead to the assumption that environmental factors, seasonal differences, type of feed, flood, draught, heavy rains, ambient temperature, humidity and level of management affects the production and reproduction traits. Management is bound to differ depending on the farm manager's capacity, his efficiency in staff supervision, his system of crop husbandry methods and the frequency of culling/ replacement in the herd.

Lactation length

The period from the date of calving to the date of the last milking is termed as lactation length. In addition to lactation days, maximum daily yield also governs the lactation length. In tropical countries environmental factors may affects the lactation length to the maximum extent. Higher lactation length around indicates the milch potential of the animals and longer peak milk yield is maintained in the more persistent buffaloes.

The overall least squares means of lactation length in Nili-Ravi buffalo was estimated as 305.72±4.95 days (Table 3). Lower estimates of least squares mean of lactation length than present study were obtained by Khan and Chaudhry (2000) as 289.5 days, Afzal et al. (2007) as 273.3±52.8 days, Khan et al. (2007) as

Table 3 Least squares mean with standard error of different production traits in Nili-Ravi buffalo

Traits	305 days or less milk yield	Total lactation milk yield	Lactation length
Overall (μ)	2603.22±47.66(214)	2691.82±56.20(214)	305.72±4.95(214)
		Parity	
First parity	2394.11^{a}±57.50(113)	2546.46^{a}±67.80(113)	324.28^{a}±5.98(113)
Second parity	2725.72^{b}±75.71(54)	2802.92^{b}±89.28(54)	308.76^{b}±7.87(54)
Third parity and above	2689.83^{b}±81.83(47)	2726.08^{b}±96.50(47)	284.11^{c}±8.51(47)
		Season of calving	
Summer season	2554.52±105.31(25)	2650.25±124.18(25)	310.45±10.95(25)
Rainy season	2669.11±51.03(124)	2748.99±60.18(124)	302.24±5.30(124)
Winter season	2586.03±66.70(65)	2676.22±78.66(65)	304.46±6.93(65)

Numbers in parenthesis indicate sample size under each class.
Means with the different superscripts in a column differs significantly.

266.6±55.15 days, Ahmad (2008) as 260.42±1.35 days, Bashir et al. (2015) as 278±0.8 days, Charlini and Sinniah (2015) as 238±78.8 days, and Chaudhary (2015) as 295.90±13.94 days in Nili-Ravi buffaloes. Higher estimates of average service period than present study were obtained by Ahmad (2008) as 340.57±61.70 days, and Hussain et al. (2006) as 369.53±8.44 days in Nili-Ravi buffaloes.

Parity had significant effect ($p<0.05$) on lactation length. The longest lactation length was observed in the first parity (324.28±5.98 days) and the shortest lactation length in the present study has been observed in the third and above parity (284.11±8.51 days). Significant influence of parity on lactation length was reported by Chaudhry (1992). Afzal et al. (2007) and Bashir et al. (2015) reported non-significant effect of parity on lactation length in Nili-Ravi buffaloes.

Season of calving had non-significant effect on lactation length in the present study. The overall least-squares mean for lactation length in Nili-Ravi buffalo for summer, rainy, and winter season was estimated as 310.45±10.95 days, 302.24±5.30 days, and 304.46±6.93 days, respectively. Naqvi and Shami (1999) in late maturing Nili-Ravi group and Bashir et al. (2015) in Nili-Ravi buffaloes reported that significant influence of season of calving on lactation length while non-significant effect on lactation length was reported by Khan and Chaudhry (2000) and Afzal et al. (2007), in Nili-Ravi buffaloes of Pakistan.

Age at service

The overall least squares mean of age at service calving in Nili-Ravi buffalo was estimated as 1418.76±16.60 days (Table 4). Parity had significant effect ($p<0.05$) on age at service. The least-squares mean for age at service for first parity, second parity and third and above parity was estimated as 881.94±20.03 days, 1404.31±26.37 days, and 1970.01±28.51 days, respectively. Season of calving had non-significant effect on age at service. The least-squares mean for age at service in Nili-Ravi buffalo for summer, rainy, and winter season was estimated as 1412.81±36.68 days, 1411.39±17.78 days, and 1432.07±23.23 days, respectively.

Age at calving

This trait is most important for dairy economics and can be defined as the period between the date of birth and date of first calving of animals. The age at first calving has a direct bearing on the lifetime performance of buffaloes. Early first calving reduces the cost of rearing the heifer and generation interval generates the first lactation record earlier as well, thereby helping early selection and consequently increasing the genetic gain. The overall average age at calving in Nili-Ravi buffalo was estimated as 1741.30±18.52 days (Table 4).

Parity had significant effect ($p<0.05$) on age at calving. The least-squares mean for age at calving for first parity, second parity and third and above parity was estimated as 1243.59±22.49 days, 1711.45±29.42 days, and 2268.85±31.80 days, respectively. Higher estimates of average age at calving in the first parity than present study were obtained by Naqvi and Shami (1999) as 1291.31±8.68 days in early maturing Nili-Ravi as well as 1308.78±16.44 days in late maturing Nili-Ravi group, Hussain et al. (2006) as 1644.87±36.31 days, Bashir et al. (2009) as 1685±9 days, Charlini and Sinniah (2015) as 1347.46±118.321 days, and Chaudhary (2015) as 1327.16±12.09 days in Nili-Ravi buffaloes.

Table 4 Least squares mean and standard error of different reproduction traits in Nili-Ravi buffalo reproduction traits

Traits	Age at service	Age at calving	Service period	Calving interval	Dry period
Overall (μ)	1418.76±16.60 (214)	1741.30±18.52 (214)	163.52±7.33 (214)	466.55±6.70 (214)	157.11±4.18 (214)
			Parity		
First parity	881.94^{a}±20.03 (113)	1243.59^{a}±22.49 (113)	182.42^{a}±8.84 (113)	483.84^{a}±8.08 (113)	167.59^{a}±5.04 (113)
Second parity	1404.31^{b}±26.37 (54)	1711.45^{b}±29.42 (54)	160.62^{b}±11.64 (54)	464.69^{b}±10.64 (54)	156.91^{b}±6.64 (54)
Third parity and above	1970.01^{c}±28.51 (47)	2268.85^{c}±31.80 (47)	147.50^{c}±12.58 (47)	451.13^{c}±11.50 (47)	146.84^{c}±7.17 (47)
			Season of calving		
Summer season	1412.81±36.68 (25)	1727.33±40.92 (25)	175.61±16.2 (25)	472.34±14.80 (25)	157.87^{a}±9.23 (25)
Rainy season	1411.39±17.78 (124)	1741.55±19.83 (124)	154.42±7.85 (124)	458.34±7.17 (124)	148.26^{b}±4.47 (124)
Winter season	1432.07±23.23 (65)	1755.01±25.92 (65)	160.52±10.26 (65)	468.98±9.37 (65)	165.21^{c}±5.85 (65)

Numbers in parenthesis indicate sample size under each class.

Means with the different superscripts in a column differs significantly.

Season of calving had non-significant effect on age at calving. The least-squares mean for age at calving in Nili-Ravi buffalo for summer, rainy, and winter season was estimated as 1727.33±40.92 days, 1741.55±19.83 days, and 1755.01± 25.92 days, respectively. The present findings were in agreement with Nawle et al. 2012, who reported non-significant effect on calving age in Murrah buffalo.

Service period

It is the period between calving and subsequent conception. The service period is one of the principal factors causing variations in the calving interval, and thus influencing the breeding efficiency in dairy buffaloes. Generally, an optimum period of 60 days is allowed as postpartum rest. However, it is also essential to optimize the service period to ensure an optimum calving interval of 13 to 14 months. This period is not only a physiological function but also greatly depends on managerial practices like heat detection and artificial insemination. Shorter the service period greater the profit from reproductive efficiency and milk production point of view.

The overall least squares means of service period in Nili-Ravi buffalo was estimated as 163.52±7.33 days (Table 4). Lower estimates of average service period than present study were obtained by Chaudhary (2015) as 150.78±16.53 days. Higher estimates of average service period than present study were obtained by Pervez et al. (1994) as 215.12±4.99 days and Hussain et al. (2006) as 208.35±24.03 days.

Parity had significant effect ($p<0.05$) on service period. Short service period was observed in the third and above parity (147.50±12.58 days) and long service period was observed in the first parity (182.42±8.84 days). Naqvi, (2000) and Ali et al. (2011) reported that parity had significant effect on service period. While Hussain et al. (2006) reported that parity had non-significant effect on service period in Nili-Ravi buffaloes.

Season of calving had non-significant effect on service period in the present study. The least-squares mean for service period in Nili-Ravi buffalo for summer, rainy, and winter season was estimated as 175.61±16.20 days, 154.42±7.85 days, and 160.52±10.26 days, respectively. Significant influence of season of calving on service period was reported by Naqvi and Shami (1999) in early maturing Nili-Ravi group, Naqvi (2000), Thevamanoharan, (2002), and Ali et al. (2011), while non-significant influence of season of calving on service period was reported by Naqvi and Shami (1999), and Hussain et al. (2006) late maturing Nili-Ravi group.

Calving interval

The calving interval is the period between two consecutive calving and comprises the service period. Since the gestation period is the least variable trait, the variation in calving interval leads to less number of calves in the lifetime and lower lifetime milk production, as well as the increased cost of replacement in the herd (Mahadevan, 1960). It is an index of reproduction and chiefly affected by lengths of the service period, lactation period, and dry period. Ideally, a minimum of 60 days of service period and 60 days of the dry period is essential for proper involution of the uterus and for proper growth of the foetus, respectively. However, if any of these periods is abnormally longer it would prolong the calving interval to an uneconomic level.

The overall least squares mean of calving interval in Nili-Ravi buffalo was estimated as 466.55±6.70 days (Table 4). Lower estimates of calving interval than the present study were obtained by many workers. The overall least square mean for calving interval in Nili-Ravi buffalo were reported by and Pervez et al. (1994) as 520.27±2.58 days, Charlini and Sinniah (2015) as 412±91.6 days, Chaudhary (2015) as 462.30±16.74 days. Higher calving interval than the present study was reported by Khan and Akhtar (1999) as 467.10±11.58 days and Hussain et al. (2006) as 473.72±3.56 days.

Parity had significant effect ($p<0.05$) on calving interval. The least-squares mean for calving interval for first parity, second parity and third and more than third parity was estimated as 483.84±8.08 days, 464.69±10.64 days, and 451.13±11.50 days, respectively.

Season of calving had non-significant effect on calving interval in the present study. The overall least-squares mean for calving interval in Nili-Ravi buffalo for summer, rainy, and winter season was estimated as 472.34±14.80 days, 458.34±7.17 days, and 468.98±9.37 days, respectively. Hussain et al. (2006) and Ghaffar et al. (1991) reported season of calving had non-significant effect on calving interval.

Dry period

The period between the dates of drying to date of next calving is termed as the dry period or dry days. The dry period is the period in which the animal has already experienced milk secretion phenomena but has ceased to produce milk due to the advanced stage of pregnancy or cessation of lactation due to other physiological reasons. This period is zero output period for the breeder, but it is the preparatory period for the mammary gland to produce in the lactations to come, after second or subsequent calving. An optimal 60 days of dry period is essential for the cow to build up her body resources for production in the next lactation.

The overall least squares means of dry period in Nili-Ravi buffalo was estimated as 157.11±4.18 days (Table 4). Lower estimates of least squares mean dry period than present study were obtained by Chaudhary (2015) as 142.99±15.81 days in Nili-Ravi buffaloes. Higher estimates of least squares mean dry period than present study were reported by Naqvi and Shami (1999) as 241.59±4.18 days, 306.39±8.78 in early and late maturing Nili-Ravi group,

Hussain et al. (2006) as 194.4±12.37 days, Bashir et al. (2015) as 258±1.6 days, and Charlini and Sinniah (2015) as 181±96.2 days in Nili-Ravi buffaloes.

Parity had significant effect ($p<0.05$) on dry period. Short dry period was observed in the third and above parity (146.84±7.17 days) and long dry period was observed in the first parity. Bashir et al. (2015) reported that parity had significant effect on dry period.

Season of calving had significant effect ($p<0.05$) on dry period. Rainy season showed short dry period (148.26±4.47 days), whereas winter season showed long dry period (165.21±5.85 days). Significant effect of season of calving on dry period has been reported by Naqvi and Shami (1999), Bashir et al. 2015 in Nili-Ravi buffaloes. Ghaffar et al. (1991) reported that season calving had non-significant effect on dry period.

Conclusions

Number of pregnancies, parity, had shown a significant effect on all production and reproduction traits whereas season of calving effected dry period in Nili-Ravi buffaloes. The present study suggests that Nili-Ravi buffaloes perform best during their first two pregnancies and parity can be included as a criteria for selection of Nili-Ravi buffaloes having superior production and reproduction. Further studies are required to compare the production and reproduction performance between Murrah and Nili-Ravi buffalo breed. This will be useful for the farmers and breeders to identify the real genetic potential of the performance traits in the Nili-Ravi buffalo and shall help in management and conservation of this useful germplasm of Punjab state.

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RESEARCH ARTICLE

Economic analysis of milk production in eastern region of India

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Abstract: India maintains the top most position in milk production due to the continuous efforts of the dairy farmers of the nation who have been consistent in dairying sector despite all the vagaries. Profitability in dairy farming is important to keep the farmer into the business. Keeping this in mind the present study was taken in eastern region of India with an aim to find out the cost and return per litre of milk. The study concluded that about fifty per cent of total feed and fodder cost was incurred on concentrate and the cost incurred on feeding buffaloes was maximum followed by crossbred and local cows. About ninety per cent of the labour cost was on own family labour. Return per litre from milk was highest for crossbred and the least for local cow and it varied positively with the herd size. It was also found that the income from sale of milk can be increased if expenditure on green fodder, dry fodder, concentrate and labour used are increased Also, the farmers in the study area are over using green fodder, optimally using dry fodder and under using concentrate and labour in milk production.

Keywords: Cost, Milk production, Marginal Value Productivity, Profit, Return

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Introduction

Dairy farming is an important source of secondary income for over 70 million milk producers, signifying its role to alleviate rural income and employment. Every second household in rural India undertakes dairy farming either as a primary or secondary means of occupation. Its role in food and nutritional security can't be overlooked in a country having one third people below poverty line. Milk is an important food item in the consumption basket of every Indian. It's important not only from consumption point of view but also from production point of view. India ranks number one in world in milk production with a milk production of 176.3 milllion tonnes (NDDB, 2019). If the country is to meet the consumption demands of the nation as well as maintain the top position as milk producing nation, then dairy farming must be a profitable business. Profit depends both on cost and yield as profit can be increased either by lowering cost of production or by improving the productivity of the animals. We are well aware that law of diminishing returns operates in dairy farming too. Hence, there is a limit to which the yield can be improved. Therefore, more emphasis should be given to lowering of the cost of milk production. Also, special attention should be given towards proper disposal of milk by eliminating the middlemen in the marketing chain, thereby, providing remunerative prices to the farmers. The present study focuses on eastern parts of the country as the region has immense scope in dairy development as it harbours about 23 per cent of total cows and buffaloes in the country. Despite of the fact that the region has about one - fourth of milch animals, it handles only about 12 per cent of the total milk produced in the country and the milk production is growing at a rate of 4 per cent.

Many studies have been carried out to find the cost and returns of milk production in specific states or districts (Chand et al. 2017; Keerthi and Paramsivam, 2019; Kumari et al. 2016; Kumawat et al. 2016; Patel and Ashwar, 2019; Singh et al. 2017; Tanwar et al. 2012). In this paper the economics of milk production has been worked out for whole of the eastern region. The paper also establishes the milk production function for the eastern region and also finds out the resource use efficiency of the various inputs. Similar study has been carried out by Bhagat et al. 2016;

Kumari and Malhotra, 2018; Lalrinsangpuii and Malhotra, 2016; Mehra et al. 2018; Vishnoi et al. 2015 etc.

Material and Methods

The eastern region of India encompasses of the states of West Bengal, Odisha, Chhattisgarh, Bihar, Assam, Eastern Uttar Pradesh and Jharkhand. Based on the per capita availability of milk, (Bihar (195 gm/day), Jharkhand (146 gm/day), West Bengal (145 gm/day), Chhattisgarh (130 gm/day), Odisha (122 gm/day) and Assam (69 gm/day)), the states of Bihar, West Bengal and Jharkhand were selected. From each state, one district was selected on the basis of highest livestock population density. Hence, the districts of Madhepura (0.88), Deoghar (0.37) and Hooghly (0.49) were selected from the states of Bihar, Jharkhand and West Bengal, respectively (The values in parentheses being the livestock population density of the districts). From each district, one tehsil was selected randomly, from which two villages were randomly selected. 300 respondents were selected according to probability proportional allocation from these six villages. Complete enumeration of these villages was done and strata were formed using cumulative square root frequency method. Three herd size categories were formed namely, small (1-3 milch animals), medium (4 and 5 milch animals) and large (>5 milch animals). Table 1 shows the sample distribution in the study area.

Every herd comprised animals of different age groups, types (crossbred, buffaloes, and local cows) and sex (male, female). Joint costs such as fixed costs and labour utilisation will be different across these categories of animals, hence apportionment of joint costs becomes necessary. For this purpose the different categories of animals were converted into homogenous animal units known as Standard Animal Units (SAUs). SAUs as suggested by Sirohi et al. 2015 (Table 2) were used for this purpose.

Total costs comprises of total variable cost and total fixed cost. Fixed cost: They do not vary with the level of output and remain unchanged over a short period of time. They included the cost of durable assets like farm inventory, cattle inventory, farm equipments etc. and were taken care of by charging depreciation on purchase cost. Depreciation was calculated using Capital Recovery Cost method. The CRC method is defined as the annual payment that will repay the cost of fixed input over the useful life of input and provide an economic rate of return on investment. The formula for estimation of CRC for farm inventory and equipments is as follows:

$$R = Z\left[\frac{(1+r)^n r}{(1+r)^n - 1}\right]$$

Where: R= Capital recovery cost, Z= Initial value of the capital asset, r = interest rate, n = useful life of the assets

The total CRC was calculated using the above formulae and was later apportioned among individual animals using SAUs. Formula for CRC estimation for cattle is same as that of above but the value of 'n' has to be ascertained differently for each animal. Generally, the productive life of crossbred is taken as 8 years (5 calvings) and of local cow and buffalo as 10 years (6 calvings). But, these cannot be used as such because using the value of the animal at the time of birth won't be appropriate. The value of the animal varies with wet-dry status, pregnancy (pregnant or non-pregnant), order of lactation, stage of lactation, etc. Hence, the estimation of CRC

Table 1 Population of dairy households and their sample sizes in the study area

State	District	Tehsil	Village	Small	Medium	Large	Total
Bihar	Madhepura	Madhepura	Gamarhia	34(46)	23(31)	6(7)	63(84)
			Bakhri	29(39)	14(19)	6(8)	49(66)
Jharkhand	Deoghar	Sarath	Gajiadih	27(36)	14(18)	7(9)	48(63)
			Gaura	31(41)	20(27)	6(8)	57(76)
West Bengal	Hooghly	Khanakul	Nabasan	27(35)	11(14)	3(4)	41(53)
			Dainan	34(44)	6(9)	2(2)	42(55)
				182(241)	88(118)	30(38)	300(397)

Note: The figures in the parentheses indicate the total dairy households in the sample village

Table 2 Standard Animal Units for eastern region in India

		Adult male	Adult female	Young stock M<1	Young stock M<1	Young stock M<1	Young stock M<1	Heifer
East	CB	1.07	1.20	0.25	0.24	0.51	0.38	0.71
	LC	0.92	1.00	0.27	0.24	0.41	0.37	0.64
	BU	1.02	0.86	0.25	0.23	0.42	0.38	0.63

Note: CB: Crossbred, LC: Local Cow, BU: Buffalo

based on the current value of milch animal is appropriate rather than considering their initial value. When current value is taken into account, 'n' has to be worked out as the length of remaining productive life.

$$n = [(\text{maximum calving} - 1) \times \text{inter calving period}] - [(\text{number of calvings done} - 1) \times \text{inter calving period}] + \text{lactation length}$$

If inter calving period and lactation length is in months then the formula needs to be divided by 12 for conversion into years. One more thing that has to be taken care of in case of animals is that Z in the formulae is net current value of the animal i.e., current value - salvage value as the salvage value in case of animals is quite high at the end of productive life.

Variable cost: These are those costs, which are incurred on the variable factors of production and can be altered in the short run. Variable cost includes four items i.e. feed and fodder cost, labour cost, veterinary cost and miscellaneous expenditure.

Feed and fodder cost included the cost incurred on feeding green fodder, dry fodder and concentrate to animals. If the concentrate was homemade then the weighted average of prices of all the ingredients was taken to ascertain the price of concentrate.

Labour was differentiated into hired labour and own labour. One man day was considered of 8 hours. For estimation of labour cost in case of women labourers, total time spent on different dairy activities was converted to man days by using conversion as:

1 day of women labour = 0.67 man day (3 women = 2 men)

Cost of grazing of animals was also included in labour cost

The expenditure on breeding and health care of the animals was covered under the veterinary expense. It included, cost of artificial insemination (AI), natural service, vaccination, medicines, fee of veterinary doctor and other related expenses. The miscellaneous expenditure included expenses on repair of fixed assets, water and electricity charges, insurance premium and any other incidental charges. These being joint costs, apportionment of the same based on SAU were done.

Later gross cost was calculated as the sum of total fixed cost (CRC), feed and fodder cost, labour cost and miscellaneous cost. Subsequently, value of dung was deducted from gross cost to arrive at net cost. Further, gross returns, net returns and cost per litre of milk were calculated. Also, in order to find out the impact of expenditure on various variable inputs on income earned from selling of milk the expenditure incurred on green fodder, dry fodder, concentrate, labour and miscellaneous inputs per farm per day were regressed on income in a dairy household from selling of milk. Different functional forms were tried and log-log form was found to be best fit. Marginal value product of these inputs was found using the formula $MVP = b_i x \frac{\bar{Y}}{\bar{X}}$ in case of log-log functional form.

Results and Discussion

In order to have a sustainable dairy farming business, the farmers must realise high milk yield. Milk yield from animals depends not only on breed and management practices but also, on feed and fodder provided to the animals. But farmers just can't go on feeding the animals with a hope to have a high milk yield. They have to keep in mind the cost incurred on feeding the animals. In the study area, animals were given green fodder in the form of chopped maize stalks and *Diancha* leaves. Dry fodder was given in the form of *bhusa* whereas concentrate was mostly homemade consisting of grains and food left over after human consumption. Table 3 shows the quantity of feed and fodder fed to each animal per day. Local cows are not given much feed and fodder as compared to crossbred cow and buffalo. Proper care of local cattle diet is not taken as their yield is low and because their yield is low they aren't fed properly. They are given mostly the maintenance ration. Thus a crossbred cow is fed 8.41 kg of green fodder, 6.12 kg of dry fodder and 4.28 kg of concentrate per day. One local cow is fed with 4.78 kg of green fodder, 1.82 kg of dry fodder and 2.03 kg of concentrate whereas a buffalo is fed with 8.21 kg of green fodder, 7.57 kg of dry fodder and 3.87 kg of concentrate on daily basis. In case of crossbred cow and buffalo, green fodder is fed in highest quantity followed by dry fodder and concentrate is the least while in case of local cow, green fodder quantity is highest followed by concentrate and the least is dry fodder. Among all the herd size categories, maximum quantity of green fodder, dry fodder and concentrate was fed by large herd size category for all the three types of animals.

Table 4 shows the cost incurred per day per animal on feed and fodder. A perusal of the table indicates that about 50 % of cost on feed and fodder is on concentrate and the least is on green fodder. Similar finding was reported by Kumawat et al. (2014) and Patel and Ashwar (2019). Among the different types of animals, cost incurred on feeding buffaloes (₹ 123.43) is the highest due to their huge appetite followed by crossbred (₹122.35) and the least is on local cows (₹ 50.22). Also, the cost incurred on concentrate and green fodder was maximum for crossbred cow while that for dry fodder was maximum for buffalo. A crossbred cow was fed ' 9.58 worth green fodder, ₹ 39.25 worth dry fodder and ₹ 73.52 worth concentrate while a local cow was fed ₹5.52 worth green fodder, ₹11.99 worth dry fodder and ₹32.71 worth concentrate. Buffaloes were also taken for grazing on a regular basis and cost incurred on that was included in labour cost. The cost incurred on green fodder, dry fodder and concentrate was ₹9.31, ₹47.76 and ₹66.36, respectively in case of buffalo. The cost incurred increases with the herd size category which may be because the larger the herd size, more is the milk for disposal and thereby, more is the income. They have more financial strength to feed the animals. Better management practices could be

another plausible reason as it ensures better feeding to animals which in turn increases the cost on feed and fodder.

Table 5 depicts the labour cost incurred per day per animal bifurcated into own family labour and hired labour. About 90 % of the labour cost is imputed cost (non-cash cost) as most of the dairy related

Table 3 Quantity of feed and fodder fed to animal daily (kg/animal/day)

Type of animal	Herd size category	Green Fodder	Dry Fodder	Concentrate
Crossbred Cow	Small	8.20	5.91	4.18
	Medium	8.33	6.40	4.38
	Large	9.96	6.61	4.61
	Overall	8.41	6.12	4.28
Local Cow	Small	4.57	1.74	1.98
	Medium	4.91	1.92	2.03
	Large	5.76	2.06	2.28
	Overall	4.78	1.82	2.03
Buffalo	Small	8.25	7.36	3.80
	Medium	7.70	7.78	3.93
	Large	9.48	8.28	4.14
	Overall	8.21	7.57	3.87

Table 4 Feed and fodder cost according to herd size category (₹/animal/day)

Type of Animal	Herd size Category	Green Fodder	Dry Fodder	Concentrate	Feed and Fodder
Crossbred Cow	Small	9.52(7.94)	38.16(31.84)	72.19(60.22)	119.87
	Medium	9.33(7.43)	40.81(32.52)	75.33(60.04)	125.47
	Large	10.66(8.31)	41.25(32.18)	76.29(59.51)	128.19
	Overall	9.58(7.83)	39.25(32.08)	73.52(60.09)	122.35
Local Cow	Small	5.40(10.90)	11.88(24.00)	32.22(65.09)	49.5
	Medium	5.60(11.15)	12.04(23.96)	32.60(64.89)	50.24
	Large	5.99(11.02)	12.51(23.01)	35.88(65.97)	54.38
	Overall	5.52(10.99)	11.99(23.88)	32.71(65.13)	50.22
Buffalo	Small	9.65(7.89)	47.01(38.44)	65.63(53.67)	122.29
	Medium	8.46(6.83)	48.30(38.98)	67.14(54.19)	123.9
	Large	9.67(7.51)	50.66(39.33)	68.49(53.16)	128.82
	Overall	9.31(7.54)	47.76(38.69)	66.36(53.77)	123.43

Note: Figures in parentheses shows the percentage of row total

Table 5 Labour cost according to herd size category and milch animal species

Type of Animal	Herd size Category	Own labour cost	Hired labour cost	Total Labour Cost
Crossbred Cow	Small	116.07(98.05)	2.31(1.95)	118.38
	Medium	96.55(77.93)	27.34(22.07)	123.89
	Large	98.33(76.31)	30.53(23.69)	128.86
	Overall	108.57(89.69)	12.49(10.31)	121.06
Local Cow	Small	32.90(96.98)	1.02(3.02)	33.93
	Medium	32.41(87.35)	4.69(12.65)	37.11
	Large	31.15(79.52)	8.02(20.48)	39.17
	Overall	32.58(92.06)	2.81(7.94)	35.39
Buffalo	Small	69.51(96.21)	2.73(3.79)	72.25
	Medium	61.89(92.91)	4.72(7.09)	66.61
	Large	57.46(90.57)	5.98(9.43)	63.44
	Overall	66.06(94.77)	3.65(5.23)	69.7

Note: Figures in parentheses shows the percentage of row total

activities are being done by the family members. In case of small farmers, the share of own family labour cost was as high as atleast 95 %. The own labour cost and hired labour cost was ₹108.57 and ₹12.49, respectively for crossbred cow, ₹32.58 and ₹2.81, respectively for local cow while, ₹ 66.06 and ₹3.65, respectively for buffalo. It was also observed that the own labour cost decreased with increase in herd size whereas hired labour cost increased with increase in herd size for all the three bovine species. The total labour cost per animal per day was found to be ₹ 121.06, ₹35.39 and ₹ 69.70 for crossbred, local cow and buffalo, respectively. As evident from figures, the labour cost was maximum for crossbred cows as they required high maintenance. As local cow had low productivity, hence they aren't given much care and hence the labour cost was least for them. Total labour charges per animal per day increased with herd size in case of both crossbred and local cows whereas in case of buffaloes, it decreased.

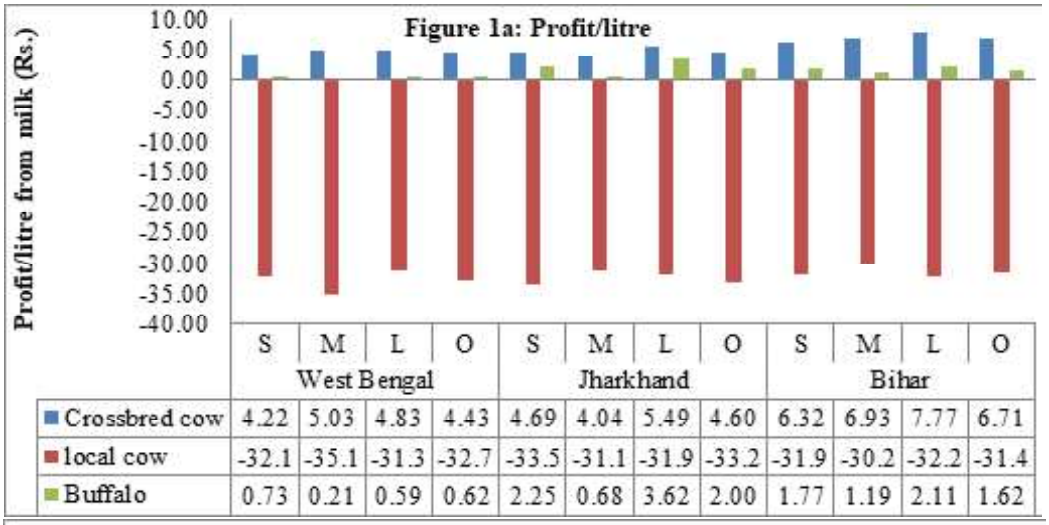
Table 6 depicts the various components of total cost incurred per day per animal in milk production. Total fixed cost varied between12 per cent to 18 per cent of the total cost. It was highest in case of crossbred (₹37.80), followed by buffalo (₹29.08) and least for local cow (₹17.83). Total fixed cost increased with increase in herd size for all the three bovine species.The share of total variable cost was very much higher than total fixed cost. Similar finding was reported in various other studies too (Chand et al. 2017; Keerthi and Paramsivam, 2019; Kumari et al. 2016; Kumawat et al. 2016; Patel and Ashwar, 2019; Singh et al. 2017; Tanwar et al. 2012). In the case of crossbred, feed and fodder cost and labour cost had an almost equal share in total variable cost. The plausible reason for equal share of feed and fodder could be high availability of labour in eastern parts of India which increases the labour cost. In case of buffaloes, more than 60 % of variable cost is feed and fodder cost indicating their higher appetite. Labour cost is highest for crossbred when compared to other type of animals as more maintenance is required for them. Value of dung was deducted from gross cost in order to arrive at net cost which is highest in the case of crossbred and least for local cow. Finally, the net cost of milk production per animal per day worked out to be ₹ 278.17, ₹100.30 and ₹ 218.81 for crossbred, local cow and buffalo, respectively. In case of crossbred cow and local cow, the net cost of milk production increased with increase in herd size whereas in case of buffalo, it was found to be highest for large herd size category followed by small and least for medium herd size category.

A perusal of Table 7 shows that price of milk is highest for buffalo milk (₹36.70/litre) owing to high fat quantity. Among all the types of animals, crossbreds are the most productive and the least are the local cows. The yield from crossbred, local cow and buffalo was found to be 13.15 litre, 1.65 litre and 6.21 litre, respectively. Such trend of yield among the different bovine species was observed by almost all the related studies (Keerthi and Paramsivam, 2019; Patel and Ashwar, 2019; Chand et al. 2017; Kumari et al. 2016; Kumawat et al. 2016; Singh et al. 2017; Tanwar et al. 2012) Also, the productivity of animals showed a positive correlation with the herd size. Cost per litre of milk was highest for local cow (' 60.94/litre) and least for crossbred (₹ 21.15/litre) because of their low and high milk productivity, respectively. Similar findings were reported by Chand et at. (2017), Keerthi and Paramsivam (2019) and Kumari et al. (2016). It was negatively correlated with herd size depicting economies to scale. Return per litre from milk was highest for crossbred cows and it varied positively with herd size. In case of local cow, return was found to negative for all herd size category. Keerthi and Paramsivam (2019) too reported high cost of milk production of local cow such that farmers incurred loss. Even though rearing of local cow is not profitable, then why do farmers still keep them? In order to find a reason for that, the total costs was bifurcated into cash and non cash costs. Cash costs included concentrate cost, hired labour cost and miscellaneous costs. All other feed and fodder other than concentrate were farm grown, hence had an imputed cost. When return over cash costs was calculated then it was found to be positive

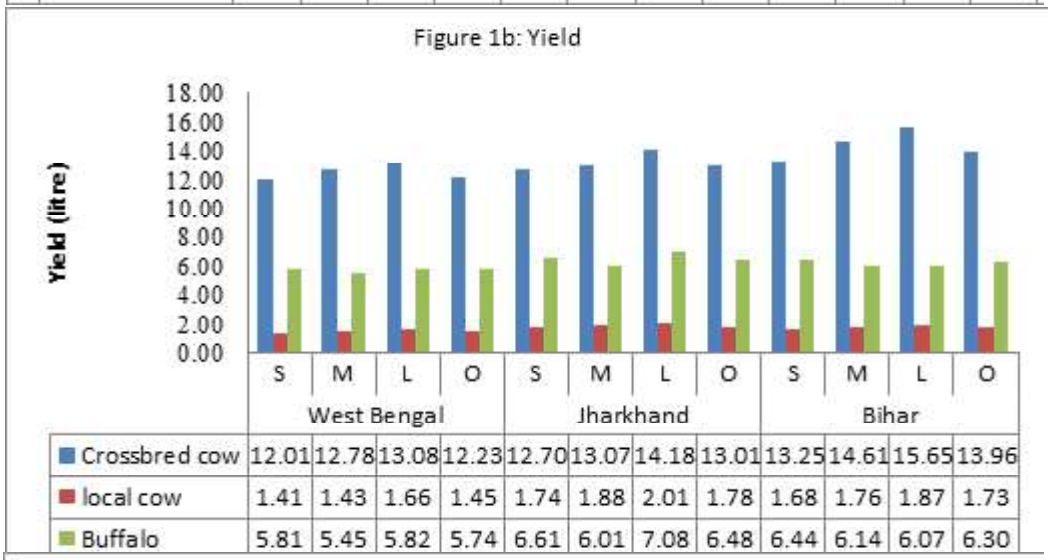
Table 6 Net cost of milk production according to herd size category and milch animal species (₹ /animal/day)

Cost	Crossbred				Local Cow				Buffalo			
	Small	Medium	Large	Overall	Small	Medium	Large	Overall	Small	Medium	Large	Overall
Total Fixed	35.21	41.84	41.62	37.80	17.14	18.17	20.93	17.83	28.33	29.57	32.12	29.08
Cost	(12.78)	(14.31)	(13.90)	(13.36)	(16.71)	(17.03)	(18.16)	(16.97)	(12.59)	(13.37)	(14.27)	(12.99)
Feed and fodder	119.87	125.47	128.19	122.35	49.50	50.24	54.38	50.22	122.29	123.90	128.82	123.43
Cost	(43.51)	(42.92)	(42.82)	(43.82)	(48.25)	(47.10)	(47.19)	(47.79)	(54.35)	(55.99)	(57.23)	(55.12)
Labour Cost	118.38	123.89	128.86	121.06	33.93	37.11	39.17	35.39	72.25	66.61	63.44	69.70
	(42.96)	(42.38)	(43.04)	(42.80)	(33.07)	(34.79)	(33.99)	(33.68)	(32.11)	(30.10)	(28.18)	(31.13)
Miscellaneous	2.07	1.15	0.73	1.67	2.03	1.15	0.75	1.65	2.14	1.18	0.73	1.72
Cost	(0.75)	(0.39)	(0.24)	(0.59)	(1.98)	(1.08)	(0.65)	(1.57)	(0.95)	(0.53)	(0.32)	(0.77)
Total	240.32	250.51	257.78	245.08	85.45	88.50	94.30	87.25	196.68	191.69	193.00	194.85
Variable Cost	(87.22)	(85.69)	(86.10)	(86.64)	(83.29)	(82.97)	(81.84)	(83.03)	(87.41)	(86.63)	(85.73)	(87.01)
Gross Cost	275.53	292.35	299.40	282.88	102.59	106.67	115.23	105.08	225.01	221.27	225.11	223.93
Value of Dung	4.70	4.79	4.52	4.71	4.73	5.00	4.54	4.78	5.30	4.94	4.58	5.12
Net Cost	270.83	287.56	294.88	278.17	97.87	101.68	110.69	100.30	219.71	216.33	220.53	218.81

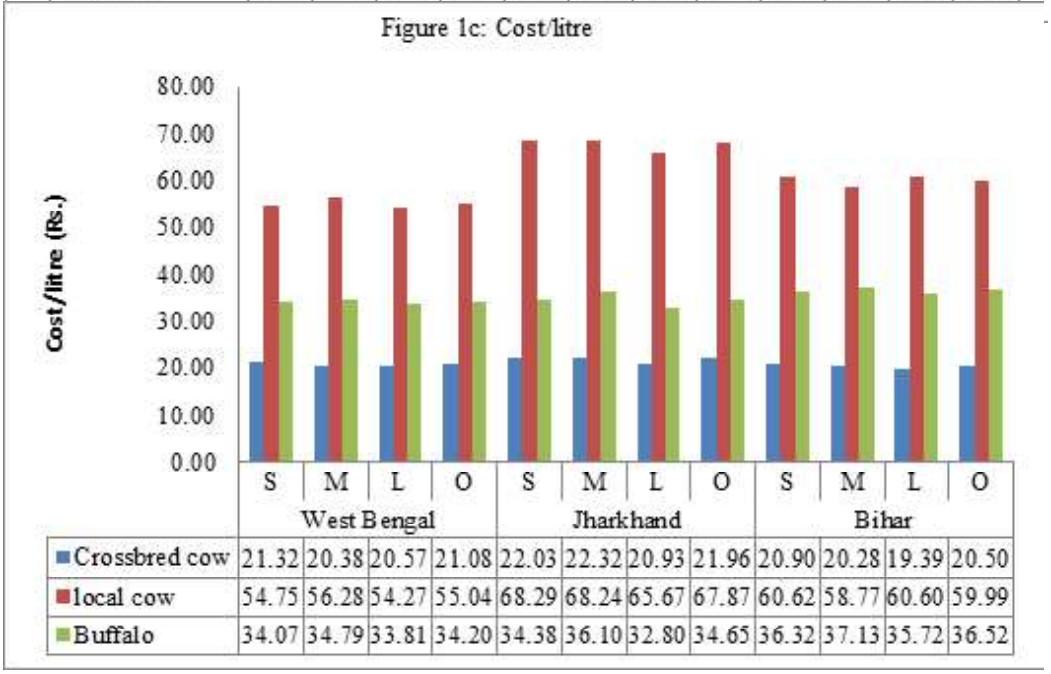
Note: Figure in parentheses shows the percentage of their respective total



	S	M	L	O	S	M	L	O	S	M	L	O
	West Bengal				Jharkhand				Bihar			
Crossbred cow	4.22	5.03	4.83	4.43	4.69	4.04	5.49	4.60	6.32	6.93	7.77	6.71
local cow	-32.1	-35.1	-31.3	-32.7	-33.5	-31.1	-31.9	-33.2	-31.9	-30.2	-32.2	-31.4
Buffalo	0.73	0.21	0.59	0.62	2.25	0.68	3.62	2.00	1.77	1.19	2.11	1.62



	S	M	L	O	S	M	L	O	S	M	L	O
	West Bengal				Jharkhand				Bihar			
Crossbred cow	12.01	12.78	13.08	12.23	12.70	13.07	14.18	13.01	13.25	14.61	15.65	13.96
local cow	1.41	1.43	1.66	1.45	1.74	1.88	2.01	1.78	1.68	1.76	1.87	1.73
Buffalo	5.81	5.45	5.82	5.74	6.61	6.01	7.08	6.48	6.44	6.14	6.07	6.30



	S	M	L	O	S	M	L	O	S	M	L	O
	West Bengal				Jharkhand				Bihar			
Crossbred cow	21.32	20.38	20.57	21.08	22.03	22.32	20.93	21.96	20.90	20.28	19.39	20.50
local cow	54.75	56.28	54.27	55.04	68.29	68.24	65.67	67.87	60.62	58.77	60.60	59.99
Buffalo	34.07	34.79	33.81	34.20	34.38	36.10	32.80	34.65	36.32	37.13	35.72	36.52

Fig. 1 Profit per litre, yield and cost per litre of milk production from crossbred, local cow and buffalo in various states

Note: S: Small herd size category, M: Medium herd size category, L: Large herd size category, O: Overall

even for local cow. Farmers usually don't maintain a farm record and hence fail to realise the imputed cost incurred. Therefore, they rear local cow despite of it being a non-profitable enterprise. Thus, the return over cash costs per litre was found to be ₹ 20.20, ₹ 8.54 and ₹ 25.97 for crossbred cow, local cow and buffalo, respectively.

Figure 1 shows the diagrammatic representation of profit per litre, yield and cost per litre of milk production from crossbred, local cow and buffalo in various states. Profit per litre from local cow was negative in all the three sample states. It was highest in Bihar (₹6.71) for crossbred cow while in case of buffalo it was highest in Jharkhand (₹ 2.00). Yield from crossbred cow was highest in Bihar (13.9 litre) while that from local cow (1.78 litre) and buffalo (6.48 litre) was highest in Jharkhand. In all the three sample states the cost per litre of milk was highest for local cow followed by buffalo and crossbred cow.

Table 7 Returns per litre from milk according to herd size category (₹/animal/day)

Cost/Returns	Crossbred				Local Cow				Buffalo			
	Small	Medium	Large	Overall	Small	Medium	Large	Overall	Small	Medium	Large	Overall
Price of milk (/litre)	26.50	26.54	26.54	26.51	28.23	28.25	28.05	28.22	36.52	37.09	36.64	36.70
Average daily milk yield	12.66	13.67	14.57	13.15	1.61	1.66	1.80	1.65	6.28	5.96	6.49	6.21
Gross return	335.44	362.70	386.76	348.63	45.56	46.85	50.44	46.44	229.47	220.89	237.64	227.86
Net Cost	270.83	287.56	294.88	278.17	97.87	101.68	110.69	100.30	219.71	216.33	220.53	218.81
Net Return	64.60	75.14	91.88	70.46	-52.31	-54.83	-60.26	-53.86	9.76	4.56	17.11	9.05
Cost/litre	21.39	21.04	20.24	21.15	60.64	61.31	61.57	60.94	34.97	36.32	34.00	35.24
Return/litre	5.10	5.50	6.31	5.36	-32.41	-33.06	-33.52	-32.72	1.55	0.77	2.64	1.46
Cash costs	76.57	103.83	107.54	87.68	35.27	38.44	44.64	37.16	70.51	73.04	75.20	71.73
Return over cash costs/litre	20.82	19.29	19.47	20.20	9.30	8.076	5.74	8.54	26.14	25.65	25.75	25.97

Table 8 Coefficients of various variables regressed on income per household per day from selling of milk

Variables	Coefficients			
	Small	Medium	Large	Overall
Intercept	3.6726*	4.4719**	6.1184**	3.4680**
	(0.2888)	(0.6956)	(1.3363)	(0.2249)
Expenditure on green fodder per farm per day	0.0528	0.1278**	0.2856**	0.1214**
	(0.0279)	(0.0481)	(0.0893)	(0.0243)
Expenditure on dry fodder per farm per day	0.2503**	0.0518	0.2952	0.2462**
	(0.0369)	(0.0929)	(0.2264)	(0.0332)
Expenditure on concentrate per farm per day	0.1351**	0.2355**	0.1765	0.1362**
	(0.0400)	(0.0792)	(0.1446)	(0.0363)
Value of labour used per farm per day	0.2387**	0.1634**	0.0560	0.2303**
	(0.0332)	(0.0479)	(0.1125)	(0.0272)
Miscellaneous expenses per farm per day	-0.1418*	0.1091	0.4930*	-0.1076
	(0.0746)	(0.1087)	(0.2262)	(0.0625)
R-square	0.6150	0.2813	0.4156	0.7156
N	182	88	30	300

Note: Figures in parentheses are the standard errors. * 5 per cent level of significance, ** 1 per cent level of significance

Table 8 shows the coefficients obtained when expenditure incurred on green fodder, dry fodder, concentrate, labour and miscellaneous inputs per farm per day are regressed on income from sale of milk. Log-log functional form was found to be the best fit. A perusal of Table 8 indicates that the expenditure on dry fodder, concentrate and labour used per farm per day had a positive and significant impact on income from sale of milk in the case of small dairy farmers whereas miscellaneous expenditure had a negative impact. Similarly, in the case of medium farmers, expenditure on green fodder, concentrate and labour used had a positive and significant impact whereas for large farmers, expenditure on green fodder and miscellaneous expenditure had a positive and significant impact. Therefore, 1% increase in expenditure on green fodder, dry fodder, concentrate and labour per farm per day leads to 0.1214%, 0.2462%, 0.1362% and 0.2303% increase in income per household per day, respectively while an increase of 1% in miscellaneous expenses per farm per day decreases the income per household per day by 0.1076%. and In nutshell, for overall category the income from sale of milk can be increased if expenditure on green fodder, dry fodder, concentrate and labour used are increased. But to what extent the expenditure should be increased? Well, this dilemma can be overcome by equating marginal value productivity of these inputs with their unit prices. The MVP of these significant inputs, i.e, expenditure on green fodder, dry fodder, concentrate and labour used were found to be 2.16, 1.01, 0.44 and 0.59, respectively. The MVPs indicate that green fodder are overused, dry fodder is used optimally, whereas, concentrate and labour are under used.

Conclusions

There is a good scope for dairy development in eastern India. The growth rate of milk production in the region is about 4 per cent and if profitability is to be maintained in the enterprise then the farmer must take care of both cost incurred in milk production and the yield with more emphasis on lowering cost of production as yield can be increased only to an extent. About fifty per cent of total feed and fodder cost is incurred on concentrate and the least is on green fodder. Cost incurred on feeding buffaloes is maximum followed by crossbred and local cows. About ninety per cent of the labour cost is imputed cost of own family labour. Return per litre from milk is highest for crossbred and negative for local cow and it varied positively with the herd size. When non-cash costs is excluded and only cash costs are taken into account then only returns from local cow milk production were found to be positive. Also, the farmers in the study area are over using green fodder, optimally using dry fodder and under using concentrate and labour in milk production.

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RESEARCH ARTICLE

Field level study to understand dimensions of antimicrobial use in dairy farms of Punjab

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Abstract: As a disease control measure on dairy farms for diseased animals, ensuring prudent antimicrobial use is a necessary step. In this perspective, Antimicrobial Resistance (AMR) has been an emerging global threat cutting across boundaries which is a serious threat to humans and dairy animals. Thus research on antimicrobial use practices and veterinarian prescribing behaviour is critical in achieving national efforts of managing AMR as far as dairy sector is concerned. In this context of antimicrobial use in dairy farming, the study was conducted in Punjab state of Northern India. Multistage stratified random sampling technique was applied to get the first hand information from 180 farmers and 60 veterinary doctors. None of the dairy farmers in the study area were following withdrawal period after their sick animal was administered with antimicrobial. Garett method of ranking on importance of treatment sources by respondents ranked the veterinary doctor first position with paravet ranked second. Prescribing behaviour of the veterinary doctors revealed prior experience of treatment and prompt results i.e. quick relief to animals as decisive factor in antimicrobial administration. Empirical research findings on dimensions of antimicrobial use in dairy farms can be vital in designing future strategies for tacking AMR in dairy sector.

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Introduction

A developing country like India has the highest bacterial infections as a result of which antibiotic use is widespread and indiscriminate; acting as a driving force towards resistance issue among dairy animals (Kuralayanapalya et al. 2019). Food producing animals which include dairy animals act as prime reservoir of zoonotic pathogens which pose severe health risk to humans. Resistant pathogenic strains in dairy animals are *Staphylococcus aureus*, S*almonella* spp., *Escherichia coli, Listeria monocytogenes* etc. often contaminating milk and milk products. *S. aureus* is frequently associated in case of mastitis which leads to its entry in the milk food chain (Sharma et al. 2018). In dairy sector, antibiotics are excessively used in treating intramammary infections which affects the quality of milk produced due to presence of antibiotic residues. This residue contaminated milk may induce intestinal alterations and allergies subsequently resulting in emergence of multidrug resistant bacteria among milk consumers (Sandholm et al. 2009). Several studies conducted from different areas of India have reported the presence of antimicrobial residues in milk which indicates the widespread antimicrobial use in dairy production systems (Moudgil et al. 2019; Lindahl, et al. 2018; Kurjogi et al. 2019). The adverse effect of residues present in milk is seen in manufacturing of dairy products like cheese and yogurt as the residues inhibit microflora of milk (Aalipour et al. 2013). National Health Policy (2017) has reiterated the lack of stringent regulations against non-therapeutic use of antibiotics. The overuse of antibiotics in animal sector has led to AMR emergence which is an unmeasured burden on India. The Policy document has identified AMR as a severe problem which requires effective actions in addressing it. By 2050, estimated economic burden due to AMR could be around $100 trillion and mortality numbers globally would be 10 million, with Asia alone contributing almost half of them at 4.7 million (O'Neill, 2018).

AMR as a global health issue can be gauged from the fact that UN high level meeting in the year 2016 focussed exclusive discussion on AMR. It was a rare instance as it was only the

fourth time a health issue was discussed after HIV, Non-communicable disease and EBOLA. In this perspective of tackling AMR, India has acted proactively by devising National Action Plan (NAP) for AMR.

NAP-AMR has outlined 5 strategic priorities to be implemented over 2017-2021 in tackling health challenge by AMR in India.

1. Improve awareness and understanding of AMR through effective communication, education and training

2. Strengthen knowledge and evidence through surveillance

3. Reduce the incidence of infection through effective infection prevention and control

4. Optimize the use of antimicrobial agents in health, animals and food

5. Promote investments for AMR activities, research and innovations

A closer look to the above five strategies from the social science lens highlights the importance of survey research in extracting the field level information from different stakeholders (farmers being primary stakeholder) with much emphasis towards dairy health care management practices. This baseline data generated can prove to be a cornerstone in plugging the gaps in tackling the AMR issue strategically in achieving the five priorities.

Kakkar et al. (2017) had opined the importance of smaller studies at regional levels in understanding the antibiotic consumption and resistant pattern due to lack of awareness on AMR and surveillance programs at national level. Though the smaller studies may not provide full understanding of the problem, but with combined efforts can act as systematic surveillance among farmers and dairy professionals in reducing antibiotic misuse. Overall research in the area of antimicrobial resistance has been limited in social science field and recently research from the farmer's perspective has gained momentum to understand the critical factors underlying AMR. Veterinary doctors inappropriate antimicrobial prescription can trigger selective pressure on bacterial infections thus increasing AMR of microorganisms in animals transferring to humans through the food chain. A number of extrinsic and intrinsic factor influence veterinarian prescribing behaviour. Norris et al. (2019) stated drug preference, professional experience, ease of administration, animal characteristics, perceived antimicrobial efficacy and cost of diagnostic tests.

Patnaik et al.(2019) on their study on veterinary doctors of Punjab reported the factors limiting them from undergoing sensitivity testing were sampling difficulties of the infections, the urgency of the situation for prescribing antimicrobial and concerns regarding the clinical relevance of in vitro tests.

The prescribing behaviour of vets engaged in treating dairy animals assumes much importance in the current antimicrobial use and resistance scenario.

To raise awareness regarding AMR concerns among dairy stakeholders, it is imperative to collect data across different regions of the country regarding antimicrobial usage and treatment patterns. The present research can help in developing evidence based policy towards regulating antimicrobial use at farmer's level. Exploring the dimensions of antimicrobial use in dairy farms through surveying farmers and veterinary doctor perspective; the two major stakeholders of the study forms the crux of this study.

Materials and Methods

The present study conducted towards understanding the field level practices that are facilitating towards AMR has followed a reductionist tradition of science. The survey research was conducted in purposively selected state of Punjab. Research design utilized for the study was both descriptive and exploratory. Multistage random sampling was followed for the study in selection of district, block, village and final respondents of the study. Three randomly districts selected for the study were Amritsar, Ludhiana and Pathankot and from each district two blocks were selected and from each block two villages. 15 farmers from each village were selected by stratified random sampling, thus totalling to 180 farmers for the study. The inclusion criterion for the farmer to be part of the study was that they should rear at least one milch animal for the last five years. The first-hand information on consultation and follow-up treatment practices on antimicrobial resistance in milk was gleaned from 180 respondents with the help of semi-structured interview schedule. Focus Group Discussions were carried out and oral histories were recorded in field diary to explore the various dimensions facilitating antimicrobial use in dairy farms at file level. Veterinary doctors working at the block level under the Govt. of Punjab providing service to dairy farmers in the selected villages were chosen for the study. A total of 60 veterinary doctors constituted as respondents of the study with 20 block level veterinarians from each district selected purposively. The data was analysed using statistical tools such as Frequency, Percentage and Garett ranking method to draw meaningful conclusions represented by stacked bar graphs, pie charts and tables.

Garett ranking method

In social science studies, Garett ranking method is widely used to rank the response data based on importance generated by survey research.

For converting ranks into percent position, the garett formula is

$$\text{Per cent position} = 100*(R_{ij}-0.5)/N_j$$

Where,

R_{ij} is the rank given for i^{th} factor by j^{th} individual

N_j is the number of factors ranked by the j^{th} individual

With the help of conversion table given by Garrett and Woodworth (1969), the percent position scores of each rank are converted into garett values. The next step is adding together the individual respondent's score for each factor and then dividing it by total number of respondents. The obtained mean scores for all the factors are arranged and ranked in descending order to identify the important ones.

Results and Discussion

Management practices for diseased animals by dairy farmers

Effective recovery of diseased animals requires certain precautions and practices to be undertaken so that the disease does not transmit to healthy animals in the herd. Isolating the sick animals is usually recommended along with disinfection of shed at regular intervals to curb disease incidence and transmission. A multiple choice question was framed to investigate the precaution taken by dairy farmers for diseased animals. The four options were isolating sick animal after being treated; disinfecting the animal shed; both isolation the animal and disinfecting the shed; none of the above practices were followed.

A perusal of **Fig.1** revealed that 17 percent farmers were isolating their treated diseased animal whereas disinfecting the shed was carried out by 22 percent of the respondents in the study area. In India most of the dairy farmers are usually small and marginal in nature and dairy animals are reared with great affection and emotional bond exists between the animal and members of the farmer family. Those dairy farmers who were not isolating their treated sick animals put forward a number a reasons. They were lack of space, difficulty in providing feed and water separately to healthy and sick animals and the animals were reared within the compound of farmer house. The researchers observed that farmers possessing small herd size were also acting as a hindrance for isolating the animal. But the main issue was the farmers were unaware of the benefits of isolating the animal to protect the healthy animals getting infected. The respondents opined that isolating the animals separately would further worsen their health indicating the compassion showed by farmers towards their herd. About 41 percent reported of following both isolating the sick animals and disinfecting the shed regularly. These respondents were possessing medium-large herds of 10-20 animals having separate animal shed. Education and extension contact played a crucial role in farmers awareness and know how following the practices of isolation and disinfection of shed for better recovery of diseased animal and preventing the spread of disease to the healthy animals of the herd.

Methods of treatment carried for dairy animals ailments

In case of diseased animals, dairy farmers have a number of choices of various treatment methods available depending on according to the financial capacity, severity of the disease and promptness of the curing methods. The different curing methods have certain bearing as far as AMR issues are concerned though homeopathy and allopathic treatment are considered novel methods. Dairy farmers prefer allopathic treatment for their animals in most cases as they are prompt in curing diseases and provide quick relief though the overuse/sub optimal use of antimicrobials can result in resistant to infections. Majority (90 %) of dairy farmers frequently preferred allopathic treatment for treatment of their animals as shown in **Fig.2.** As reported by dairy farmers, ayurveda and homeopathy were never a preferred choice for treatment of dairy animals. Focus group discussion conducted with dairy farmers revealed that those possessing indigenous buffaloes and cattle were more resistant to disease infections. During the interaction with farmers, few innovative and progressive farmers stated that they had tried ayurveda and homeopathic treatment for their animals but they were largely unsuccessful and their efficacy was quite low compared to allopathic treatment. Nair et al. (2015) reported the utility of ayurveda treatment practices as it reduced the number of antibiotic positive milk samples by 18 to 49% in India. Chand et al. (2016) in their study to compare the efficacy of homeopathic and allopathic treatments against Foot and Mouth disease in cattle reported that treatment of FMD affected animals with homeopathic drugs (*Kalium iodatum*, Calendula, and Sulphur) led to better recovery of animals in terms of reducing temperature, increasing appetite and ruminal motility, and rapid healing of the oral mucosal and foot lesions. Hence it is suggested that similar comparative research studies are needed in cases of other diseases of milch animals so that ayurveda and homeopathy can act as potential alternative treatment for animals against prevailing allopathic treatment practices. The visibility and scope of alternate treatment methods are highly necessary to reduce to reliance on antibiotic use thus minimizing the residues in milk making it safe for human consumption.

Treatment guidelines follow-up by dairy farmers

To understand the aspects farmers were following after their dairy animal is treated with antimicrobials helps to know the gaps between the recommended practices and actual practice taking place at field level. The farmers were asked on a 3 point continuum regarding three crucial after treatment recommended practices of discarding the milk of treated animal(withdrawal period), purchasing the full recommended dose and completing the course of antimicrobial. Surprisingly, in the study area none of the farmers were following withdrawal period i.e. discarding the milk of treated animal **(Fig. 3).** Withdrawal period is the time between the last dose of antimicrobial treated and the time when the milk can be safely consumed. Following the withdrawal period reduces the

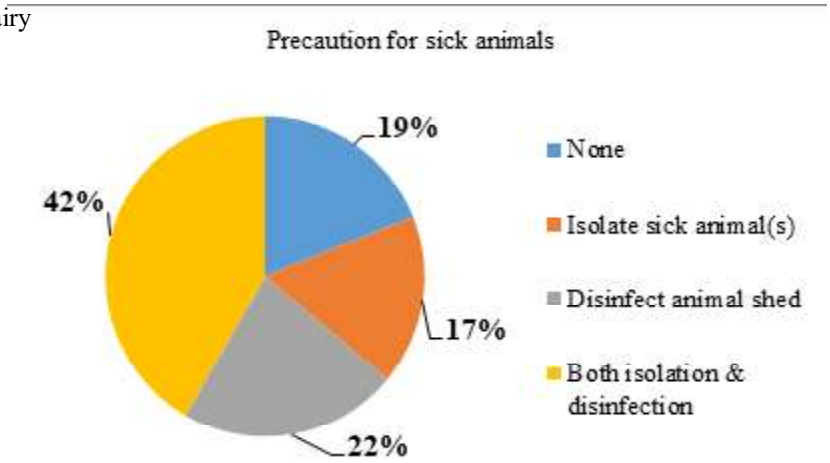

Fig.1 Management practices for diseased animals by dairy farmers

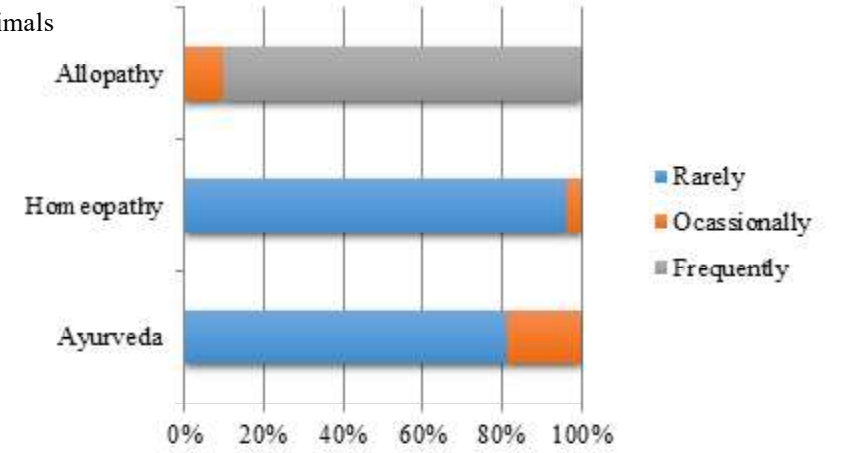

Fig.2 Methods of treatment carried for dairy animals ailments

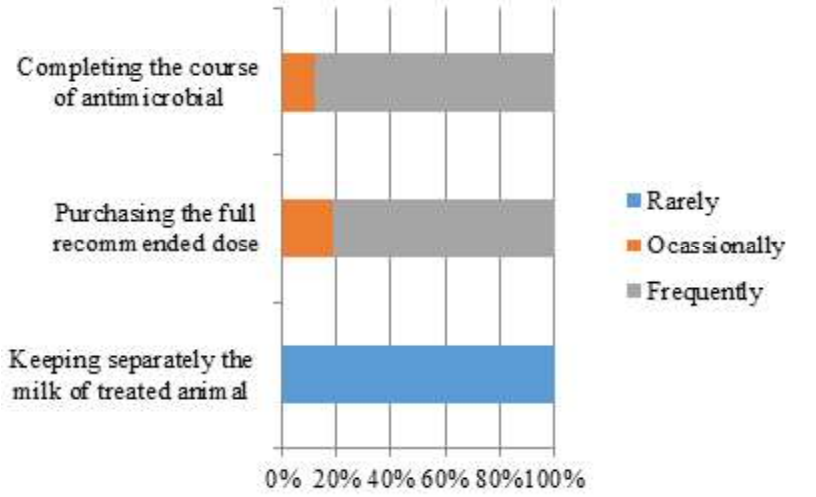

Fig.3 Treatment guidelines follow-up by dairy farmers

drug to safe tolerance levels so that residues in milk in not consumed by humans. The researchers observed that the farmers were unaware regarding withdrawal period. The researchers informed the farmers regarding the ill effects of residues in milk and benefit of following withdrawal period. The farmers reiterated that following withdrawal period was not feasible as they would incur heavy financial loss by discarding the milk. An open ended question was asked to farmers whether they would follow the withdrawal period if premium price would be provided for residue free milk by milk co-operatives. The question provided mixed

Fig.4 Prescribing behaviour of antimicrobials by veterinarians

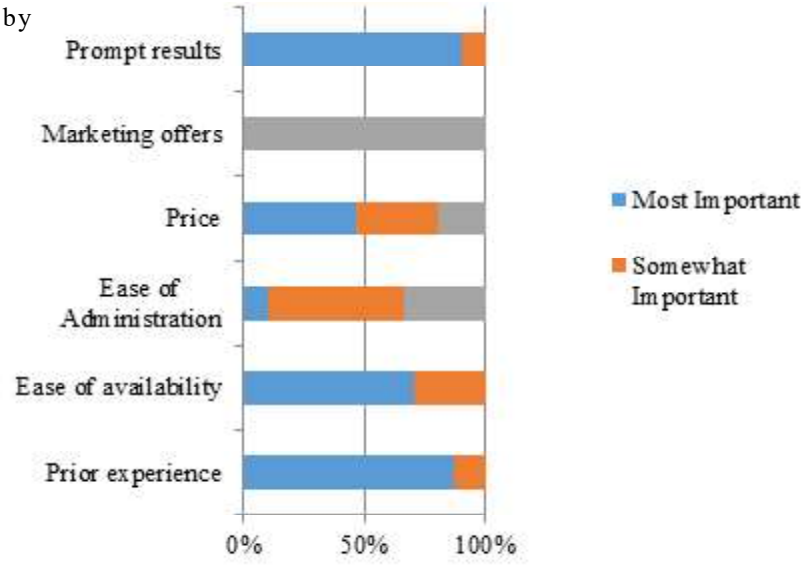

Table 1 Ranking of treatment sources based on their credibility

Sources of Treatment	Mean Garett Score	Rank
Veterinary Doctor	72.13	1
Paravet	61.40	2
Over - the- Counter(OTC)	46.72	3
Private Milk vendor	41.63	4
Other Dairy farmer	39.08	5
Milk Co-Operative official	37.01	6

reaction from the farmers. The farmers with large herds agreed to the proposition of following the withdrawal period if such a policy would be formulated. Small farmers were not interested in proposition of receiving premium price for residue free milk as most of them sold milk in the unorganized sector. Eltayb et al. (2012) reported a technique that by following pasteurization and electrochemical oxidation of raw milk with oxytetracycline at 100 mg/ml, milk of antibiotic treated animal can be made suitable for feeding calves. Practically this procedure is difficult at farmer level due to lack of awareness and know-how and further research is needed to make the above technology accessible to the farmers and understanding the efficacy of the technique.

A perusal of **Fig. 3** reveals that 93.70 percent of the respondents reported of purchasing the full recommended of medicines prescribed. The farmers consulting the veterinarians for treating animal diseases charged fees which include the antibiotics provided to them during and after the treatment. It was observed that 90.74 percent of farmers reported of completing the antimicrobial course prescribed for their dairy animals. A critical observation of the results shows 93.70 percent purchasing the full recommended dose and 90.74 percent completing the prescribed antimicrobial course. This difference can be attributed to the cases of farmers using left over medicines in subsequent cases of similar diseases or ailments. In depth discussion with the farmers, it was observed that farmers were saving medicines so that they could be utilized later. A study by Sawant et al. (2005) found only 24 percent of respondents completing the antimicrobial prescribed course; thus failure in completion the course could lead to sub-optimal use of antimicrobials which is a major factor of AMR

Ranking of treatment sources based on their credibility

Treatment to animals of dairy farmer is provided by number of persons or sources. Understanding the credibility associated with each treatment source helps us to know whom the farmer trust regarding the health care management practices of diseased animal. Six treatment sources were identified after consulting dairy experts and pilot study with farmers of non-sampling area. The respondents were asked to rank the treatment sources in ascending order from 1 to 6 based on the credibility of treatment sources in health care management aspect. Garett ranking method was employed to analyse the ranking data generated from the respondents. Veterinary doctor was ranked first position by the dairy farmers as the most credible source of providing treatment (Table. 1). The farmers reported of consulting the veterinarian when their animal gets sick. Bergevoet (2019) studied the antibiotic use in Dutch farms; found the sow farmers considered veterinarian followed by feed supplier as the most important source of information on antibiotic use and treatment. Similar studies conducted on antibiotic use in farms from different regions have invariably reported the veterinarian to be the most crucial treatment source for diseased animals among various treatment sources (Ison and Rutherford, 2014; Jones et al. 2015). Paravets were ranked the second position which can be attributed due to their personal cosmopoliteness. The researchers found that farmers of Pathankot district were possessing small herd of dairy animals and were more dependent on paravets for treating their animals. The paravets were easily assesible to farmers and

charged less compared to veterinary doctors. Due to lack of strict guidelines, availability of antibiotics over the counter (OTC) was widely prevalent in the study area. Hence the OTC persons were ranked third by the farmers. Self-prescription was also seen in case of progressive farmers, who have gained skills in treating the animals with years of experience in dairy farming. The farmers selling milk in the unorganized sector to milk vendors (*dudias*) reported they consulted sometimes vendors in case of minor ailments. A good observation by the researchers which reflects in the rankings was the low credibility of vendors and milk co-operative persons in providing treatment advice to farmers. These sources of treatment are unreliable and consulting them would result in misuse of antibiotics. The high credibility of veterinary doctor can act as the most important stakeholder in changing the behaviour of farmers towards antibiotic use. It would be highly useful to include the veterinarian and possibly the paravet in creating self-awareness regarding AMR issue and disseminating information to farmers by undertaking improved and easy practices of maintain hygiene and cleaning the shed regularly and vaccination the animals regularly. These small steps can have a large effect in controlling the disease in animals ultimately reducing the overall antibiotic use.

Prescribing behaviour of antimicrobials by veterinarians

Veterinary doctors are an indispensable part of the research on antimicrobial use because of the treatment they provide to the sick dairy animals. The veterinarians were asked to respond to the statements on factors of prescribing behaviour on a 3 point continuum of importance they felt towards each statement. The two prominent factors were prompt result i.e. quick relief to sick animals and prior experience of drugs and treating similar diseases. A perusal of **Fig. 4** indicates that 90 percent of respondents stated prompt result is a decisive factor for prescribing antimicrobials whereas prior experience was stated by 86 percent respondents. The importance given to prior experience in choosing and administering the antimicrobial by vets can be attributed to their knowledge on the aetiological agent and its susceptibility profile. Gibbons et al. (2013) in their study on veterinary practioners of Ireland found that 95.7 percent of the respondents considered prior experience of treatment 'often' or 'always' in making decisions on prescribing drugs. Ease of availability of antimicrobial was also kept in mind while administering or prescribing antimicrobial by 70 percent of the respondents. Providing quick relief to the diseased animals was the prime motto perceived by the veterianians, hence price of the antimicrobial was not that a decisive factor. Still 46 percent of the respondents had considered the price of antimicrobial while prescribing them. The veterinarians stated marketing offers by various marketing representatives have never influenced their decision of prescribing antimicrobial. Veteriarians in the study area reported the antimicrobials they frequently prescribed for treating various diseases of dairy animals were Ceftiofur, Oxytetracycline, Ceftriazone, Gentamycin, Enrofloxacin, Sulphadimiene, Marbofloxacin, Ceftizozim, Streptopenicilin, Methoxazole etc. Briyne et al. (2013) reported the factors strongly influencing prescribing behaviour of veterinarians in Europe were ease of administration, risk of developing antibiotic resistance, sensitivity tests and experience of the respondents.

Conclusions

Field study was undertaken to understand dimensions of antimicrobial use in dairy farms which is very crucial to formulate policies if at all the risk due to antimicrobial resistance is to be tackled. Allopathic treatment was mostly preferred by dairy farmers in the present study. However with concerns over antimicrobial resistance, developing effective and safer alternatives to antimicrobial therapy in viral/bacterial infections is essential. Veterinarian was ranked the most credible source for treatment of dairy animals followed by paravet as evident from the preferential ranking by the dairy farmers. In this aspect, the veterinarians can do a useful job by treating the animals with those antimicrobials which has short withdrawal time. The paper also highlighted the nature of veterinary doctor antimicrobial prescribing decision and the choice of prescribed antimicrobial. Though the veterinarians relied more on prior experience for treating animals, sensitivity tests should also be conducted in choosing the right and most effective antimicrobial. Institutional facilities should be created at veterinary hospitals and dispensaries along with refresher training so as to facilitate sensitivity tests and aware the veterinarians regarding AMR issues in dairy sector. The existing uses of antimicrobial agents can be improved through increased utilization of veterinary professional services, the introduction of enhanced infection control measures, improved point-of-care diagnostic tests. Innovative research on dimensions of antimicrobial use in dairy farms and subsequent cost effective interventions coupled with primary stakeholders like farmers and veterinary doctors can pave way for tackling the AMR problem in a big way.

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RESEARCH ARTICLE

Constraints faced by dairy farmers in hill region of Uttarakhand

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Abstract: Dairy farming has been an indispensable activity in history of human civilization which is not only important from economic point of view but it has nutritional importance too as an alternative food for ever growing human population. This activity is carried out in millions of households across the state and provides employment to the marginal and landless farmers. Despite this, Milk production of state is 1.656 MT, contributing 1.15 percent to dairy industry. Research studies pinpoint toward possibility of improving dairy industry with empowering dairy farmers. For this purpose, lacunae in dairy farming at farmer level especially in hill areas of Uttarakhand needs to be studied thoroughly. Against this backdrop, the present study was conducted with the objective to study socio-economic, psychological and communication characteristics of dairy farmers and elicit the constraints faced by dairy farmers. The study was carried out in Almora district of Uttarakhand, selected purposively. Two districts i.e., Dwarahat and Tarikhet blocks were selected by simple random sampling. The present study reported that unavailability of green fodder round the year, low productivity of animal, non-remunerative prices of milk were major three constraints faced by dairy farmers. Furthermore, other constraints found were high cost of concentrate mixture, unavailability of resource person, lack information about government schemes, unavailability of concentrate mixture, improper disposal animal waste, occurrence of diseases among animal, Unavailability of drinking water and poor conception rate in Artificial Insemination.

Keywords: Constraints faced by dairy farmers, Dairy Farmers, Hill Region, Information seeking behavior

Introduction

In India, agriculture sector is vast and diversified which contributes on an average 16 per cent to GDP and 10 per cent to export earnings (Himani, 2014). As an inevitable part of agriculture, animal husbandry has been an integral part of farming from the time of its evolution 10,000 years ago. Animals provide nutrient-rich food products, draught power, dung as organic manure and domestic fuel, hides and skin, and are regular source of cash income for rural households (Chinnadurai et al. 2018). In this context, Dairy farming has been an indispensable activity in history of human civilization which is not only important from economic point of view but it has nutritional importance too as an alternative food for ever growing human population.

Dairy farming is one of the most important economic activities in the rural mountainous areas of Uttarakhand where dairy is closely intervened with farming system. Dairy farming is considered as lifeline in rural areas of hilly region of Uttarakhand where people have been performing it traditionally for their livelihood security. This activity is carried out in millions of households across the state and provides employment to the marginal and landless farmers. Presently, Uttarakhand is coming up with a vast web of small-scale dairy and milk collection centers. Milching cow and buffalo are reared at all altitudes and they have high potential to develop dairy farming. The other important factors that promote dairy farming in the Uttarakhand Himalaya are vast forest (59.7%), grazing land (3.4%) and ample water (Sati, 2016). These factors make the state potential area for milk production but bigger question arises is why dairy farmers of Uttarakhand state are still contributing only 1.15 percent to dairy industry and the annual average income of livestock owners in the state is ¨ 13,560 (ULDB, 2001) only.

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Statistically, the population of cattle in the state is 29,93,828 which contributes 0.999 percent to country's cattle population. Milk production of state is 1.656 MT contributing only 1.15 percent to dairy industry. Around 90 percent of dairy owners in Uttarakhand operate dairy at small and medium scale. Despite being the world's largest milk producer, India's productivity per animal is very low, at 987 kg per lactation, compared with the global average of 2038 kg per lactation. No doubt, there are many public and private institution in India who are generating number of technologies with huge investments but most of these technologies and improved practices remain confining to limited periphery of research stations. Research studies indicate towards possibility of improving dairy industry with empowering dairy farmers. For this purpose, lacunae in dairy farming at farm level especially in hill state Uttarakhand needed to be studied thoroughly.

Material and Methods

The present study was carried out in Almora district of Uttarakhand state. Almora district was purposively selected as locale of the study because from livestock wealth and milk production point of view, it is a well-endowed district with highest milk production and dairy animal population among all hill districts (NDDB, 2018). Two Blocks i.e., Dwarahat and Tarikhet were randomly selected through Simple Random Sampling using chit method. Two villages were selected from each selected block through Simple Random Sampling using chit method for accessing information needs of dairy farmers. Total 120 respondents were selected from all the four villages through Probability Proportionate to Size (PPS) sampling. The data was collected with the help of pre-tested well-structured interview schedule. Pre-testing of schedule was done in Naini village of district Almora with 30 respondents. The respondents were asked open ended about their problems related to dairy farming. All the issues raised by respondents were listed down and considered as their constraints. The data were coded, tabulated, analyzed and were interpreted with appropriate statistical tools and techniques in the light of objectives of the study. Frequency, percentage, arithmetic mean, standard deviation was used to analyze the data for meaningful interpretation.

Result and Discussion

Socio-economic, psychological and communication characteristics of the dairy farmers

Presented below are the Socio-Economic, Psychological and Communication Characteristics of the Dairy Farmers (Table 1). The results revealed that majority of the dairy farmers (62.50%) belonged to middle age group while 20.83 per cent belonged to old age group. It further reveals that maximum respondents (27.5%) were having education up to middle class followed by 24.17 per cent had education upto high school. It was found that 21.67 per cent farmers were illiterate while 15 per cent farmers had primary education. Majority of the respondents (70%) belonged to nuclear family while 30 per cent of the respondents belonged to joint family. Study indicated that majority of the respondents

Table 1 Distribution of respondents according to their socio-personal characteristics (n= 120)

Characteristics	Categories	Frequency	Percentage
Age	Young (<38)	20	16. 67
	Middle (38-64)	75	62.50
	Old (>64)	25	20.83
Education	Illiterate	26	21.67
	Primary Education	18	15
	Education upto middle	33	27.5
	High School	29	24.17
	Intermediate	10	8.33
	Graduation	4	3.33
	Post-graduation	0	0
Family Type	Nuclear	84	70
	Joint	36	30
Family size	Small (<4 members)	17	14.17
	Medium (4-8 members)	90	75
	Large (>8 members)	13	10.83
Annual Income	Low (< Rs. 87000)	1	0.83
	Medium(Rs. 87000- Rs. 290000)	100	83.33
	High(> Rs. 290000)	19	15.84
Occupation	Agriculture as primary occupation	85	70.83
	Other than agriculture	35	29.17
Size of Land Holding	Marginal (<1ha)	120	100

Herd size	Small (<2)	32	26.67
	Medium (2-3)	64	53.33
	Large (>3)	24	20
Type of dairy animal	Indigenous cattle	147	45.24
	Crossbred cattle	116	35.68
	Buffaloes	62	19.08
Farm implements	Sickle / reaper	120	100
	Pick axe	120	100
	Agriculture plough	120	100
	Shovel	120	100
	Iron beans	117	97.5
	Bhakar (Wooden box)	13	10.83
	Chaff cutter	9	7.5
	Sprayer	6	5
Communication media	T.V.	120	100
	Telephone/Cellular phone	120	100
	Newspaper	6	5
	Radio	5	4.16
	Computer	3	2.5
	Internet	0	0
Transportation material	Motorcycle/ Scooty	22	18.33
	Car (Four-wheeler)	15	12.5
	Bicycle	4	3.33
Material possession	Low (<7)	0	0
	Medium (7-8)	88	73.33
	High (>8)	32	26.67
Milk Production	Low (<3 *l*)	13	10.83
	Medium (3-6 *l*)	95	79.17
	High (>6 *l*)	12	10
Achievement Motivation	Low (<13)	17	14.17
	Medium (13-18)	86	71.66
	High (>18)	17	14.17
Scientific Orientation	Low (<13)	4	3.33
	Medium (13-17)	71	59.17
	High (>17)	45	37.5
Attitude towards dairy farming	Low (<9)	24	20
	Medium (9-11)	84	70
	High (>11)	12	10

(75%) had medium family size followed by 14.17 per cent had family size of less than four and remaining 10.83 per cent respondents had large family size. It was reported that majority (83.33%) of the respondents had medium annual income followed by 15.84 per cent having high annual income and 0.83 per cent having low annual income. majority of the respondents (70.83%) had agriculture as their primary occupation while 29.17 per cent were performing occupation other than agriculture as primary occupation. All the respondents had marginal land holding of less than one hectare and none of the farmer had small, semi-medium, medium or large land holding. Majority of the respondents (53.33%) had medium herd size followed by 26.67 per cent respondents owned small herd size and only 20 per cent owned large herd size. All the respondents had sickle, pick axe, agricultural plough and shovel, none of the respondent possessed land leveler and 7.5 per cent respondents possessed chaff cutter and 5 per cent respondents possessed sprayer. For storage of agriculture harvest, it was reported that 97.5 per cent respondents possessed iron beans, 10.83 per cent possessed *Bhakar* and none of the respondents possessed *kothar/kangi* and copper bottom. All of the respondents had mobile phone and television at their home and none of them had internet facility. Also, 4.16 per cent respondents possessed radio, 2.5 per cent possessed computer and 5 per cent possessed newspaper at their home. Regarding transport possession, 12.5 per cent farmers possessed vehicle, 18.33 possessed two-wheeler and 3.33 per cent possessed bicycle. Majority of the respondents (73.33%) had medium overall material possession followed by 26.67 percent of respondents who had high overall material possession. Majority of the farmers (79.17%) were found to have medium milk

production per day i.e. 3-6 liters followed by low i.e. 10.83 per cent and high i.e. 10 per cent milk production. Majority of the respondents (71.66%) had medium level of achievement motivation while similar proportion of respondents (14.17%) had low and high level of achievement motivation. Majority of the respondents (59.17%) had medium level of scientific orientation followed by 37.5 per cent having high scientific orientation and few respondents (3.33%) had low scientific orientation. Majority of the respondents (70%) have medium attitude towards scientific dairy practices followed by 20 per cent having low attitude and 10 per cent having high attitude towards dairy farming.

Information seeking behaviour

The information seeking behaviour of the farmers was studied under four categories included in Bhairamkar (2009) scale adopted for the present investigation. The scale was modified after pre-testing of interview schedule. As per pre-testing of the scale veterinary officers/doctors, private medicine dealers, milk cooperative societies and *kisan mela* was added in the scale and agricultural assistants, agricultural supervisors, agriculture officers, meetings, discussion, demonstration, farmer's rally, agriculture campaigns, farm tour and workshops were removed from the scale.

The study revealed (Table 2) that majority of the respondents (70.83%) 'Occasionally' seek information from friends and relatives indicating that friends and relatives were contacted by all the respondents occasionally or always. Similarity was found with the findings of Sunetha and Ansari (2014) who reported that 83 per cent farmers always seek information from friends and relatives. The study revealed that majority of the respondents

Table 2 Distribution of respondents according to the use of different sources for seeking information (n=120)

S. No.	Information sources	Always		Occasional		Never	
		No.	%	No.	%	No.	%
A.	Personal Localite						
1	Friends and relatives	35	29.16	85	70.83	0	0
2	Progressive farmers	0	0	0	0	120	100
3	Neighbors	34	28.33	86	71. 66	0	0
4	Others	0	0	0	0	120	100
B.	Personal Cosmopolite						
1.	Single Window System						
a	Veterinary officer/Doctor	60	50	60	50	0	0
b	Private medicine dealer	0	0	0	0	120	100
c	Milk Cooperative Society	5	4.16	22	18.33	93	77.5
2.	Panchayat Samiti						
a	Gram Sevak	0	0	0	0	120	100
b	Rural Development Officer	0	0	0	0	120	100
c	Agril. Extension Officer	0	0	0	0	120	100
d	Block Development officer	0	0	0	0	120	100
3.	Agriculture University						
a	Agril. Assistant	0	0	0	0	120	100
b	Subject matter specialist	0	0	0	0	120	100
c	Scientist	0	0	0	0	120	100
4.	Bank						
a	Agril. Officer	0	0	0	0	120	100
b	Branch officer	0	0	0	0	120	100
c	Branch Manager	0	0	0	0	120	100
C.	Mass Media						
1	Newspaper	0	0	0	0	120	100
4	Agril. Films	0	0	0	0	120	100
5	Radio	0	0	0	0	120	100
6	Television	0	0	0	0	120	100
7	Kisan call center	0	0	0	0	120	100
D.	Extension Education Methods						
1	Field day	0	0	0	0	120	100
2	Field visit	0	0	0	0	120	100
3	Kisan mela	15	12.5	13	10.83	92	76.66

(71.67%) 'Occasionally' seek information from neighbors whereas 28.33 per cent respondents 'always' seek information from neighbors. So, it can be concluded that neighbors were contacted by all the respondents occasionally or always. Data regarding information seeking behaviour from personal cosmopolites that 50 percent 'always' visited veterinary doctors for seeking information whereas rest 50 per cent respondents 'occasionally' seek information from veterinary doctor. It shows that veterinary doctors are key source of information for respondents and from them respondents seek information occasionally or always. Moreover, majority of the respondents (77.5%) respondents 'never' seek information from milk cooperative union while 18.34 per cent respondents seek information 'occasionally' from milk cooperative union and only 4.17 per cent respondents seek information 'always' from them. Furthermore, there were no private medicine dealers from whom they can seek information. It was further reported that respondents 'never' seek information from Panchayat Samiti, Agriculture University and Bank. So, it can be concluded that among personal cosmopolites, veterinary doctors and milk cooperative union are key sources of information from which farmers seek information regarding dairy farming. Data regarding utilization of mass media for information seeking that all of the respondents 'never' seek information from any of the mass media. Although all of them had one or another mass media but they didn't used it for seeking any king of information regarding agriculture or dairy. This may be because mass media is used for entertainment purpose only and respondents rely on interpersonal sources for seeking information regarding agriculture and allied activities. The study also indicated that among extension education methods, *Kisan Mela* was the only source of information for farmers from which they sought information. It was found that majority of the respondents (76.67%) 'never' seek information from *Kisan Mela* while 12.5 per cent 'always' seek information from *Kisan Mela* while 10.83 percent 'occasionally' seek information from *Kisan* Mela. It was observed that many of the farmers regularly or occasionally visited *Kisan Mela* which used to be held twice or thrice annually at the beginning of season.

Constraints faced by the respondents

Constraints refer to the problems which are faced by farmers in successful operation and management of dairy activity. Dairy production in area had numerous problems hindering the smooth progress of dairy sector in area. The constraints were ranked on the basis of frequency and percentage of respective dairy farmers, who expressed them as constraints in dairy farming. On the basis of present study farmers claimed the following constraints which are enlisted rank wise in Table 3. On the basis of expressed opinion of the respondents the constraints were listed down and it was reported that there were eleven constraints in dairy farming of which, three major constraints were unavailability of green fodder round the year(100%) , low productivity of animal (70%), non-remunerative prices of milk (55%). Other constraints reported were high cost of concentrate mixture (50%), unavailability of resource person especially veterinary doctor in nearby area (50%), lack information about government schemes (45%),unavailability of concentrate mixture (36.67%), improper disposal animal waste (35.83%), occurrence of diseases among animal (34.17%), Unavailability of drinking water (30%) and poor conception rate in Artificial Insemination (15%).

In present study, it was found that farmers don't grow fodder in their fields purposively for dairy purpose. Grasses grow in the fields naturally, residual crop harvest of finger millet, wheat, maize were used as fodder occasionally. Mostly they relied upon forest for green fodder and in winters there was acute shortage of green fodder. Small and fragmented land holding, unavailability of irrigation water, unavailability of fodder seeds was major reason for not growing fodder in their field. Consequently, it was reported that dairy animals were under fed which resulted in, late maturity of animal, high mortality, infertility and low milk production. Gupta

Table 3 Distribution of respondents according to different constraints faced by them in dairy farming (n=120)

S. No.	Constraints	Respondents		Rank
		Frequency	Percentage	
1	Unavailability of green fodder round the year	120	100	I
2	Low productivity of animal	84	70	II
3	Non-Remunerative prices of milk	66	55	III
4	Unavailability of resource person especially veterinary doctor in nearby area	60	50	IV
5	High cost of concentrate mixture	60	50	V
6	Lack information about government schemes	54	45	VI
7	Unavailability of concentrate mixture	44	36.67	VII
8	Improper disposal of animal waste	43	35.83	VIII
9	Occurrence of disease among animal	41	34.17	IX
10	Availability of drinking water for animal	36	30	X
11	Poor conception rate in Artificial Insemination	18	15	XI

(2003) also reported Unavailability of green fodder as a major constraint in dairy farming in hill region. Similar results were found by Sharma et al. (2018) in Nainital district of Uttarakhand.

Unavailability of veterinary doctor in nearby area was another major constraint where farmers travelled more than ten or fifteen kilometers along with their animal for the treatment and expert advice. It was found that doctors were not present in their posted areas many times and this created problem for villages in accessing information or any kind of service. Yet another constraint was lack of information on government schemes and subsidies. It was found that farmers were unaware of recent advances in different aspects of dairy. Moreover, there was acute unawareness of government support and subsidies. Sharma et al. (2018) also reported Insufficient veterinary doctors or attendants (72%) as one of the major constraints among dairy farm women in Nainital District.

Non-availability of water was constraints for many of villagers especially during summer season. Villagers used to travel long distance for getting drinking water for them and their animal. It was reported that hand pumps were there in villages but animal didn't drink that water. In case of dairy farmers who had buffaloes, they faced problem shortage of water for drinking as well as for bathing of animal. For dairy farmers who had large herd size and buffaloes faced severe problem regarding availability of water.

Another major constraint was low productivity of dairy animal. This could due to the fact that dairy farmers had local breed of animal, there was shortage of quality fodder and they were following peer feeding practices for dairy animal. On discussion with respondents, it was found that dairy farmers sell was selling milk either to milk union or to people in cities. In both the cases they were found dissatisfied with the price they were getting for milk.

Other than the above-mentioned constraints they faced problem with the availability of concentrate feed and high cost of concentrate feed. It was reported that concentrate feed was an important part animal diet which they preferred crucial for high milk production. It was regular part of animal diet and they used to buy concentrate mixture either from market or from milk cooperative union. It was reported that there was unavailability of concentrate feed in milk cooperative union who provided it to them at minimal price. In such condition they bought it from market at high cost. For dairy farmers who had low milk producing animal and who didn't earn much from dairy, it was a tough task. The results are in consonance with the findings of Sharma et al. (2018) who reported Low availability and high cost of concentrates (66%) and Low productivity of animal (78%) as constraints in dairy farming.

Furthermore, farmers had problem with proper disposal of animal waste. In absence of disposing-off area near animal shed, they didn't know what to do with animal waste especially during rainy season. It was found that dairy farmers lack proper knowledge about compost making and *gobar* gas production. Sharma et al. (2013) also observed that the disposal of the fresh cow dung was the major problem among dairy farmers. Also, few respondents expressed their dissatisfaction from Artificial Insemination services due to poor conception of animal from AI. Last but not least, respondents expressed that occurrence of diseases among animal created problem for them. In rainy season, the case of disease infestation was more. Bloating and Food and Mouth disease were major obstruction for them. Beside this, non-availability of doctors during this period made situation worse for them.

The present findings are in consonance with previous research studies. Rajpoot et al. (2018) reported low price of milk and milk products (83%), lack of veterinary facility in village (70%), respondents not having cross breed/superior animals (72.5%), feed and green fodder /roughages management (63%) as the constraints faced by dairy farmers while adopting animal management practices. Similarly, another study by Chaurasiya et al. (2017) reported that major constraints expressed by dairy farmers were lack of veterinary facilities in the village (68.75%). Another study by Rathod et al. (2011) highlighted that majority of farmers (87%) faced non-availability of fodder round the year, inadequate knowledge about feeding (76%), lack of timely Artificial Insemination (AI) facility (72%), low conception rate through artificial insemination (57%) as the constraints in dairy farming. Irregular disposal of dung and animal waste, non-availability of timely treatment facilities, shortage of green fodder particularly during summer months, non-remunerative price of milk and lack of provision for clean drinking water was indicated as constraints in dairy farming by Singh et al. (2017). Similarly a study in Gujrat by Kathiriya et al. (2014) reported that more than 70 percent respondents had difficulties in getting medical aids, lack of technical knowledge about feed, fodder and health management, lack of artificial insemination facilities at village level, lack of quality fodder and lack of medicinal facilities in the villages as constraints in dairy farming among rural women.

Conclusions

The study concluded that dairying was one of the major activities performed by farmers in hill region of Uttarakhand for livelihood security in consistent manner. So, present study highlighted that in interior region of hills, people seek information more from personal localite than any other source of information. Hence, percolation of need based information among personal localites is utmost important for dairy improvement. Furthermore, it was indicated that veterinary doctors and milk union play a very crucial role in dissemination dairy related information to farmers. The study provided a comprehensive view of constraints faced by dairy farmers in hill region of Uttarakhand state, which were unavailability of green fodder round the year, low productivity

of animal, non-remunerative prices of milk, high cost of concentrate mixture, unavailability of resource person especially veterinary doctor in nearby areas, lack information on government schemes ,unavailability of concentrate mixture, improper disposal animal waste, occurrence of diseases among animal, Unavailability of drinking water and poor conception rate in Artificial Insemination. Data suggested that efforts are needed for awareness of personal localites on different aspect of improved dairy practices as they act as link for other dairy farmers. It is also emphasized that we still have long way to go in availability feed and fodder round the year, improvement of animal breed and awareness of dairy farmers on government schemes and subsidies. Also, with better prices and consistent veterinary services dairying can be made more profitable for dairy farmers in hills of Uttarakhand state.

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RESEARCH ARTICLE

Clean milk production practices and its impact on the prevention and control of mastitis in Tiruchirapalli district of Tamil Nadu

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Abstract: Mastitis is one of the major infectious diseases affecting dairy cows and causing economic loss. Majority of the rural dairy farmers are not aware of preventing mastitis through management practices. Lack of adoption of clean milk production practices is the major reason for mastitis. So the focus of study was imparting knowledge on clean milk production practices to dairy farmers from selected villages in Tiruchirapalli district along with management of mastitis in dairy animals through capacity building programme. After the adoption of clean milk production practices, there was remarkable reduction in the incidence of mastitis from 19.33 per cent to 2.4 per cent. The keeping quality of milk was also improved by enhancement in methylene blue dye reduction time (MBRT). This was an indirect indication of improved microbial quality of raw milk. The subclinical mastitis was monitored with somatic cell count (SCC) and the quarters of udder health by California mastitis test (CMT) respectively. The mastitic animal was treated effectively with suitable antibiotic by conducting antibiotic sensitivity test (ABST) and thereby protected the dairy farmers from huge economic loss.

Keywords: Capacity building, Clean milk production, Mastitis, Prevention, Control

Introduction

The bovine Mastitis is an economically important disease in lactating animals across the world followed by lameness and infertility under field condition and causes considerable financial losses to Dairy industry (Green et al, 2004). The annual economic loss due to mastitis has been calculated to be Rs.7165.51 crores. Among this, subclinical mastitis has been estimated to account for 57.93 percent (4151.16 crores) of the total economic loss due to mastitis (NAAS, 2013).

Mastitis severely affects the milk quality and production of the cow and may spread to other cows in the herd. Deterioration of milk quality is a major sequel of Mastitis. Mastitis is characterized by inflammation of udder tissue causing pathological changes in udder parenchyma and characterized by physical, chemical and microbiological changes in milk (Radostitis *et.al,* 2000). Further, the mastitic milk is unsuitable for consumption and is one of sources of communicable diseases such as tuberculosis, brucellosis, staphylococcal toxemia, septic sore throat, gastroenteritis, etc., (Kalorey., 2001). Although notable progress has been made in prevention of mastitis by appropriate hygienic measures, enhanced control of milking techniques and antibiotic treatment of dry cow, but it is still necessary to treat a large number of cows which suffer from mastitis. The multiplicity of the cause and emergence of indiscriminate and prolonged use of antibiotics in absence of antibiogram is a major hurdle in the control of mastitis (Mathialagan and Kumaresan, 2015).

Hence, the proposed study is aimed to impart knowledge on clean milk production practices to dairy farmers through capacity building programme which will create awareness about subclinical, clinical mastitis, prevention and management of mastitis in dairy animals. The dairy cows suffering from mastitis are going to be treated with suitable antibiotics based on Antibiotic Sensitivity Test (ABST) so that the trained beneficiaries will be protected from huge economic loss by improving the raw milk quality.

Materials and Methods

Study area

The study was conducted in Tiruchirapalli district of Tamil Nadu. Three blocks which have recorded maximum numbers of case of mastitis were identified viz. Andhanallur, Mannachanallur and

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Manikandam, from the endemic record maintained at Animal Disease Intelligence Unit, Tiruchirapalli. Four villages from each block, totally twelve villages were selected for this field study. Fifty dairy farmers from each village, constitute a total of six hundred beneficiaries were selected on the basis of having at least two dairy animals out of which one should be in lactation.

Capacity building programme

The two day capacity building programme included both on farm and institutional training programme at TANUVAS-Veterinary University Training and Research Centre, Tiruchirapalli for the dairy farmers on clean milk production practices and the demonstrations on cleaning of dairy cattle, disinfection and sanitation of shed, milking methods, cleaning of udder, post teat dipping, strip cup test-mastitis detection were also conducted. Inputs like mastitis detection kit, post-teat dip container, povidone iodine, plastic hand sprayer, potassium permanganate, plastic bottles, test tubes for milk sample collection and reading materials in local vernacular were distributed to all the beneficiaries.

Assessment of raw milk quality and detection of mastitis

The Methylene Blue dye Reduction Test (MBRT) was performed in dairy farm since it is a quick method for determining the bacterial load, sanitary condition and keeping quality of milk. The test also indicates the hygienic condition of milk during its production and handling.

The most significant abnormality found in milk due to subclinical mastitis is the increase in Somatic cell count (SCC). Since cell numbers in milk are closely associated with inflammation of udder health, these somatic cell counts currently constitute an important criterion for the measurement of milk quality as an international standard (Hillerton, 1999).The milk from suspected animals were collected and examined for somatic cells count using Ekomilk Horizon Milk Analyzer.

The California Mastitis Test (CMT) was extremely used to identify the specific quarters infected with clinical and subclinical mastitis. The test was conducted on a white plastic paddle with four cups corresponding to four teats. Milk from each teat is flown directly into the cups and equal amount of reagent added along with bromocresol purple as an indicator. The positive case revealed gel formation and in negative case there was no precipitation (Sandholm, 1995).

Antibiotic Sensitivity Test

The milk sample from suspected mastitis animals were collected aseptically and sent to the laboratory at Veterinary University Training and Research Centre, Tiruchirapalli for antibiotic sensitivity test using Muller-Hinton agar plates and Antimicrobial sensitivity discs. Based on zone of inhibition, suitable antibiotic therapy was suggested by the specialists to cure the animal suffering from mastitis (Erskine et al, 2003).

A teacher made knowledge test was developed in a simple vernacular language and the same was employed thrice viz., before training, immediately after training and 90 days after training to assess their retention of knowledge learned during the training. The enhancement in the knowledge level is the difference between knowledge retention score and the pre-training knowledge score. A semi-structured interview schedule was prepared and employed for data collection. Descriptive statistics were employed to interpret the findings.

Results and Discussion

Status of clean milk production at field level

General Cleanliness

Table1 shows that 75.83 per cent of respondents were cleaning their cattle shed daily. Similarly, 60.00 per cent of them were cleaning their dairy cows daily. Most of the respondents were cleaning their milch cows ranged from daily basis to five days interval. Each and every respondent was cleaning the milk utensils before milking. These findings were in accordance with Gautam and Mohammad, 2019.

Clean milk production practices

The Pre-exposure and post-exposure knowledge tests were conducted to all the 600 beneficiaries focusing on the objectives of clean milk production before the commencement and immediately after completing the training programme. The retention level of gained knowledge was tested 90 days after the completion of capacity building programme.

Table 2 shows that only 5.33 per cent the dairy farmers were following the practice of Cleaning of udder with potassium permanganate solution before milking. In contrast, Patel et al. (2014) revealed that splashing of water on udder or teats and washing of hand before milking was followed by most (97.50%). After washing the udder, around 4.33 percent wipe it with clean cloth. Only 8.17 per cent knew that cleaning of hands of milkman before milking was important. Patil et al. (2009) also suggested that majority of the dairy farmers did not know that the milker should be healthy and free from bad habits. This might be because of their ignorance about milker's personal hygiene and spreading of zoonotic diseases through human.

There are various types of cleaning and sanitation agents that have been specially designed to clean and disinfect milk-handling equipment (Lore et al. 2006). The study revealed that only 12.17 per cent were practised cleaning agent for milking utensils. Few respondents (10.83%) were sun drying their utensil after cleaning and most of them were not practicing the same. Majority of the

respondents were not giving concentrate feed at the time of milking except 2.5 per cent.

Full hand milking method was practiced by 15.33 per cent and majority them 84.67 per cent preferred stripping and knuckling method of milking, because of small size of their animal's teats and shorter milking time. Similar finding of high rate of Knuckling practice (74.4%) was also reported by Sinha et al. (2010). A very few dairy farmers (1.83%) had knowledge regarding knuckling method leads to teat injury. Post teat dipping with Iodine solution was practiced only by two dairymen out of six hundred.

Not even a single dairy farmer had the knowledge regarding animal should stand for 15- 20 minutes after milking so that teat canal gets closed and the soil bacteria could not enter teat canal which leads to mastitis. Nearly half of the famers (47.67 %) were providing roughage feeding material to their animals and rest of the respondents were not even conscious about the benefits of providing roughages to animals since it made the animal to stand for half an hour and during that time teat canal got closed.

Usage of sieve during transfer of milk from one can to another was practiced moderately by 37.17 per cent and rest were not aware that sieve could prevent the entry of dirt and extraneous matter. Only (16%) were used steel utensil for milking purpose and remaining (84%) were using plastic or aluminium cans or brass utensils. Out of 600 respondents only four persons were able to detect subclinical mastitis by making milk strip in their hand palm and examined with taste buds, revealed sour taste since chloride content was more in subclinical mastitis.

During this project, we have encountered only seventeen (2.83%) well informed farmers who employed milking machine in their farm. The majority of the dairy farmers were told that milking machine was costlier and it could cause damage to udder. But in practical, milking machine is safe and faster and convenient when compare to human hand milking as it minimize milk contamination through milker and at the same time cleaning and maintenance also simple. Nowadays, Government subsidy is available in some states and farmers can make use of it and avoid dependency of milkman.

Table 1 General cleanliness of dairy farm (n=600)

S. no.	Parameter	Daily (%)	2 days interval (%)	5days interval (%)	More than week (%)
1.	Cleaning of cattle shed	455(75.83)	140(23.33)	5(0.83)	0
2.	Cleaning of dairy cow	360(60.00)	125(20.83)	115(19.17)	0
3.	Cleaning of milking utensils	600(100.00)	0	0	0

Table 2 Knowledge level of dairy farmers on clean milk production practices (n=600)

S.No.	Technologies / practices	Pre-exposure (%) adoption	Post-exposure (%) knowledge	Retention (%) of knowledge
1	Cleaning of udder with Potassium permanganate solution before milking	32(5.33)	535(89.17)	485(80.83)
2	Wiping the udder with clean cloth	26(4.33)	502(83.67)	433(72.17)
3	Cleaning of milkman hands before milking	49(8.17)	514(85.67)	449(74.83)
4	Usage of cleaning agent for utensils	73(12.17)	547(91.17)	496(82.67)
5	Sun drying of utensil after cleaning	65(10.83)	529(88.17)	471(78.50)
6	Providing concentrate to animal during milking	15(2.50)	510(85.00)	478(79.67)
7	Knowledge of Full hand milking method	92(15.33)	524(87.33)	469(78.17)
8	Knowledge regarding Knuckling method leads to teat injury	11(1.83)	547(91.17)	493(82.17)
9.	Post teat dipping with Iodine olution	2(0.33)	538(89.67)	476(79.33)
10.	Knowledge regarding animal should stand for 15- 20 minutes after milking so that teat canal gets closed	0(0.00)	501(83.50)	482(80.33)
11.	Provision of roughages after milking	286(47.67)	559(93.17)	502(83.67)
12.	Usage of sieve during transfer of milk from one can to another	223(37.17)	543(90.50)	498(83.00)
13.	Usage of stainless steel utensils for milking purpose	96(16.00)	526(87.67)	487(81.17)
14.	Detection of subclinical mastitis	4(0.67)	510(85.00)	473(78.83)
15.	Usage of milking machine	17(2.83)	508(84.67)	462(77.00)

Constraints faced by the dairy farmers pertaining to mastitis

The results of Table 3 revealed that only 5.33 per cent of the dairy farmers were aware of mastitis prevention methods and none of them were aware about subclinical mastitis detection test. The dairy farmers revealed that if a cow was affected with mastitis, they had to spend more money for the treatment because it was costlier (83.50%). They also perceived that most of the time the animal was not responded to treatment (77.17%) and if the mastitis was not cured, then the particular quarter could become dormant (31.17%), thereby they used to get huge economic loss (90.33%). Similar results were reported by Byarugana et al. (2008) and also Mathialagan and Kumaresan (2015) who stated that none of the dairy farmers knew about subclinical mastitis and most of them considered mastitis was a major constraint for wealth creation through milk production.

Impact of clean milk production training on incidence of mastitis and raw milk quality

The analysis of benchmark survey revealed that 116 (19.33%) of the beneficiaries had faced the problem of mastitis and the same has been controlled through the implementation of the project. Table 4 shows the impact of the adoption of mastitis detection and prevention technique as perceived by the scheme beneficiaries.The beneficiaries reported that there was a drastic reduction in the occurrence of mastitis cases from 19.33 per cent to a level of 2.4 per cent after adoption of the mastitis detection, prevention and control techniques. This indicates that the mastitis prevention and control training methodology was highly effective. Riekerink et al. (2010) reported that most of Canadian dairy farms adopted important mastitis prevention practices such as clean milk production practices since mastitis was highly related to farm management activities.

Interestingly, the keeping quality of raw milk was also improved by increase in Methylene blue dye reduction time (MBRT) from 2-3 hours to 4-5 hours. This is an indirect indication of improved microbial quality of raw milk.

Sub clinical mastitis is characterized by increase in Somatic cell count without any observable changes in milk and udder and it is a chief cause of heavy monetary losses to dairy farming (Jadav et al. 2010).The original limit for Somatic cell count of a healthy quarter as suggested by the International Dairy Federation is 5,00,000 cells per ml (Jadhav et al. 2013) From Table 4, the microscopic examination of the milk samples revealed that there was significant reduction in the somatic cell count

Table 3 Constraints faced by the dairy farmers pertaining mastitis (n=600)

S.No.	Constraints	Yes (%)
1	Not aware of the subclinical mastitis detection test	596(99.33)
2	Treatment is costly	501(83.50)
3	Lack of training on mastitis prevention methods	568(94.67)
4	Animal not responded to treatment	463(77.17)
5	Awareness that affected quarter becomes dormant	187(31.17)
6	Heavy economic loss	542(90.33)

Table 4 Impact of training on incidence of mastitis and raw milk quality

Incidence of mastitis in milch cows		Methylene Blue dye Reduction Test (MBRT) n=86		Somatic Cell Count (SCC) n=86		California Mastitis Test (CMT) n=61	
Before adoption	After adoption	Before adoption	After adoption	Before adoption	After adoption	Fore quarter mastitis	Hind quarter mastitis
		hours		lakhs /ml			
116 (19.33%)	14 (2.4%)	2-3	4-5	6-8	4-5	23(37.7)	38(62.3)

Table 5 Sensitivity of antibiotics towards mastitis organism based on ABST (n=14)

Type of reaction	Name of the antibiotics					
	Enrofloxacin	Tetracycline	Gentamicin	Cipro floxacin	Penicillin	Cephalosporin
Sensitivity	9	12	13	10	7	11
Resistance	5	2	1	4	7	3
Sensitivity(%)	64.28	85.71	92.85	71.42	50.0	78.57

after implementing clean milk production strategies in dairy farm.

The California mastitis test is used to identify the specific udder quarter infected with clinical and sub clinical intra-mammary infection and is an indicator of mastitis as well as heard monitoring programme (De, 2010). The present study revealed that around 62.3 per cent of mastitis was diagnosed in hind quarters and 37.7 per cent in fore quarters of udder.

Sensitivity of antibiotics towards mastitis organism based on ABST

The dairy farmers were started to follow the clean milk production practices after the training programme which eventually leads to reduction in the incidence of mastitis from 116 to 14 during the one year period of study. Table 5 shows the antibiotic sensitivity pattern of infected milk samples. The results indicated that the mastitic pathogens were sensitive to Gentamicin, Tetracycline, Cephalosporin, Ciprofloxacin, Enrofloxacin and Penicillin as descending order of sensitivity. These results were communicated to the training beneficiaries and the concerned veterinarians who have treated the affected animals. The affected cows were treated at the earliest with specific antibiotic based on antibiogram and thereby saved the farmers from huge economic loss.

Conclusions

The capacity building programme was conducted in the selected villages pertaining to clean milk production practices which had great impact in reduced incidence of mastitis in dairy cattle. The dairy farmers were trained on mastitis detection and inputs were supplied for the detection of mastitis in the initial stage itself. The keeping quality of raw milk was improved with enhanced methylene dye reduction time. There was also considerable reduction in the somatic cell count in raw milk by the adoption of clean milk production practices and it was an indirect indication of improved microbial quality of raw milk. The study revealed that there was a drastic reduction in the occurrence of mastitis cases from 19.33 per cent to 2.4 per cent after adopting knowledge and skills on the mastitis detection and control techniques. The affected animals were treated with suitable antibiotic based on Antibiotic Sensitivity Test and saved the dairy farmers from economic loss. It was concluded that imparting knowledge on clean milk production practices to dairy farmers will eventually lead to prevention and control of mastitis under field condition.

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RESEARCH ARTICLE

Adoption of recommended dairy farming practices by farmers in Maharashtra under Kamdhenu Dattak Gram Yojana

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Abstract: The present study aimed to measure the extent of adoption of recommended dairy farming practices by farmers of Pune and Nashik divisions of Maharashtra which were selected based on livestock population and milk production in the year 2018-19. From each division two districts were selected randomly and from each district two blocks were selected randomly. From each block one adopted and one non-adopted village under the Kamdhenu Dattak Gram Yojana scheme were selected from each village 15 dairy farmers were selected on random basis. Overall, 240 dairy farmers were selected, as the respondents for this study constituting 120 beneficiarie farmers and 120 non-beneficiaries of the Kamdhenu Dattak Gram Yojana. The quantitative and qualitative data were collected through interview schedule, discussion, observation and available secondary sources. Findings indicated that the most of beneficiaries (43.33%) were in medium level overall adoption and majority of non-beneficiaries (62.50%) had fallen in low level of overall adoption category on recommended dairy farming practices covering breeding, feeding, health, general management and waste management practices. The overall adoption index of recommended dairy farming practices of beneficiaries (75.31) was found to be more compared to non-beneficiaries (66.75) of the scheme and found highly significant at 5 per cent level of probability using 'Z' test. Based on adoption index, maximum adoption of dairy farming practices was found in case of Breeding practices, whereas minimum adoption was found in case of Waste management practices in both beneficiaries and non-beneficiaries of the scheme. The results clearly indicated that the Kamdhenu Dattak Gram Yojana had created positive impact on the adoption level of the beneficiaries of scheme by adopting higher recommended dairy farming practices compared to the non-beneficiaries of the scheme.

Keywords: Adoption index, adoption, dairy farming practices, *Kamdhenu Dattak Gram Yojana*

Introduction

Dairy is having potential to improve the economic condition of the rural poor to fulfill the national commitment. This vast potential of dairy farming can be harness by efficient management of livestock production which, in turn, needs updated or latest scientific, economic and technical information to the farmers. Adoption and diffusion of dairy innovations is very essential to enhance the productivity of livestock and in turn income to dairy farmers' upto desired extent (Parihar et al. 2019). The slow pace of adoption of improved dairy farming practices is attributed to various factors. A firsthand knowledge of these factors to the extension personnel would create the speedy adoption of dairy innovations in the villages.

Therefore, a study entitled "Impact of *Kamdhenu Dattak Gram Yojana on Dairy Farmers in Maharashtra: An Appraisal from Farmers Perspective"* was conducted with specific objective to measure the adoption of the recommended dairy farming practices by the respondents in Maharashtra. The *Kamdhenu Dattak Gram Yojana* was launched by the Maharashtra government in October, 2010. The aim of the scheme was implementation of various essential activities of animal husbandry relevant to the breeding of animals, health care and disease control, general management and feed-fodder production in the selected village/cluster of villages in all the districts with a focused approach and mission mode basis. Under this programme, each Animal Health Centre of State Animal Husbandry Department has to adopt one village

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which is having 300 or above breedable cattle and buffalo population. Based on which a large number of technologies suitable for farming situations have been implemented in adopted villages for the overall development of dairy farmers by increasing the income from dairying.

Materials and Methods

Study area

The present study was conducted in the Pune and Nashik divisions of the state which constitute more than 2/3rd bovine population of the state comprising mainly crossbred's cows and buffaloes which contribute 68.69 per cent of total milk production of state. The milk production of Pune and Nashik division are 41.80 percent and 26.88 percent, respectively of the total milk production of state in 2017-18 (GOM, 2019). The *Kamdhenu Dattak Gram Yojana* was launched initially on pilot basis in these divisions of Maharashtra since 2011-12. The state government has set target for implementation of this scheme through animal husbandry and dairy development department of state.

Study approach and sampling methods

A triangulation approach using both qualitative and quantitative methods were used in the study. A multistage sampling procedure was followed. Pune and Kolhapur districts from Pune division and Nashik and Ahmednagar districts from Nashik division were selected randomly. From each district two blocks were selected randomly. From each block one *Kamdhenu Dattak Gram Yojana* adopted village and one non-adopted village were selected randomly. From each village 15 dairy farmers were selected randomly. Thus, in total, 120 dairy farmers from adopted villages under *Kamdhenu Dattak Gram Yojana* and 120 dairy farmers from non-adopted villages were selected using Random Sample Technique for this study.

Method of data collection

Data were collected using a well-structured interview schedule during October, 2018 to March, 2019 period. In the present study, adoption was measured by collecting all the relevant items related to recommend dairy farming practices with consultation veterinarians and SAUs in the study area, and also concerned literature and previous research studies. The selected items were divided in following heads: feeding, breeding, health care, general management and waste management practices. The response of the respondents were taken against each of the practice on a three point continuum representing '*Adopted'(A), 'Partially adopted' (PA), and 'Not adopted*' *(NA)* adopted with scores of 2, 1and 0, respectively.

Data analysis

The respondents were categorized into low, medium and high categories of adoption on the basis of Cumulative square root frequency method in different aspects of adoption as well as overall adoption of improved dairy farming practices. An adoption index was calculated for comparison of adoption of different recommended dairy farming practices among beneficiaries and non-beneficiaries of the scheme. The following formula was used to measure the adoption index of different aspects of improved dairy farming practices.

$$\text{Adoption Index} = \frac{\text{Score obtained by an individual}}{\text{Maximum obtainable score}} \times 100$$

Results and Discussion

The level of adoption of recommended dairy farming practices by beneficiaries would give an indication about how much impact taken place due to implementation of *Kamdhenu Dattak Gram Yojana*.

Adoption level of breeding practices

The results in the Table 1 revealed that half of beneficiaries (50.00%) were found under medium level of adoption. Whereas, 25.00 percent beneficiaries were found in equally both high and low level of adoption categories. On the other hand, in case of non-beneficiaries, the majority (60.00%) of respondents had low level of adoption of breeding practices followed by 24.17 and 15.83 per cent non-beneficiaries were found in medium and high level of adoption of breeding practice. This improvement among the beneficiaries might be due to motivation and technical help by the animal husbandry department officials to more adoption of breeding practices under *Kamdhenu Dattak Gram Yojana*. The overall adoption index of beneficiaries (81.83) is found higher compared to non-beneficiaries (73.08) of scheme. Similarly, Divekar et al. (2016) also observed parallel adoption index (81.40) to that of beneficiaries of present study in breeding management practices.

Adoption level of feeding practices

In the feeding practices, Table 1 revealed that most of the beneficiaries (47.50%) were found in medium level of adoption and 41.67 per cent of non-beneficiaries had low level of adoption of feeding practices. Whereas, about 28.33 and 24.17 per cent of beneficiaries were found in low and high level of adoption of the breeding practices, respectively. In case of non-beneficiaries, 39.17 per cent of respondents were found in medium level of adoption. Only 19.17 per cent of non-beneficiaries had high level of adoption of breeding practices. Milk production mainly depends upon feeding of concentrate mixture, getting knowledge of preparation of balance feed and silage making through demonstration by veterinary officials and sufficient mineral mixture feeding to animals.

Adoption level of health practices

Adoption of better healthcare practices is keeping animals from diseases free, consistent in milk production and good energy level of animal. It could be noted from Table 4.6 that the most of the beneficiaries (39.17%) had medium level of adoption of health practice followed by 37.50 per cent of the beneficiaries were found in low level of adoption. In case of non-beneficiaries, the majority (60.00%) of the non-beneficiaries had low level of adoption. This is mainly due to the veterinary officials of state government in beneficiary villages strictly following yearly schedule of vaccination, deworming by organizing camps at same time in village to contained disease infection spread to other animals. The overall adoption index of beneficiaries was found (78.96), which agreement with result reported by Divekar et al. (2016) in adoption index of different health practices by farmers was found 81.33 in Gujarat.

Adoption level of general management practices

The data presented in Table 1 inferred that in case of beneficiaries, the most of the beneficiaries (40.83%) had medium level of adoption followed by 35.00 and 24.17 per cent were found in to low and high level of adoption of management practices, respectively. On the other hand, the majority of the non-beneficiaries (57.50%) were having low level of adoption of management practices. The high adoption of management practices by beneficiaries might be timely information of practices provided under the scheme. Maintaining daily account keeping and milk production records by dairy farmers might help to monitor loss and profit in dairy farming and milk yield of animal. Whereas, non-beneficiaries of scheme were less aware of these management practices and ignorance of record keeping and milk yield monitoring of animals.

Adoption level of waste management practices

The results presented in Table 1 revealed that 37.50 and 30.83 percent of the beneficiaries were found under medium and high level of adoption of waste management practices, respectively. About 31.67 per cent of the beneficiaries had low level of adoption of waste management practices. In case of non-beneficiaries, most of them (45.00%) were found in medium level of adoption followed by 36.67 per cent had low level of adoption of waste management practices. Whereas, only 18.33 per cent of the non-beneficiaries were found under high level of adoption of waste management practices. Beneficiaries had more adoption of dung pit availability for FYM making, biogas production and vermin-compost making compared to non-beneficiaries. The reasons expressed by beneficiaries of scheme of adopted villages regarding more adoption due to more awareness through demonstration on waste management practices such as dung pit making which, vermi-compost making and biogas production and its importance (Ponnusamy and Devi, 2017). Another reason for more adoption FYM and vermi-compost, it used in their own field and also get additional income by selling remaining FYM and vermi- compost in market. The reasons cited for more adoption of Biogas in adopted villages by farmers were the biogas was required only installation cost and saved other cooking gas cost and also gives clean energy to them. The veterinary officials

Table 1 Distribution of respondents on the basis of adoption of recommended dairy farming practices

Sl. No.	Particulars	Category	Beneficiaries	Non-beneficiaries
1	Breeding practices	Low	30(25.00)	72(60.00)
		Medium	60(50.00)	29(24.17)
		High	30(25.00)	19(15.83)
2	Feeding practices	Low	34(28.33)	50(41.67)
		Medium	57(47.50)	47(39.17)
		High	29(24.17)	23(19.17)
3	Health practices	Low	45(37.50)	72(60.00)
		Medium	47(39.17)	25(20.83)
		High	28(23.33)	23(19.17)
4	General management practices	Low	42(35.00)	69(57.50)
		Medium	49(40.83)	26(21.67)
		High	29(24.17)	25(20.83)
5	Waste management practices	Low	38(31.67)	44(36.67)
		Medium	45(37.50)	54(45.00)
		High	37(30.83)	22(18.33)
6	Overall practices	Low	42(35.00)	75(62.50)
		Medium	52(43.33)	23(19.17)
		High	26(21.67)	22(18.33)

Figures in parenthesis indicate percentage of the respondents

Table 2 Extent of adoption of recommended dairy farming practices based on Adoption Index (AI) among beneficiaries and non-beneficiaries

S. No	Particulars	B (n=120)		NB (n=120)		MeanDifference	Percentdifference	'Z' value
		AI	Rank	AI	Rank			
1	Breeding	81.83	I	73.08	I	8.75	11.97	4.66*
2	Feeding	76.98	III	70.83	II	6.15	8.68	2.15*
3	Health	78.96	II	67.19	III	11.77	17.52	3.52*
4	General Management	73.70	IV	65.99	IV	7.71	11.68	2.04*
5	Waste management	56.94	V	48.89	V	8.06	16.48	1.86**
Overall Adoption Index		75.31		66.75		8.56	12.82	2.94*

*** Significant at 1 per cent level of significance*

** Significant at 5 per cent level of significance*

of scheme helped by linking the beneficiaries to get subsidies from allied state government department to installation of biogas plant.

Overall adoption of recommended dairy farming practices

Most of beneficiaries (43.33%) and majority of non-beneficiaries (62.50%) had fallen in medium and low adoption category in all aspects (breeding, feeding, health, management and waste management) regarding improved dairy farming practices (Table 1), respectively.

The findings also indicated that, 35.00 and 21.67 per cent of the beneficiaries of scheme were having low and high level of adoption in all aspects of recommended dairy farming practices, respectively. On the other hand, among non-beneficiaries, 19.17 per cent had medium level of adoption and only 18.33 per cent non-beneficiaries had high level of adoption in all aspects of recommended dairy farming practices. This indicated that adoption level of beneficiaries were higher as compared to non-beneficiaries.

Comparison of respondent's extent of Adoption of recommended dairy farming practices based on Adoption Index (AI)

A perusal of Table 2 shown that in case of beneficiaries, the breeding practices were ranked first with adoption index (AI) 81.83, which was followed by health practices and feeding practices which were ranked II and III with adoption index 78.96 and 76.98, respectively. While general management practices were ranked IV with adoption index 73.70 and waste management practices had ranked last with adoption index 56.94. The overall AI of recommended dairy farming practices was 75.31 of beneficiaries. On the other hand, among the non-beneficiaries, maximum adoption in the breeding practices which ranked first with adoption index (AI) 73.08 of recommended dairy farming practices. The feeding practices and health practices were ranked II and III, with adoption index 70.83 and 67.19, respectively. While the general management practices and waste management practices had 65.99 and 48.89 adoption index which ranked IV and V, respectively in extent of adoption of recommended dairy farming practices.

It also observed that, in both beneficiaries and non-beneficiaries, the adoption index was higher in breeding practices as most of the respondents had adopted the recommended breeding practices like keeping crossbred dairy animal; practicing AI in the animals at proper time of heat; practicing pregnancy diagnosis between 45-120 days after service; breed improvisation through selective breeding at farm level and practicing cow served within 60-90 days after calving. Whereas, adoption index was lowest in waste management practices due to the reason that recommended waste management practiced like dung pit availability for waste management (Ponnusamy *et al*, 2019); biogas production and vermi-compost making practices were not adopted by the most of dairy farmers. It could be observed from Table 1 that the overall adoption index of recommended dairy farming practices of beneficiaries (75.31) was found to be more compared to non-beneficiary (66.75). This results is in conformity with finding of Raut (2009) reported the overall adoption index scientific dairy farming practices by farmers was found 65.88 which was line with adoption index of non-beneficiaries of *Kamdhenu Dattak Gram Yojana* scheme. According to Mande and Thombre (2009) feeding practices for cow possessed more adoption index (57.36) followed by feeding practices for newly born calf (54.83), breeding practices (52.83) and health care practices (40.20). The overall adoption index was 52.17 in Latur district.

The score of adoption index of recommended dairy farming practices of beneficiary dairy farmers over non-beneficiary dairy farmers was found highly significant at 5 per cent level of probability. The beneficiaries had higher extent of adoption which might be due to higher knowledge of improved dairy farming practices, the information and the service provided by the scheme officials on time to farmers lead to creation of desire, interest and conviction to adopt recommended dairy farming scheme due to implementation of *Kamdhenu Dattak Gram Yojana*.

Conclusions

The study concluded that the most of beneficiaries (43.33%) in medium level of overall adoption and majority of non-beneficiaries (62.50%) had fallen in low level of overall adoption category in overall aspects (breeding, feeding, health, management and waste management) regarding recommended dairy farming practices. The overall adoption index of recommended dairy farming practices of beneficiaries (75.31) was found to be more compared to non-beneficiary (66.75) of the scheme. The *Kamdhenu Dattak Gram Yojana* had created positive impact on the adoption level of the beneficiaries of scheme. Based on the findings and observations, it can be recommend that there is need to increase exposure visits, demonstrations of different technologies, organizing campaigns, create more awareness through various extension literature as well as by providing on campus training programmes on improved dairy farming practices to dairy farmers which motivate them to higher adoption in non-adopted villages of scheme. Secondly, exposure and use of ICT tools such as mobile internet and various social media platforms for easy asses to good quality dairy farming practices and information of different scheme of government to both in adopted and non-adopted villages of *Kamdhenu Dattak Gram Yojana.* In order to increase dairy farming production and productivity, it is essential to provide input services (breeding, feeding, management and health care) at the farmers' door and create awareness among the dairy farmers about the new technologies through a strong Animal Husbandry Extension Network system.

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SHORT COMMUNICATION

Studies on production of low calorie lassi

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Abstract: Lassi containing the artificial sweeteners was compared with control sample containing sugar for quality parameters. Aspartame was used at 80, 90 and 100 ppm, and sucralose at 20, 25 and 30 ppm levels for total replacement of sugar during production of low calorie lassi. Based on the physico-chemical and sensory quality parameters, lassi added with 90 ppm aspartame and 25 ppm sucralose were found to be acceptable compared to control lassi. To achieve the mouthfeel of the finished product as that of control lassi, a bulking agent i.e. sorbtiol was added at three different levels i.e. 7, 7.5 and 8.0% respectively to the selected levels of sweetener. It was found that 7.5 per cent sorbitol level was more suitable than the lassi prepared with 7.0 and 8.0 levels of sorbitorl with respect to physico-chemcial and sensory quality. Satisfactory quality low calorie lassi was prepared by using artificial sweeteners viz . sucralose and asparatame along with sorbitol.

Keywords: Aspartame, Lassi, Low calorie lassi, Sucralose, Sorbitol,

Lassi is considered as digestive, nutritive and useful in gastrointestinal ailments. The health benefits of lassi are the result of biologically active components that are present in native milk and also due to suitably modulated activities produced through the action of lactic acid bacteria, and used for the treatment of diarrhea, dysentery, chronic specific and non specific colitis, piles and jaundice (Padghan et al. 2015). Diabetes is a heterogeneous primary disorder of carbohydrate metabolism and it affected around 171 million people worldwide in 2000 and this number may increase to at least 366 million by 2030. According to India's current national survey, more than 20% of the Indians are overweight or obese. Every fifth diabetic in the world is an Indian and India is the diabetes capital with 6.22 crore diabetic patients, which will reach to 8 crore in 2030 (Vairagade et al. 2016). Dairy products are occupying the major part in human diet every day, however they become a setback for health conscious, diabetic and obese people due to the presence of sugar and fat in sufficient quantity. Milk products traditionally used contain 8-10% sugar which provides 32.8- 41Kcal/100gm. The sugar in milk and milk products

contribute to overweight and acts as promoting factor for diabetes. To overcome the disorders, generally sugar is replaced with artificial sweeteners to reduce the calorific value of the products. Several texturizers such as pectin and gums have been proposed for use in low calorie beverages to compensate the texture loss and mouth feel (Saha and Bhattacharya, 2010; Berry, 2013). Artificial Sweeteners are also known as intense sweeteners because they are many times sweeter than the natural sugars like cane sugar. Artificial sweeteners are synthetic sugar substitutes but may be derived from naturally occurring substances, including herbs or sugar itself (Sushim Chaudry, 2015). Recently Indian government has permitted the use of artificial sweeteners in traditional dairy products. Based on the requirements of the consumers in the dairy industry the present investigation was undertaken to develop a low calorie lassi beverage using low calorie artificial sweeteners along with bulking agent for the diabetic and calorie conscious people.

For preparation of low calorie lassi, food grade sorbitol syrup (70% TSS) was procured from Vintop Products Pvt. Ltd., Mysore Road, Bangalore, intense sweetener aspartame (sugar free) was procured from Changzho Nioutang Chemical Pant Co. Ltd., Niutang Town, China whereas sucralose was procured from Shandong Kanbosweet Inc., Shandong, China. Lassi (control) was prepared as per standard procedure of Aneja et al. 2002 by using milk standardized to 3.5% fat and 8.5 % SNF. Initially the milk is preheated to 60-65°C and homogenized @2000 psi in I

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stage and 500 psi in II stage then apply heat treatment @ 90°C for 10 min and cooled to 30°C. Then add dahi culture @ 2 per cent level and incubate about 37°C around 12 hours. Then break the curd and blend for 50 per cent pasteurized water and mix thoroughly with agitator. Then add 12 % sugar as sweetening agent and 0.2 per cent vanilla used and packed in PET/PP cups and stored at 4±1°C.

Preparation of lassi with low calorie sweeteners and bulking agent**:** The procedure of lassi preparation was same as that of control sample as explained above; however, sugar was replaced with sucralose and aspartame. Aspartame (80ppm, 90ppm and 100ppm) and sucralose (20ppm, 25ppm, and 30ppm) were used to completely replace sugar. Further to improve the mouth-feel and viscosity of the product, sorbitol was used as bulking agent at the rate of 7.0%, 7.5%, and 8.0% along with the two artificial sweeteners.

The lassi was evaluated for physico-chemical and sensory characteristics. Fat, SNF content in raw milk and lassi were determined by Gerber method and Rose-Gottlieb method as described in IS: 1224-1958 and A.O.A.C, 2000 respectively. Viscosity of lassi (cp at 25°C) was determined at 20 ºC using Brookfield viscometer. pH of lassi was determined by a digital pH meter ElicoPvt Ltd., Hyderabad .Titratable acidity of lassi was determined as per the method given in (IS: 1479, Part-1,1960). The acceptability of lassi prepared by substituting sugar with low calorie sweeteners along with bulking agent was evaluated by conducting sensory evaluation by a panel of trained judges using 9-point hedonic scale. The results were analysed by statistical R core team.

On perusal of results presented in Table 1 it was observed that there was no significant difference between sweeteners and between sweetener levels and control in pH and acidity. It was observed that the initial pH and acidity of the milk did not get influenced by the addition of two low-calorie sweeteners. Artificial sweeteners added to yoghurt did not cause greater rise in acidity or reduction in pH during storage (Sabahat Yaqub et al. 2018). The results are also corroborated by the findings of El. Tahra et al. (2015) who reported that incorporation of honey at 2%, 4% and 6% w/v in yoghurt did not significantly influence the pH and lactic acid levels of the product. The viscosity was greater in control when compared to the artificial sweeteners added lassi. Statistically it was found that there was no significant difference between sweeteners however a significant difference ($p<0.01$) was found between control and sweeteners levels. It is evident that the lassi made with two low calorie sweeteners at different levels had significantly lower mean viscosity than the control wherein the sugar directly contributed to the viscosity. The present findings are in agreement with the results observed during the preparation of low calorie chocolate flavoured milk made with aspartame stevia and sucrose in different proportions by Aziz et al. (2012) and wherein the viscosity of control *lassi* was higher when compared with *lassi* by incorporation of concentrated and lactose hydrolyzed whey**.**

The mean flavour score of control lassi containing 12% sugar was 8.5. Acceptable sweetness in 25 ppm sucralose added lassi was observed by the panellists. This was equal to the perceptiveness of the lassi made with sugar without any after taste compared to the other two levels viz., 20 and 30 ppm added lassi. It was observed from the mean flavour scores of sucralose

Table 1 Influence of sweeteners' level on physico – chemical and sensory quality (1-9 score) of lassi without bulking agent

Level of sweetener added (ppm)	Sample attributes						
Sucralose	pH	T.A* (% LA)	Viscosity, (cp at 25ºC)	Flavour score	Color & Appearance score	Consistency score	O A*score
Control	4.50[a]	0.75[a]	242.00[a]	8.50[a]	8.44[a]	8.56[a]	8.46[a]
20 ppm	4.50[a]	0.75[a]	123.20[b]	7.92[b]	8.42[a]	7.60[b]	7.86[b]
25 ppm	4.52[a]	0.75[a]	128.00[b]	8.50[a]	8.42[a]	7.82[a]	8.26[a]
30 ppm	4.50[a]	0.75[a]	125.20[b]	8.08[a]	8.42[a]	7.70[b]	8.00[b]
CD value	NS	NS	104.2	0.56	NS	0.77	0.41
Aspartame			Sample attributes				
Control	4.50[a]	0.754[a]	242.80[a]	8.50[a]	8.50[a]	8.56[a]	8.48[a]
80 ppm	4.50[a]	0.754[a]	124.80[b]	7.96[b]	8.44[a]	7.70[b]	8.04[b]
90 ppm	4.50[a]	0.754[a]	126.20[b]	8.54[a]	8.40[a]	8.30[a]	8.26[a]
100 ppm	4.51[a]	0.754[a]	122.60[b]	8.00[b]	8.38[a]	7.60[b]	8.04[a]
CD value	NS	NS	105.70	0.49	NS	0.62	0.37

*T.A – Titratable acidity *OA – overall acceptability

All values are average of five trials

Control – Lassi added with sugar @ 12% level on milk basis

NS – Non significant

Similar superscripts in a column indicate non-significant at the corresponding critical difference ($P<0.05$)

Table 2 Influence of sorbitol level on physico – chemical and sensory quality (1-9 score) of lassi with low calorie sweeteners

Level of sorbitol added	Attributes of lassi with Sucralose (25ppm)							Attributes of lassi with Aspartame (90ppm)						
	pH	T.A* (% LA)	Viscosity, (cp at 25°C)	Flavour score	Color & Appearance score	Consistency score	O A* score	pH	T.A* (% LA)	Viscosity (cp at 25°C)	Flavour score	Color & Appearance score	Consistency score	O A* score
Control	4.47^{a}	0.756^{a}	280.6^{a}	8.50^{a}	8.28^{a}	8.30^{a}	8.34^{a}	4.47^{a}	0.756^{a}	280.6^{a}	8.50^{a}	8.28^{a}	8.30^{a}	8.34^{a}
7.00%	4.46^{a}	0.758^{b}	304.2^{b}	8.44^{a}	8.50^{b}	8.38^{b}	8.44^{b}	4.46^{a}	0.758^{b}	304.2^{b}	8.44^{a}	8.50^{b}	8.38^{b}	8.44^{b}
7.50%	4.46^{a}	0.758^{b}	313.4^{b}	8.46^{a}	8.40^{b}	8.50^{b}	8.46^{b}	4.46^{a}	0.758^{b}	313.4^{b}	8.46^{a}	8.40^{b}	8.50^{b}	8.46^{b}
8.00%	4.46^{a}	0.758^{b}	393.2^{c}	8.08^{b}	8.44^{b}	8.60^{b}	8.37^{b}	4.46^{a}	0.758^{b}	389.2^{b}	8.08^{b}	8.44^{b}	8.60^{b}	8.37^{b}
CD value	NS	0.01	79.8	0.34	0.16	0.23	0.1	NS	0.01	79.8	0.34	0.16	0.23	0.1

*T.A – Titratable acidity *OA – overall acceptability
All values are average of five trials
Control – Lassi added with sugar @ 12% level
NS – Non significant
Similar superscripts in a column indicate non-significant at the corresponding critical difference (P<0.05)

added lassi that there was a significant difference between the sweetener levels and control. These findings are in agreement with the results of low calorie sterilized flavoured milk made with 75ppm sucralose (Natraj, 2008) wherein a higher level of sucralose was added to the mix to meet sterilization losses. The colour and appearance mean score values were more or less stable between the three levels of sucralose added lassi. However, there was no significant difference between levels when compared to control. Further, it was observed from the mean consistency scores of sucralose added lassi that there was a significant difference between the levels and control, as sugar is contributing ingredient to the consistency. The observations are in conformity with the findings of Arora et al. (2008) who reported insufficient water binding capacity of artificial sweetener which resulted in a product with lower consistency. In addition, there was a significant difference between the levels and control observed with the mean overall acceptability scores of sucralose added lassi.

The average mean flavour score for aspartame added lassi without bulking agent for the level of 90 ppm aspartame was found to be best compared to the level of 80 and 100ppm. It was observed from the mean flavour scores of aspartame added lassi that there was a significant difference between the levels and control. However, 80 and 100 ppm aspartame added lassi were found to be having low and higher degree of perceptible sweetness respectively and was not comparable to that of control. The results are comparable with the findings of Arora et al. (2008). The colour and appearance mean score values of the three levels of aspartame as well sucralose added lassi had no significant difference between levels when compared to control. Evidently, Arora et al. (2008) reported that the colour and appearance score were significantly lower ($p<0.05$) for artificially sweetened kalakand as compared to control. It was observed from the mean consistency scores of aspartame added lassi that there was a significant difference between the levels and control. Evidently, addition of sugar in control contributed a higher mean consistency scores than the aspartame added lassi without sugar. The mean overall acceptability scores of aspartame added lassi showed that there was a significant difference between the levels and control. The results are comparable with the study done by Rathod et al. (2013) indicating that with increase in the level of aspartame, there is a significant difference between the levels and control and Arora et al. (2008) studied that sucrose was successfully replaced with the sweetener aspartame for the preparation of lassi and flavoured milk. Aspartame at a level of 0.07% in flavoured milk and 0.08% in lassi on milk basis scored highest in terms of sweetness perception and resembled control prepared with sucrose.

Sorbitol is a polyhydric alcohol which contributes to all aspects of sweetener functionality including sweetness, bulking and freezing point depression. As it has the same calorie value of sucrose it cannot be used for calorie reduction. However, in diabetic dairy products it replaces sugar from the aspect of

providing some of the bulk and mouth feel and also some sweetness. The average flavour score for 7.5% incorporation of sorbitol was 8.46, which was higher than 7 and 8% level of addition (Table 2). Some amount of sweetness is contributed to the lassi through sorbitol, the optimum perceptible sweetness was appreciated by the judges in 7.5% level incorporation which is slightly lower than that of control. Statistically, it is supported by showing a significant difference between the 8 % sorbtiol added lassi and control lassi. The mean colour and appearance, consistency and overall acceptability scores were significantly different between the sorbitol levels and control. It is evident from the study that sorbitol contributed good body and texture and mouth feel to product compared to the sugar containing control lassi. Further, an acceptable and appreciable colour and appearance of the product was observed in lassi made with sorbitol than sugar made lassi. Jayaraj Rao and Pagote, (2018) suggested use of 40 brix sorbitol solution containing 14.3 mg/L Asparatame for low calorie rasogolla. Further Narendra Raju and Dharampal, (2011) confirmed that use of asparatame and acesulfame-k at 0.38g/Kg and 0.35g/Kg along with 14g/Kg of maltodextrin gave highly acceptable quality of Mishti dahi. Nataraj (2008) standardized the level of sorbitol content in development of low calorie sterilized flavoured milk and found that addition of 8 per cent sorbitol recorded an optimum sensory scores than 7.5 % level. Based on the sensory attributes in the above study, 7.5% sorbitol and 25ppm sucralose added low calorie lassi were found to be highly desirable when compared to others.

The average flavour score for 7.5% incorporation of sorbitol was 8.46 which was higher than 7 and 8% level incorporated products. Some amount of sweetness was contributed to the lassi through sorbitol, the optimum perceptible sweetness was appreciated by the judges in 7.5% level incorporation which was slightly lower than that of control. Statistically it was supported by showing a non-significant difference between the sorbtiol levels and control. The mean colour and appearance, consistency and overall acceptability scores were significantly different between the sorbitol levels and control. Based on the above study, low calorie lassi made with incorporation of 7.5% sorbitol with 90 ppm aspartame showed acceptable perceptible sweetness. Finally, based on the above study it was found that the following flow diagram can be evolved for production of low calorie lassi made with two artificial sweeteners along with the bulking agent sorbitol

Milk (Fat 3.5%, SNF 8.5%)
↓
Preheating 60°C
↓
Homogenization (I stage 2500 psi and II stage 500 psi)
↓
Curd setting
↓
Addition of 0.009% aspartame and 7.5% sorbitol and flavor

or

Addition of 0.0025% sucralose and 7.5% sorbitol and flavor
↓
Making Lassi
↓
Packaging in PET/PP cups
↓
Storage (4+1°C)

The calorific value of 25 ppm sucralose + 7.5% sorbitol, 90 ppm Aspartame + 7.5% sorbitol added low calorie lassi and control lassi made with 12% sugar (on milk basis) were found to be 66.8, 66.20 and 86.92 Kcal of energy per 100 g respectively. Further, it was found that on calculation there ~~is~~ was 23% and 23.8% reduction in calorific value of the above two low calorie lassi respectively than compared to control.

Conclusion

Based on the physico – chemical and sensory evaluation, it was found that lassi made with 7.5% sorbitol plus 25 ppm sucralose and 7.5% sorbitol plus 90 ppm asparatame were highly acceptable compared to the control.

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SHORT COMMUNICATION

An assessment on inclusion of private input dealers in the public extension services delivery system

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Abstract: The present study examined the amenability & readiness of private input dealers in deliverance of dairy and other livestock extension services. "Q Methodology" was adopted in Kancheepuram and Villupuram districts of Tamil Nadu state with 26 identified input dealers, handling dairy and other livestock input items, during the year 2016. The attentiveness of the respondents towards their inclusion in the public extension services system', were estimated subjectively with the help of twenty five attitude statements which were moderated out of forty five statements. Principle Component Analysis (PCA) was used to categorize the statements under four identified discourses based on the obtained Eigen values. The four defined discourses *viz.,* "Risks and opportunities in the endeavour", "Socio-economic empowerment in the undertaking", "Elements of collaboration in the endeavour" and "Possible refraining behaviour / escapism in the process"; in a nutshell had cued positive agreement of input dealers to the statements supporting to their inclusion in public extension service stream.

Keywords: Extension Services Delivery, Private Input Dealers, Q Methodology, Principal Component Analysis

Input service providers in animal husbandry extend valuable services to the clients to maintain health and enhance productivity. Private input dealers, an integral part of livestock services, form a wide category as far as animal husbandry is concerned. The importance and utilization of experienced private sector players would strengthen the livestock services infrastructure, credit facilities, insurance, health care & prophylaxis and identified areas of value addition and processing of livestock products including milk, meat, skin etc. Efforts to tap the potential of private input dealers are in process through some initiatives by the central and state authorities and one such effort is the "Diploma in Agricultural Extension Services for Input Dealers (DAESI)" program by National Institute of Agricultural Extension Management (MANAGE), Hyderabad. In this backdrop, the ability of private companies and dealers in rendering services to the livestock owners was felt to be thoroughly examined for utilizing their services in nation's agrarian cause. "Q Methodology" was followed and the attitudes of the respondents were estimated subjectively to assess the priorities & readiness of private input dealers in deliverance of livestock extension services. This subjectivity analysis was carried out in purposively selected Villupuram and Kancheepuram districts of Tamil Nadu involving 26 identified input dealers.. The past experience of the researcher in the districts as a Veterinary Services Execute in a reputed pharma company and subsequently as a Veterinary Officer with the state government; were pondered and this formed the basis to purposively select the districts, as rapport was felt as a critical factor to tap the respondents' subjective views on the research component. Only 26 input dealers were identified during the course of the research process due to limitations in identifying vivid input dealers in the study area.

Subjectivity is judgment based on individual personal impressions, feelings and opinions rather than external facts. This can be considered mind-dependent, because one is not using a fact, they are using their personal opinion (Amandaroseboyd, 2011). The increasing credibility of qualitative

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approaches for research has opened up new debates about methodology and rigor. The transition to a more subjective, reflexive approach to research brings benefits and with the loss of scientific rigor comes the gain of eliciting true meaning, by recreating the experiences of others through co-operative enquiry. Schultz (1994) argues that an openly subjective approach allows the researcher to be a real partner with informants, and to openly use his or her own experiences and reflections in order to uncover valuable meaning; adding value to the methodology. Q Methodology was adopted to subjectively analyse the input dealers' view on their inclusion in the mainstream extension services delivery system., as the method has been considered as a robust and relatively novel method in subjectively presenting the thoughts. It also is the method which has a combination of qualitative and quantitative entities (Gjalt de Graaf, 2007).

Q Methodology was developed by William Stephenson, an Englishman; Q methodology provides a foundation for the systematic study of subjectivity, a person's viewpoint, opinion, beliefs, attitude, and the like (Brown, 1993). In a Q methodological study, people are presented with a sample of statements about some topic, called the Q-set. Respondents, called the P-set, are asked to rank-order the statements from their individual point of view, according to some preference, judgment or feeling about them. By Q sorting, people give their subjective meaning to the statements, and by doing so reveal their subjective viewpoint. These individual rankings (or viewpoints) are then subject to factor/principal component analysis. By correlating people, Q factor analysis gives information about similarities & differences in viewpoint on a particular subject and the factors resulting from Q analysis thus represent clusters of subjectivity that are operant (Smith, 2001). As per Van Excel and de Graff (2005), the means and process of Q method can be explained like "A person is presented with a set of statements about some topic, and is asked to rank-order them (usually from 'agree' to 'disagree'), referred to as 'Q sorting.' The statements are matters of opinion only (not fact), and the fact that the Q sorter is ranking the statements from his or her own point of view is what brings subjectivity into the picture. The analytical process of Q method reduces the data based on principal components analysis (PCA) or factor analysis (FA). However, instead of correlating variables (as in regular PCA and FA), in Q the respondents are correlated in order to elucidate the relationships between them. The final results consist of a small number of sets of sorted statements, which are different from each other and summarize the perspectives existing among the

Table 1 Rotated component matrix

	Rotated Component Matrix[a]			
	Component			
	1	2	3	4
VAR00001				
VAR00002	-.447		-.589	
VAR00003			.602	
VAR00004		.765		
VAR00005			.737	
VAR00006		-.773		
VAR00007	-.903			
VAR00008			-.840	
VAR00009	.706			
VAR00010	.566			.596
VAR00011				.483
VAR00012				-.748
VAR00013	-.630			
VAR00014		-.716		
VAR00015	.765			
VAR00016	.680		-.470	
VAR00017		.822		
VAR00018			.857	
VAR00019		-.783	.428	
VAR00020		.783		
VAR00021	-.823			
VAR00022	.605			-.522

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 5 iterations.

respondents (Zabola, 2014). The results of a Q methodological study can be used to describe a population of viewpoints and not, like in other method called R method; a population of people (Risdon et al. 2003).

The "Q" set for this study consisted of 25 statements which was moderated among 45 identified statements by the researcher in consultation with fellow scientists and input dealers in the field; who ultimately did not fall in the "P" set – respondents. The identified statements (Q Set) were numbered and presented along with the distribution of respondents (P Set) with the frequencies up on their degrees of agreement (highly agree to highly disagree). The analysis of the Q sorts is purely technical, objective procedure – and is therefore sometimes referred to as the scientific base of Q. First, the correlation matrix of all Q sorts was calculated. This matrix represents the level of agreement/disagreement between the individual sorts, that is, the degree of similarity/dissimilarity in points of view between the individual Q sorters. The correlation matrix then was subjected to principal component analysis, with the objective to identify the number of natural groupings of Q sorts by virtue of being similar or dissimilar to one another, to examine how many basically different Q sorts are in evidence.

Principal component analysis (PCA) is a statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. The number of principal components is less than or equal to the number of original variables. PCA is mostly used as a tool in exploratory data analysis and for making predictive models. PCA can be done by Eigen value decomposition of a data covariance (or correlation) matrix or singular value decomposition of a data matrix, usually after mean centering (and normalizing or using Z-scores) the data matrix for each attribute. The results of a PCA are discussed in terms of component scores, also called as factor scores and loadings i.e., the weight by which each standardized original variable should be multiplied to get the component score. The factors (out of 22 statements from 25) in each group deliver the most important information to reconstruct four discourses: four different views of private livestock input dealers about the inclusion of themselves in public extension system. Here, in each discourse description, first a label is presented; some relevant statements for the discourse are then presented, together with the idealized score of the four discourses. Then, a narrative of the discourse is presented. The composite sorts are used to interpret and describe the discourses and respondents' explanations on their ranking of statements have been discussed on the basis of interaction made with them. Discussion would have emphasis on the variable having a minimum significant score of 0.6, as it has been fixed on analyzing the factor values across the table 1.

The interpretation and conclusion in Q method is done by explanations based on the increased factor loadings and sign of the value obtained from the Q sorts, after computing Principal component analysis and has been presented in the table 2. To sum up the analysis, statements having obtained high factor loadings (both + and - values) in all the discourses had to be presented and general views and insight of the respondents on the present topic of interest had to be analyzed in all possible dimensions. In the first factor discourse "Risks and opportunities in the endeavor, among the seven presented variables, statement about risky nature of the process' possessed the maximum factor score of -.903 followed by statement on procedural intricacies' and one on government policies', both had factor scores -.823 and 0.765 respectively with sign difference. The respondents' had a keen view and acceptance on the negative side that the inclusion venture may not go risky, in other words the input dealers did not have a concern over the risk

Table 2 Categorized variables (statements) and their Eigen scores

	Discourse A : Statements pertinent to the label "Risks and opportunities in the endeavor"	
Variable	Statement	Score
Var 7	This venture seems to be too risky for me	-.903
Var 9	Chances of discontinuance and absenteeism by input dealers is more in this alliance	0.706
Var 13	Strict implementation of extension strategies can result in non-inclusion of third parties like us	-.630
Var 15	The government has started significant number of welfare schemes related to livestock & animal husbandry in the recent years	0.765
Var 16	Time spent in this collaboration will not be in proportion to the money earned if I concentrate on the business which I am in now	0.680
Var 21	Frequent meetings and other procedural intricacies shall make this combination difficult to achieve the benefits	-.823
Var 22	Combined training for the input dealers with farmers is an important pre-requisite to the possibility of including them in to the mainstream extension service delivery system	0.605
	Discourse B: Statements pertinent to the label "Socio-economic empowerment in the undertaking"	

Variable	Statement	Score
Var 4	Input dealers can be utilized by the government for their efforts leading to widespread technology transfer	0.765
Var 6	Surge of information and ease in attaining them is going to be very useful for the needy farming community	-.773
Var 14	Young and middle age entrepreneurs can make this collaboration effective	-.716
Var 17	Special status shall be provided to input dealers involved in public service	0.822
Var 19	Number of collaborative efforts should be minimal for effective functioning	-.783
Var 20	Veterinarians often guide us in stock maintenance and market orientation for more profitable business	0.783
	Discourse C: Statements pertinent to the label "Elements of collaboration in the endeavor"	
Variable	Statement	Score
Var 2	Government - private input dealers' collaboration can be a fruitful venture for people cause	-.589
Var 3	Public's recognition is difficult to achieve in this mode of operation	0.602
Var 5	Comfort zone in my business will be in stake if I get involved in this activity	0.737
Var 8	Information availability in this global era is easy and abundant not only for farmers but for all	-.840
Var 18	The collaboration between government and private input dealers shall be effective if it is incentive based	0.857
	Discourse D: Statements pertinent to the label "Possible refraining behavior / escapism in the process"	
Variable	Statement	Score
Var 10	Service is not the primary motto for any businessman including livestock input dealers like me	0.596
Var 11	Line department officers may render deserving respect and regard to input dealers in this collaboration	0.483
Var 12	Manpower lacuna is the important factor for the prevailing situations in extension services delivery	-.748
Var 22	Combined training for the input dealers with farmers is an important pre-requisite to this initiative	-.522

involved in the inclusion process. The degree of disagreement to procedural intricacies and agreement on the variable, innumerable initiatives by the governments carries impressive factor load.

The second factor discourse "Socio-economic empowerment in the undertaking", among the six presented variables in the second group, statement about special status to the input dealers possesses the maximum factor score of 0.822 which implies that the input dealers if collaborated, should be given special status by means of recognizing them with honours and awards, a very positive step in the direction. Statement on incentive-based collaboration' topped the group with an impressive factor score 0.857 in the third factor discourse "Elements of collaboration in the endeavor" referring the private input dealers', depicting the importance of the financial element in the inclusion process. The fourth factor discourse "Possible refraining behaviour / escapism in the process" had highlighted the statement on manpower paucity and prevailing situations in extension services delivery' with factor loading -.748. In a nutshell the statements and the discourses categorized and analyzed in the study cues on positive agreement of input dealers supporting to their inclusion in public extension service stream which can be pondered up on by the planners and policy makers in implementing the theme on pilot basis and furtherance to its augmentation.

Conclusions

Role of extension personnel is crucial in fostering collaboration and networking between and among different agencies so as to facilitate farming situations to stand at par with the market-driven economy (Ponnusamy and Pachaiyappan, 2018). Networking is all about involvement of multiple partners with same objective, and this reduces the efforts in scrambling extension services to the needy. Of late, the central and state governments have started numerous special schemes and efforts in empowering animal husbandry farmers and these schemes need multiple partners in the value chain for an effectual and speedy reach. Private input dealers, particularly the shop owners who directly deal with the livestock farmers in terms of OTC (over the counter sales) can very-well be a partner in outreaching the government's initiative. The research findings of this study also suggest the integration of input dealers in the value chain development process which

can also be mediated through DAESI benefactors and other noted dealers on trial basis. Social recognition and incentive-based collaboration of the governments and input dealers, the concepts emerged from subjectivity analysis points confirmed the input dealers' agreement in the inclusion process; which might entice valid consideration by the implementing authorities thereby augmenting win-win situation for all the stake-holders in livestock services delivery system.

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SHORT COMMUNICATION

Sire evaluation based on first lactation milk yield traits in HF X Gir halfbred

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Abstract: The data on first lactation milk production performance of 422, HF X Gir halfbred cows maintained at Research Cum Development Project on Cattle, MPKV, Rahuri were utilized for estimation of least squares means, correlation among the traits and heritability of traits. Breeding values of sires were estimated by REML computer programme. The overall least squares means (kg) of first lactation monthly test day milk yield (from 1st to 6th month) (FLMTDMY), first lactation peak milk yield (FLPMY) and first lactation 300 days milk yield (FL300 DMY) were 10.54 ± 0.14, 10.57 ± 0.13, 10.10 ± 0.12, 9.48 ± 0.12, 8.93 ± 0.11, 8.23 ± 0.11, 14.22 ± 0.14 and 2438.98 ± 38.49 respectively. Phenotypic and genotypic correlations among the traits were positive and significant (P<0.01). Heritability of traits was low to moderate and ranged from 0.06 to 0.25. Sire number HG-412 and HG-129 had highest breeding values for FLMTDMY and H-28I and HG-15 had highest breeding value for FLPMY and FL300DMY and ranked first.

Keywords: FLMTDMY, FLPMY, FL300 DMY , HF X Gir halfbreds, Sire evaluation

To bring about improvement in milk production of cattle, it is must to execute breed improvement program for genetic evaluation of males and females through selection of superior animals of high genetic merit. Based on first lactation milk production, the animal can be selected for future breeding. Use of monthly part lactation yields will be useful in selecting cows during early younger age resulting in reduced generation interval, increased intensity of selection attributed to the availability of more number of records on daughters having monthly records. The usefulness of part lactation records depends upon the accuracy with which sires are evaluated on the basis of these records besides the genetic correlations between part lactation milk yield records and 305- day milk yield. Literature available on part lactation milk yield also revealed that these milk yields can be used for prediction of 305-day milk yield as high genetic association between monthly part lactation milk yield and complete milk production records was observed Mundhe et al. (2018). Sire evaluation is most essential for selection of sire and for genetic improvement of cattle. It is also needful as majority of genetic improvement can be made through selection of males rather than females. Success of breeding programme depends on how early and accurately young bulls are evaluated. Systematic evaluation of bulls need complete records of daughters at least for first lactation. Hence, the present investigation was undertaken with the objective of estimation of breeding value of sires using first lactation monthly milk yield, peak milk yield and 300 days milk yield of the daughters.

The data for research were collected from pedigree, history and milk recording sheets of 422 HF X Gir cows maintained for period of 43 years from 1974 to 2016 at Research Cum Development Project on Cattle, Mahatma Phule Krishi Vidyapeeth, Rahuri.

The data of first lactation 1st to 6th monthly test day milk yield (FLMTDMY), first lactation peak milk yield (FLPMY) and first lactation 300 days milk yield (FL300DMY) were estimated by least squares technique (Harvey, 1990) by considering period of calving and season of calving effects. Period of calving were grouped as P_1 (1974-80), P_2 (1981-87), P_3 (1988-94), P_4 (1995-2001), P_5 (2002-08) and P_6 (2009-16). Season of calving were divided as S_1 (Rainy), S_2 (Winter) and S_3 (Summer). Duncan's Multiple Range Test (DMRT) as modified by Kramer (1957) was used to make pairwise comparison among least squares means. Data were corrected for significant effects of non-genetic factors and used

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for estimation of genetic parameters as suggested by Gacula et al. (1968) and sire evaluation. Heritability of traits was estimated by Paternal half sib correlation method, genetic correlations were computed by Paternal half sib analysis of co-variance method and phenotypic correlations among traits were estimated according to procedure suggested by Snedecor and Cochran (2004). The HF X Gir half bred sires were evaluated on the basis of their breeding values estimated by Restricted Maximum Likelihood (REML) computer programme using univariate model (Meyer, 1998).

The least squares analysis of various means of first lactation monthly test day milk yield, peak milk yield and 300 days milk yield of HF X Gir half bred are presented in Table 1,2 and 3. The overall mean first lactation test day milk yield from 1st to 6th month was 10.54 ±6.14 kg. The analysis of variance indicated that the effect of period of calving on monthly test day milk yield was significant (P<0.01). Similar results were reported by Rashia Banu (2010) in Karan Fries cattle. The results showed that first lactation monthly test day milk yield from 1st to 6th month of cows calved during P_1 (1974 to 1980) was significantly higher than those calved in rest of the periods. The influence of season of calving on monthly test day milk yield of HF X Gir cows was non-significant. The monthly test day milk yield was highest in cows calved during winter season in all months except first month. This might be due to favorable climate and adlib fodder available during winter season.

The overall mean first lactation peak milk yield in HF X Gir halfbred was 14.22 ±0.14 Kg. The effect of POC on FLPMY was significant (P<0.01). The peak milk yield of cows calved during P_1 (1974 to 1980) was significantly higher (16.95±0.24 kg) than those calved during rest of periods. Peak milk of cows calved during P_2 (1981 to 1987), P_5 (2002 to 2008) and P_6 (2009 to 2016) did not differed significantly from each other and significantly higher than calved in P_3 (1988 to 1994) and P_4 (1995 to 2001) periods. The variation due to season of calving in peak milk yield of Gir halfbred was non-significant. The FLPMY was

Table 1 Least squares means of monthly test day milk yield (kg)

Source of variation	N	T_1	T_2	T_3	T_4	T_5	T_6
Population mean (μ)		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
	421	10.54 ± 0.14	10.57 ± 0.13	10.10 ± 0.12	9.48 ± 0.12	8.93 ± 0.11	8.23 ± 0.11
Period of calving							
P_1 (1974-1980)	1126	12.69 ± 0.23^a	13.63 ± 0.22^a	12.90 ± 0.21^a	12.02 ± 0.20^a	11.41 ± 0.19^a	10.32 ± 0.19^a
P_2 (1981-1987)	665	11.10 ± 0.32^b	10.79 ± 0.31^b	9.95 ± 0.29^b	9.21 ± 0.28^b	8.76 ± 0.27^b	8.30 ± 0.26^b
P_3 (1988-1994)	666	9.51 ± 0.32^d	9.39 ± 0.31^e	9.31 ± 0.29^b	8.71 ± 0.28^b	8.15 ± 0.27^b	7.66 ± 0.26^{bc}
P_4 (1995-2001)	73	9.48 ± 0.30^e	9.67 ± 0.29^d	9.23 ± 0.28^b	8.78 ± 0.27^b	8.35 ± 0.25^b	7.52 ± 0.25^c
P_5 (2002-2008)	60	9.81 ± 0.34^c	9.74 ± 0.33^c	9.36 ± 0.31^b	9.06 ± 0.30^b	8.43 ± 0.28^b	7.89 ± 0.28^{bc}
P_6 (2009-2016)	31	10.66 ± 0.47^b	10.23 ± 0.45^b	9.88 ± 0.43^b	9.09 ± 0.41^b	8.45 ± 0.39^b	7.71 ± 0.38^{bc}
Season of calving							
S_1 (Rainy)	1109	10.40 ± 0.26	10.32 ± 0.24	9.83 ± 0.23	9.31 ± 0.23	8.90 ± 0.21	8.30 ± 0.21
S_2 (Winter)	1153	10.57 ± 0.21	10.78 ± 0.20	10.35 ± 0.19	9.76 ± 0.19	9.16 ± 0.18	8.43 ± 0.17
S_3 (Summer)	1160	10.66 ± 0.21	10.62 ± 0.20	10.13 ± 0.19	9.37 ± 0.19	8.71 ± 0.18	7.97 ± 0.17

Means under each class in the same column with different superscript differed significantly

Table 2 Effect wise least squares means of first lactation peak milk yield

Source of variation	N	Mean(kg) ± SE
Population mean (μ)	421	14.22 ± 0.14
Period of calving		
P_1 (1974-1980)	126	16.95 ± 0.24^a
P_2 (1981-1987)	65	13.93 ± 0.34^b
P_3 (1988-1994)	66	12.71 ± 0.34^d
P_4 (1995-2001)	73	13.29 ± 0.32^c
P_5 (2002-2008)	60	13.83 ± 0.36^b
P_6 (2009-2016)	31	14.62 ± 0.49^b
Season of calving		
S_1 (Rainy)	109	14.08 ± 0.27
S_2 (Winter)	153	14.36 ± 0.22
S_3 (Summer)	160	14.23 ± 0.22

Means under each class in the same column with different superscript differed significantly

Table 3 Effect wise least squares means of FL300DMY

Source of variation	N	Mean(kg) ± SE
Population mean (μ)	421	2438.98 ± 38.49[a]
Period of calving		
P_1 (1974-1980)	126	3310.33 ± 63.99[a]
P_2 (1981-1987)	65	2652.09 ± 88.39[b]
P_3 (1988-1994)	66	2087.19 ± 88.56[c]
P_4 (1995-2001)	73	2065.57 ± 84.03[c]
P_5 (2002-2008)	60	2265.28 ± 94.08[c]
P_6 (2009-2016)	31	2253.38 ± 129.04[c]
Season of calving		
S_1 (Rainy)	109	2444.82 ± 71.04
S_2 (Winter)	153	2447.63 ± 59.42
S_3 (Summer)	160	2424.48 ± 59.13

Means under each class in the same column with different superscript differed significantly

highest in cows calved during winter season (14.36 ±0.22kg) followed by summer and rainy season. The results showed declined FLPMY in cows calved during later period. The higher monthly test day milk yield in HF X Gir cows calved in initial period (P_1) might be due to fact that during this period maximum number of cows were F_1 halfbred having hetrotic effect. However, FLTDMY in later period declined as cows during later period were interbreeds of HFX Gir in those cows hetrotic effect might have been slightly declined.

The first lactation 300 days milk yield in HFX Gir halfbred was 2438.98±38.49kg. The difference due to period of calving in FL300DMY was significant (P<0.01). These results were in agreement with Bhadauria and Katpatal (2003) reported in Friesian X Sahiwal halfbred. Whereas, non-significant effect of period of calving on second lactation 300DMY was reported by Mote and Bhoite (2018) in FG halfbred. The mean FL300DMY of cows calved during P_1 (3310.33±63.99 kg) was significantly higher than cows calved in rest of the periods. The 300 days milk yield of cows calved during P_2 (2652.09 ±88.39kg) was significantly higher than those calved in P_3 to P_6 periods which were at par with each other. The influence of SOC on 300 days milk yield in HF X Gir halfbred was non-significant. Non-significant effect of season of calving on 300 days milk yield was in accordance with Mote and Bhoite (2019) in Friesian X Gir half bred cattle. However the milk yield was highest in cows calved during winter season (2447.63 ±59.42kg) and lowest in cows calved in summer season.

The heritability of monthly test day milk yield viz. T_1, T_2, T_3, T_4, T_5 and T_6 in HF X Gir halfbred was 0.12±0.12, 0.11±0.12, 0.09±0.11, 0.06±0.11, 0.09±0.12 and 0.09±0.13 respectively. These results indicated that the heritability of monthly test day milk yield was low. Lower heritability estimates of MTDMY indicated higher role of environmental fluctuation on the trait. Similar low heritability of MTDMY was reported by Kumar et al. (2018) in crossbred cattle. The heritability of FLPMY and FL300DMY was 0.25 ±0.14 and 0.12±1.00 respectively.

The genetic correlations among various monthly test days milk yield were positive and significant (P<0.01) in HF X Gir halfbred. Similar results were observed by Kumar et al. (2018) in crossbred cattle. The genotypic correlations between FL300DMY (0.48±0.45) and peak milk yield as well as between monthly test day milk yield and 300DMY were positive and significant (P<0.01).

The Phenotypic correlations among the entire test day milk yield ranged from 0.51±0.03 (T_1 with T_6) to 0.84±0.01 (T_5 with T_6). Phenotypic correlation of peak milk yield with all monthly test day milk yield were positive and significant (P<0.01). The results indicated that in HFX Gir halfbred the genotypic and phenotypic correlations among FLMTDMY, FLPMY and FL300DMY were positive and significant.

Among the HF X Gir halfbred sires the highest EBV (16.82) based on FLPMY was noted for sire number H-281 and ranked first however the lowest EBV (13.27) was observed for sire number H-242 and ranked last. On the basis of FL300DMY the sire number HG-272 had highest EBV (2810.17) and ranked first, whereas the lowest EBV (2319.45) was observed for sire number HG-45 and raked last.

Conclusions

In HF X Gir halfbred the effect of period of calving was significant while the season of calving was non-significant on MTDMY, FLPMY and FL300DMY. Heritability of all MTDMY and FL300DMY was observed to be low. However the medium h^2 of FLPMY indicated that judicious selection of sires to improve the productivity of HF X Gir halfbred. The sire number H-325 had highest estimated breeding value for MTDMY while the sire number H-281 and HG-272 had highest estimated breeding value for FLPMY and FL300DMY, respectively.

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SHORT COMMUNICATION

Effect of feeding total mixed ration blocks on productive performance of crossbred dairy cattle

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Abstract: A feeding trial of four weeks was conducted using forty crossbred dairy cattle reared under intensive system. The animals were divided into 2 groups, based on milk yield, parity and stage of lactation and fed either a commercially available total mixed ration (TMR) blocks or conventionally available compounded cattle feed (in form of pellet) and green grass separately at different times of the day. The results indicated that the total daily milk yield was significantly lower ($p<0.01$) in the group fed TMR blocks when compared to the group fed conventionally. Feeding TMR blocks or conventional feeding with compounded cattle feed did not have any significant effect on the milk composition *viz* fat, solids not fat (SNF), lactose and protein content. The results suggested that feeding TMR blocks showed negative impact on milk production when compared to conventional feeding system with compounded cattle feed.

Keywords: Crossbred dairy cattle, Conventional feeding, Milk yield, Milk composition, Total mixed ration

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The common practice usually followed in our country to feed dairy cows is to offer them with concentrates and roughages separately. To meet the high nutritional demands during early and mid lactation, large quantities of concentrates are fed at the expense of high-fiber forages leading to intermittent drops in ruminal pH. As per the current guidelines, the risk of sub-acute ruminal acidosis increases when ruminal pH drops below 5.6 for more than 3 h/d (Plaizier et al. 2008) or below 5.8 for more than 5 to 6 h/d (Zebeli et al. 2008). Hence, feeding the animals in the form of total mixed ration (TMR) aids in alleviating such problems and helps to maintain a constant ruminal pH and thus leading to a better ruminal environment. There are variable reports on impact of feeding TMR on milk production and its composition. Some researchers (Bargo et al. 2002; Kishore et al. 2013; Ferland et al. 2018; Sarker et al. 2019) observed enhanced productivity upon feeding of TMR whereas others indicated that milk production or its composition were not influenced (Hundal et al. 2004; Khan et al. 2010; Kumar et al. 2015; Saikia et al. 2015). Also, currently many commercial TMR blocks are available in the market with unspecified nutritional quality and studies on its influence on the productivity of the crossbred dairy cattle when compared to conventional feeding system are scanty. Hence, the present study was conducted to explore the effect of feeding a commercial total mixed ration (TMR) block *vs.* conventional feeding system on milk yield and its composition in crossbred dairy cattle.

Forty crossbred dairy cattle were divided into two groups of twenty each based on milk yield, parity and days in lactation and were assigned randomly to one of the two feeding systems. One of the groups (TMR group) was fed with TMR blocks based on hay (procured from Kerala Feeds Ltd., Kerala) along with 5 kg green grass whereas the control group (conventionally fed group) was fed with concentrate feed (compounded cattle feed in form of pellets manufactured at feed plant under Department of Animal Nutrition, Kerala Veterinary and Animal Sciences, University) and roughages separately. The TMR blocks (as per manufacturer's recommendations) and concentrates (as per Package of Practices Recommendations 2016, Kerala Veterinary and Animal Sciences University), as per the animals' requirement based on milk yield were offered to the respective groups before morning and afternoon milking and orts were recorded after 24 hour consumption. The control group was fed with 25 kg of

Table 1 Proximate composition (% on DM basis) of feed stuffs

	Total mixed Ration Block	Compounded cattle feed	Green grass
Crude protein	14.86	18.53	5.68
Crude fibre	15.93	5.62	35.44
Ether extract	2.18	2.2	2.00
Total ash	11.48	13.5	6.44
Nitrogen free extract	43.85	53.12	44.23
Calcium	1.00	1.20	0.50
Phosphorus	0.58	1.04	0.28

Table 2 Effect of feeding TMR on milk production and milk composition

Parameter	Control group	TMR group
DM Intake (kg/day)**	$11.21^{a} \pm 0.20$	$12.46^{b} \pm 0.29$
Milk yield (kg/day)		
First day of trial	10.45 ± 0.48	10.25 ± 0.52
Week I*	$10.40^{a} \pm 0.18$	$9.86^{b} \pm 0.19$
Week II**	$10.36^{a} \pm 0.17$	$9.43^{b} \pm 0.19$
Week III**	$10.09^{a} \pm 0.18$	$9.04^{b} \pm 0.16$
Week IV**	$9.84^{a} \pm 0.19$	$8.90^{b} \pm 0.17$
Milk composition (%) on 15th day of trial		
Fat	3.38 ± 0.19	3.40 ± 0.14
SNF	7.96 ± 0.08	7.87 ± 0.08
Protein	3.13 ± 0.03	3.10 ± 0.03
Lactose	4.21 ± 0.05	4.16 ± 0.04
Milk composition (%) on 22nd day of trial		
Fat	3.18 ± 0.10	3.23 ± 0.12
SNF	8.02 ± 0.09	7.86 ± 0.07
Protein	3.13 ± 0.03	3.09 ± 0.03
Lactose	4.22 ± 0.04	4.16 ± 0.04

*Means bearing different superscripts in the same row differ significantly ($p<0.05$)
**Means bearing different superscripts in the same row differ significantly ($p<0.01$)

.green grass (Napier hybrid and Guinea grass) separately in three offerings. The animals were provided clean and fresh water ad libitum. All the animals were maintained under uniform managemental conditions. The TMR block, compounded cattle feed and grass was analyzed at Animal Feed Analytical and Quality Assurance Laboratory, Namakkal (AOAC, 2012). Ca and P estimation was done as per AOAC 927.02 and 965.17, respectively.

Daily milk yield was recorded and composition of milk was determined at weekly intervals using Ekomilk Ultra pro milk analyzer (Milkana KAM 98-2A). The data was analysed using MS Excel 2007 and 'Independent sample T test' was used for comparing the means.

Proximate composition of the TMR block and compounded cattle feed used in the study are presented in Table 1. The nutrient composition of the TMR blocks used in the study complies with the nutrient specifications for TMR for lactating cattle as mentioned by Kishore et al. (2017). Daily dry matter intake (DMI) of the animals in both the group is provided in the Table 2. DMI was significantly higher in TMR group when compared to the control group. Similar results have been reported by Sarker et al. (2019) who could also observe a significant improvement in DMI in Red Chittagong cows when feed was offered as TMR blocks when compared to the conventional feeding system. In contrary to this, Hundal et al. (2004) and Kumar et al. (2015) concluded that feeding system does have any significant effect on daily dry matter intake.

The average milk yield on the first day of the trial and average daily milk yield on weekly basis are presented in Table 2. The milk yield was significantly lower in the TMR group when compared to the control group during all the four weeks of the study. The results were not in accordance with the observations of the researchers who either reported a significant improvement in milk production upon feeding TMR (Bargo et al. 2002; Kishore et al. 2013; Ferland et al. 2018; Sarker et al. 2019) or of those who couldn't observe any significant impact on milk production on feeding TMR (Hundal et al. 2004; Khan et al. 2010; Kumar et al. 2015; Saikia et al. 2015). In the present study, both TMR and

compounded cattle feed were procured from two different sources and hence the constituent ingredients might be different which would have resulted in lower milk production in TMR group when compared to control group, though both the groups were fed in congruence with their requirements. The reduction in milk production in the TMR group might also be due to the fact that the animals in the present study were accustomed only to green grass as the roughage source and hence the change in the roughage source may have reduced the milk production.

The effect of feeding TMR on milk composition has been shown in table 2. No significant difference could be observed in milk composition viz fat, solids not fat (SNF), protein and lactose content in both the groups either on 15th day or on 22nd day of the trial. The results were in confirmation with those of Saikia and Saikia (2015) who could not observe any effect of different feeding systems on milk constituents. Similarly, Khan et al. (2010) and Hundal et al. (2004) reported that milk constituents except lactose were not influenced by different feeding systems. However, Bargo et al. (2002) and Sarker et al. (2019) reported an increase in fat, protein and SNF per cent with TMR feeding.

Conclusion

Hence, from the present study it could be concluded that lower milk production was observed due to feeding of commercially purchased TMR blocks when compared to conventional feeding system.

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SHORT COMMUNICATION

Assessment of genetic diversity using mitochondrial DNA variation in Gir cattle of India

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Abstract: The present study is carried out with the aim to study the genetic diversity of 60 Gir cattle in the newly adopted herd of NDRI on the basis of mitochondrial D-loop hyper variable region. The animals were purchased from 4 major locations-IGFRI-Jhansi, Bhilwara, Ajmer and Kisangarh. After aligning the sequences a total of 53 haplotypes were identified. The Overall haplotype Diversity was observed to be 0.996±0.003 which shows that the population is diverse. Nucleotide diversity ranged from 0.693 ±0.35 to 0.707± 0.35 with overall diversity of 0.704±0.34 across all 60 sequences and the average no. of nucleotide difference varied from 279.45±125.42 to 285.16±128.36. F_{ST} value is significantly different from 0 for all pair wise combinations representing significant amount of Genetic differentiation. F_{ST} estimates between the population shows that animals from Ajmer and Bhilwara are more genetically differentiated (0.02987) while animals from Kisangarh and Bhilwara are genetically more closer (0.00440). All the above results show substantial variability between populations. These findings can be used for designing proper breeding and management strategies for Gir cattle in NDRI Herd.

Keywords: Genetic Diversity, Gir cattle, Genetic Improvement, Mitochondrial DNA

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The most important component of any breed improvement programme is genetic diversity which is the major cause of response to selection or genetic gain and plays a major role in improving livestock. Maintaining genetic diversity should be an important task in livestock breeding as it strengthens a population by increasing the likelihood that at least some individuals will be able to survive major disturbances and it enables the population less susceptible to inherited disorders and climate change. Animal genetic resources consist of species that are of agricultural and economic importance to man and food production systems depend heavily on utilization of locally adapted animal species. Genetic diversity and population structure need to be understood for guiding breed development programs en route for meeting the current production which allows sustained genetic improvement, to facilitate adaptation, and to device proper measures of utilization and conservation of livestock breeds (Notter, 1999; Dalvit et al. 2008). Thus, assessment of population structure and genetic diversity is an important basic tool for genetic improvement through modern breeding strategies (Toro et al. 2009; Sharma et al. 2013; Chung et al. 2017). Various technologies to study genetic diversity include PCR-RFLP, mini-satellites, micro-satellites, mitochondrial DNA analysis and SNP chips. The mtDNA has proved to be valuable in the study of genetic diversity as it shows maternal inheritance and changes rapidly than single copy nuclear DNA in mammals (Brown et al. 1982). The D-loop is the major control region for mitochondrial DNA (mtDNA) expression. The rate of nucleotide substitution in mtDNA is five to ten times higher than that of nuclear DNA (Brown et al. 1979). The mtDNA polymorphisms have been widely used to investigate the structure of populations, interspecies variability and identification of maternal lineages and postnatal growth (Bradley et al. 1998; Troy et al. 2001; Liu et al. 2004; Malau-Aduli et al. 2004; Yoon et al. 2005; Odahara et al. 2006; Lei et al. 2007). Considering the importance of cattle in Indian agriculture, few efforts have been made to evaluate the genetic diversity. Therefore, the present work was undertaken to quantify the genetic diversity of Gir breed of cattle in the National Dairy Research Institute, Karnal, Haryana. The objectives of this study was to use mitochondrial DNA hyper variable region polymorphisms to characterize the within-breed genetic diversity and to use the molecular information supplied to elucidate the

genetic diversity of this breed in order to establish adequate breeding strategy.

Blood samples were collected from the standing 60 female cattle which had completed their first lactation. Around 10 ml of venous blood was collected aseptically from the jugular vein of the animals in a 15ml vaccutainer tube under sterile condition using 0.5 ml of EDTA as an anticoagulant. Blood samples were stored in -20° C until DNA isolation.DNAs were extracted according to the manufacturer's standard protocol of Wizard genomic DNA purification Kit (Promega, USA). Partial mitochondrial D-loop hyper variable region was amplified using the polymerase chain reaction. The reported primer with forward 5'-CCCAGGCAAGAGGTAATGTA-3' and reverse primers 5'-TGTCCTGTGACCATTGACTG-3' (Bhuiyan et al. 2007) was used to amplify 588 bp from the hyper variable region of D-loop. PCR amplification was carried out in a 25 µl reaction volume containing 100ng of genomic DNA, 1× PCR master mix buffer (50 mM KCl, 10mM Tris-HCl, pH 8.3,1.5 mM MgCl2, 200 µM dNTPs, 1 U *Taq* polymerase) (Promega, USA) and 0.5pM of each primer. Amplification was performed in a C1000 Thermal cycler (Applied Biosystems,USA) thermal cycler using a 10 min denaturation step followed by 30 cycles of 30 sec at 94°C, 30 sec at 62°C, 1 min at 72°C and a final extension at 72°C for 10 min. The size of amplification product was checked by loading5 µL PCR product on to a 1.8 % agarose gel containing 0.5 µL/mL ethidium bromide. Sequencing was done directly by Sanger DNA Sequencing method (Apical Scientific Sdn Bhd, Malaysia). The sequences of the PCR product were analyzed using Chromas software. The sequences were edited and corrected by aligning forward and reverse sequences using BLASTN. Sites representing a gap in any of the aligned sequences were excluded from the analysis. Comparison of 60 D-loop sequences of a 588-bp hyper variable region-I (HVR-I) fragment of mtDNA control region obtained from 60 Gir cattle from 4 major locations was done. Mean number of nucleotide differences (k), nucleotide diversity (π) within cattle breeds, nucleotide haplotype diversity (Hd) ,Pair wise FST value and molecular Diversity index were calculated by Arlequin 3.1(Excoffier et al. 2006).

Alignment of 60 sequences of Gir cattle illustrated 53 different haplotypes in this investigation. Identical sequences were considered as the same haplotype.The populations from Bhilwara and Ajmer had highest no. of haplotypes with 18 and 17 respectively. The overall haplotype diversity was 0.98 throughout the population. Overall haplotype Diversity shows the population is diverse.The nucleotide diversity ranged from 0.693 to 0.707 (Table 1) with an overall nucleotide diversity of 0.704.The average no. of nucleotide differences (k) was 284.067 with a maximum of 285.169 in Kisangarh and minimum of 279.465 in population from Bhilwara. Tajima's D value was found to be 0.37889, which showed that all populations evolved naturally and possesses greater genetic diversity.

In total, on an average 1092.5 variable substitutions were determined in the mtDNAs from 53 haplotypes of Gir cattle. Among them, 690 substitutions were transitions and 402.5 weretransversions (transitions/transversions rate = 1.72) indicating a heavy bias towards transition substitution that has previously been reported for bovine mtDNA (Loftus et al. 1994; Mannen et al. 1998; Henkes et al. 2005; Bhuiyan et al. 2007 and Sharma et al. 2015).

Table 2 represents Global Fixation values between the populations. FST value are significantly different from 0 for all pair wise combinations representing significant amount of Genetic differentiation between population.The mean sequence divergence values ranged between 0.029 to 0.004 among different populations. FST estimates between the population shows that animals from Kisangarh and Bhilwara are more genetically differentiated while animals from Ajmer and Bhilwara are genetically closer values for pair wise FST was significant for populations of Ajmer and Bhilwara. Similarly, AMOVA revealed

Table 1 Genetic Diversity indices of Gir cattle of different locations

Locations	No. of Sequences	Nucleotide Diversity (pi)	Avg. Nucleotide Differences(k)	Haplotype Diversity(Hd)
Jhansi	13	0.704± 0.36	283.80±129.95	1.000±0.030
Ajmer	12	0.702 ± 0.36	283.24±130.49	0.984±0.040
Bhilwara	18	0.693 ±0.35	279.45±125.42	1.000±0.018
Kisangarh	17	0.707± 0.35	285.16±128.36	1.000±0.020
Overall	60	0.704±0.34	284.06±123.15	0.996±0.003

Table 2 Global Fixation values between the populations

	Jhansi	Ajmer	Bhilwara	Kisangarh
Jhansi				
Ajmer	0.012			
Bhilwara	0.020	0.029		
Kisangarh	0.006	0.006	0.004	

that percent of variation among the populations was 0.61 % while within the population it was 99.39 %.

Conclusions

This study involves detailed analysis of the genetic diversity and indicates existence of genetic diversity and population structure in Gir cattle. It could generate the baseline information which will assist in formulating effective breeding strategies in future for overall genetic improvement of Gir cattle in the NDRI herd. However a well defined breeding plan is a must to maintain the existing genetic diversity which will help the future bull mother farm to assist in dissemination of high merit germplasm to the farmer's herd.

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SHORT COMMUNICATION

Compositional and fatty acid analysis of Kankrej cows' milk

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Abstract: A study was conducted to assess the composition and fatty acid profile of milk obtained from Kankrej Cows. Milk samples from twenty Kankrej cows (average 60 days in milk; 9.5 kg/d of milk yield and 355 kg of body weight) were collected to determine milk composition and fatty acid profile. Mean milk fat, solids not fat, protein and lactose were 4.15, 8.55, 3.40 and 4.48%, respectively. In Kankrej cow milk saturated fatty acids (SFA) accounted for 73.17% and unsaturated fatty acids (UFA) accounted for 26.83% of total fatty acids. In UFA, concentration monounsaturated fatty acids (MUFA) was 22.94% and polyunsaturated fatty acids (PUFA) was 3.89%. The ratio UFA/SFA was 0.37 which ranged from value 0.28 to 0.47. Among the SFA, palmitic acid (C16:0; 31.22%) was present in the highest level followed by stearic acid (C18:0; 16.02%) and myristic acid (C14:0; 13.45%). Oleic acid (C18:1) was ranged between 13.43 to 25.31%. The average value for C4:0 and C6:0 were 2.07 and 1.84%, respectively. Linoleic acid (C18:2) and linolenic acid (C18:3) were ranged between 1.12 to 5.42% and 0.65 to 2.18%, respectively. Results indicated that milk composition and fatty acid profile of Kankrej cow are comparable to other Indigenous breeds.

Keywords: Fatty acid profile, Kankrej cow, Milk composition

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India is the largest producer of milk in the world with 187.7 million tonnes production in 2018-19 (BAH&FS, 2018-19). Dairying is an important activity in Indian economy contributing about 27 per cent of the agricultural gross domestic product which is around 4 per cent of the national GDP (Singh et al. 2019). Milk and milk products are the major source of nutrients in the human diet as they are rich source of fat, protein and carbohydrates. Milk fat is also carrier of the naturally present fat-soluble vitamins (A, D, E and K) as well as β-carotene. The main lipids in dairy fat are the triacylglycerides, accounting for more than 98% of total fat. Triacylglycerides composition is extremely complex as more than 400 different fatty acids are present in milk fat (Hanuš et al. 2018). Milk fat consists of about 70% of saturated fatty acids (SFA) and 30% unsaturated fatty acids (UFA). Among UFA, 25% is monounsaturated fatty acid (MUFA), 2.3% is polyunsaturated fatty acid (PUFA) and 2.7% is trans-fatty acid (Meena et al. 2019). The milk fatty acids are preferentially hydrolysed and transferred directly from the intestine to the bloodstream. Afterwards, they are rapidly metabolized in the liver and used as energy source for active cells. They also contribute to the regulation of cell metabolism and play an important role in intracellular signalling (Schönfeld and Wojtczak, 2016). The fatty acid composition of milk fat greatly influences the physico-chemical, functional and nutritive properties of milk. Composition of milk fat varies from species to species, feed consumed by lactating animal and lactation status.

Nowadays, not only the nutritional value of milk but also composition and fatty acid profile of milk have attracted interest of consumers. However, very limited research has been conducted with regards to milk fatty acid profile of Indigenous cows (Saroj et al. 2017). Out of the total 192.49 million cattle population, 142.11 million was contributed by Indigenous cattle with a decline of 6% in the total indigenous cattle population (20th Livestock Census, 2019). Among the Indigenous cattle, Kankrej is one of the important breeds of cattle in India which is mainly found in the region of north Gujarat and neighboring districts of Rajasthan. Though, Kankrej is dual purpose breed, but also good milk producers (Ekka et al. 2014; Gupta et al. 2019). The average milk yield of Kankrej cattle is around 1,738 kg with fat content around minimum 2.9 to maximum 4.2% (NBAGR, 2019). However, Kankrej cows maintained at Livestock Research Station, Sardarkrushinagar

Dantiwada Agricultural University, Gujarat had average standard (305 days) lactation yield of 2501 litres during the year 2018 (Anonymous, 2019). Knowledge of milk fatty acid composition offers an opportunity to know the concentrations of various fatty acids and bioactive lipids present in milk. To the best of our knowledge only one study (Bharwade et al. 2017) has reported the fatty acid profile of Kankrej cow milk. Therefore, this study was planned to evaluate milk fatty acid profile of Kankrej cows.

Milk samples from twenty lactating Kankrej cows (average 60 days in milk; 9.5 kg/d of milk yield, 355 kg of body weight) were collected during the month of March (2019) from the cows at Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India. It is located in semi arid region of north Gujarat having latitude of 24.35 North and longitude of 72.59 East and at an elevation of 189 meters above the mean sea level. All animals were maintained under uniform management conditions and feeding regime. Cows were fed with commercial concentrate, lucerne, maize fodder and jowar hay to meet the nutrient requirement. The feed was offered twice daily in equally divided doses, while clean drinking water was made available *ad lib*. The chemical composition of feeds and fodder used in this study is given in Table 1.

Analysis of milk fatty acid profile was conducted at ICAR-Central Institute for Research on Goats, Makhdum, India. Fat was separated from each collected sample and was methylated using sodium methylate according to O'Fallon et al. (2007). Fatty acid methyl esters were analyzed using a gas chromatograph (Thermo Scientific Ceres 800) with flame ionization detector and capillary column (60m×0.25mm×0.20mm). The initial oven temperature was 120°C, held for 5 min, subsequently increased to 240°C at a rate of 2°C min^{-1}, and then held for 60 min. Nitrogen at a flow rate of 1 ml/min was used as the carrier gas. Both the injector and the detector were set at 260°C. The split ratio was 30:1. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards and were expressed as percentage of total fatty acids.Milk samples were also analyzed for milk composition (fat, solids not fat, protein and lactose) using EKOMILK Ultra Pro Milk Analyzer (Everest Instruments Pvt. Ltd.). The mean, standard error (SE), minimum and maximum values for individual fatty acid, groups of fatty acids and milk components were estimated using R (R core team, 2019).

The average values for milk fat, solids not fat, protein and lactose were 4.15, 8.55, 3.40 and 4.48%, respectively (Table 2). In agreement with the current findings, earlier studies reported similar values for milk components in Kankrej cows (Joshi et al. 2018; Gami et al. 2019). Gajbhiye et al. (2019) reported 4.16, 8.86, 3.28 and 4.83% of fat, solids not fat, protein and lactose in milk of Gir cows. The mean concentration of PUFA linoleic acid (C18:2) and linolenic acid were 2.60 and 1.28%, respectively in Kankrej

Table 1 Chemical composition (% DM basis) of feeds and fodder

Composition	Concentrate	Lucerne	Green maize	Jowar hay
Dry matter	95.55	89.73	15.64	19.92
Crude protein	18.59	2.29	6.16	18.76
Crude fibre	6.58	37.58	27.43	22.64
Ether extract	2.43	1.36	1.16	2.93
Ash	8.25	10.51	8.56	9.38
NFC	35.53	28.22	40.32	27.80
NDF	35.2	57.62	43.80	41.13
ADF	19.05	45.17	33.76	21.15

NFC: Non-fiber carbohydrate, calculated by equation: NFC (% of DM) =100 – (CP + NDF + EE + ash); NDF: Neutral detergent fiber; ADF: Acid detergent fiber.

Table 2 Milk composition and concentrations of fatty acid groups (%) in milk of Kankrej cows (n=20)

Fatty acid group	Mean	SE	Minimum	Maximum
Milk composition (%)				
Fat	4.15	0.07	3.13	5.09
Solids not fat	8.55	0.04	7.72	8.90
Protein	3.40	0.02	3.06	3.59
Lactose	4.48	0.02	4.03	4.68
Fatty acid groups (%)				
Saturated fatty acids (SFA)	73.17	0.62	68.23	78.31
Unsaturated fatty acids (UFA)	26.83	0.62	21.69	31.76
Monounsaturated fatty acids (MUFA)	22.94	0.56	19.04	28.85
Polyunsaturated fatty acids (PUFA)	3.89	0.33	2.16	6.75
UFA/SFA	0.37	0.01	0.28	0.47

Table 3 Milk fatty acids profile (%) of Kankrej cows (n=20)

Fatty acid	Isomer	Mean	SE	Minimum	Maximum
Butyric acid	C4:0	2.07	0.10	1.26	2.86
Caproic acid	C6:0	1.84	0.18	0.89	3.60
Caprylic acid	C8:0	0.97	0.08	0.26	1.64
Capric acid	C10:0	2.01	0.13	1.07	3.00
Lauric acid	C12:0	2.00	0.17	0.60	3.40
Myristic acid	C14:0	13.45	0.37	10.92	16.65
Myristoleic acid	C14:1	0.68	0.05	0.22	1.09
Pentadecanoic acid	C15:0	0.56	0.05	0.20	0.94
Pentadecenoic acid	C15:1	0.29	0.10	0.08	1.75
Palmitic acid	C16:0	31.22	0.62	26.86	36.63
Palmitoleic acid	C16:1	2.35	0.22	1.01	5.17
Heptadecanoic acid	C17:0	0.64	0.05	0.22	1.32
Heptadecenoic acid	C17:1	0.43	0.09	0.14	1.53
Stearic acid	C18:0	16.02	0.47	11.67	18.31
Oleic acid	C18:1	19.18	0.68	13.43	25.31
Linoleic acid	C18:2	2.60	0.26	1.12	5.42
Linolenic acid	C18:3	1.28	0.10	0.65	2.18
Arachidic acid	C20:0	0.48	0.09	0.12	1.42
Behenic acid	C22:0	1.92	0.21	0.95	5.53

cow milk. SFA accounted for 73.17% and UFA accounted for 26.83% of total fatty acids. In UFA, concentration MUFA was 22.94% and PUFA was 3.89% (Table 2). The ratio UFA/ SFA were ranged from 0.28 to 0.47, with average value of 0.37. Saroj et al. (2017) reported that milk of Sahiwal and crossbred cows contain 61.35 and 67.43% of SFA, respectively. The SFA in milk fat of African Indigenous cows were ranged from 60.9 to 78.4% (Myburgh et al. 2012). The SFA contents of 78.4 *vs.* 74.5% were reported in milk of Jersey as compared to milk of Mafriwal (Yassir et al. 2009). Kirchnerová and Vršková (2015) reported SFA, UFA and PUFA were 69.34, 30.66 and 3.55%, respectively in the milk of Simental dairy cows. In cow milk PUFA account for as little as 3% of all fatty acids (Markiewicz-Kęszycka et al. 2013); however, Frelich (2009) has found more than 4% of PUFA in Czech Fleckvieh and Holstein cows. Saroj et al. (2017) reported 1.94 and 1.53% of PUFA in milk of Sahiwal and crossbred cows, respectively. The PUFA in milk fat of African Indigenous cows were ranged from 1.68 to 3.06%, respectively (Myburgh et al. 2012). Discrepancies in milk fatty acid profile of dairy cows among various studies might be due to type of breed, diet and season of the experiment.

Concentration of individual milk fatty acid in Kankrej cows is given in Table 3. Among the SFA, palmitic acid (C16:0; 31.22%) was present in the highest level followed by stearic acid (C18:0; 16.02%) and myristic acid (C14:0; 13.45%). The average value for short chain fatty acids (SCFA) C4:0 and C6:0 were 2.07 and 1.84%, respectively. Oleic acid (C18:1) a MUFA was ranged between 13.43 to 25.31% with mean value of 19.18%. Linoleic acid (C18:2) was recorded between 1.12 to 5.42% with mean value of 2.60% and linolenic acid (C18:3) was ranged from 0.65 to 2.18% having average of 1.28%. Very long chain fatty acids C20:0 and C22:0 were found to be 0.48 and 1.92%, respectively. In agreement with the present findings, Bharwade et al. (2017) in milk fat of Kankrej cows recorded 33.18 and 10.87% palmitic acid (C16:0) and myristic acid (C14:0), respectively. Similar values for palmitic acid (C16:0) and myristic acid (C14:0) were reported in milk of Sahiwal and crossbred cows (Saroj et al. 2017). Palmitic acid (16:0) was accounted for approximately 30% by weight of the total saturated fatty acids. While, oleic acid (18:1) accounting for 23.8% by weight of the total unsaturated fatty acids (Samková et al. 2018). Also, different studies suggests that monounsaturated fatty acids (MUFA) content of milk fat was is similar in sheep, cow, and goat milk and may range from about 20% to about 35%. Among the MUFA group, the oleic acid (C18:1) is characterized by the highest content (Krizova et al. 2017; Samková et al. 2018).

Conclusions

Results indicated that milk composition and fatty acid profile of Kankrej cow are comparable to other Indigenous breeds. Milk fatty acids concentration is depends upon the interrelationship among dietary lipid supply, rumen fermentation, and metabolic changes occurring in liver, blood, and finally in mammary gland. Further studies are required to know the non-genetic factors influencing milk fat composition in cows.

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