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Preservation approaches for milk and milk products: A Review

Tarun Verma, Ankur Aggarwal*, Abhishek Dutt Tripathi, Dinesh Chandra Rai and Sukriti Jaspal

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Abstract: This review article summarizes the effect of formalin and other recommended preservatives on the chemical composition of milk and milk based other traditional dairy products including khoya, paneer, ice cream and other dairy products as well as its impact on the shelf life extension. Formalin (0.4%) is the only legally permissible preservative according to the Food Safety and Standard Regulations, 2011. Formalin is an antibacterial compound commonly used in preservation of organ specimens. It degrades nucleic acids by alkylation of amino, carboxyl or hydroxyl groups. All microorganisms including spore forming ones are rendered inactive. Although formalin's preservative action is quite effective, there are conflicting and contradictory data in the literature regarding the influence of formalin in terms of estimating major milk ingredients i.e. milk fat, protein, lactose etc. which is primarily used in the commercial production of dairy products. There is a need to produce a viable formalin substitute. For the preservation of dairy products mercuric chloride, potassium dichromate, alcohol, chloroform, ether, hydrofluoric acid, bronopol and other preservatives can be efficiently used. In view of the below discussion, it is clear that the use of preservatives other than formalin for milk and milk product samples intended for analytical purposes needs to be reconsidered.

Keywords: Antimicrobial Properties, Formalin, Milk, Preservatives, Shelf Life Extension, Traditional Dairy Products,

Introduction

Dairy farming accounts for nearly a quarter of the agricultural gross domestic product (GDP) which accounts for about 4.35 % of the overall GDP in India whereas milk production in India increased at a compound annual growth rate (CAGR) of approximately 6.2 % in the year 2020-21, reaching 209.96 million tonnes (Economic Survey, 2022). Due to the high demand for milk and its scarcity, unscrupulous dealers have engaged in practices such as adulterating milk with chemicals or removing the natural fat and replacing it with cheaper oils and fats. The Food Safety and Standards Act of 2006 (FSSA, 2006) was enacted to combat such activities performed by local food business operators in the dairy sector and it established minimum criteria for all types of dairy products. Any food sample which fails the prescribed threshold criteria is considered to be contaminated / adulterated and its individual seller is accountable and has to face criminal charges. Penalties and punishments for violating food regulations differ depending on the severity of the hazard. In this case, a preservative with a longer shelf life is required since the samples are kept at ambient temperature which may allow for excessive microbial growth and changes in the sample composition rendering the sample unsuitable for analysis. To preserve the stored sample, the physical or chemical preservation method can be used. Physical method of preservation requires considerable economic investment and maintenance facilities and it can only be used to store food for a shorter duration. As a result, the only option is to use chemical preservatives to extend the shelf life of milk and milk products without compromising their qualities.

Minimum chemical preservation requirements for dairy products

When preservative added to foods where chemical preservatives can inhibit, delay or stop the process of fermenting, acidification or other degradation according to the Food Safety and Standard Act Rules (FSSR, 2011). The "testability of sample" is the minimum needed for acceptable chemical preservation for dairy products. This means that the milk and milk product sample should retain its original composition starting from sampling up to the analysis and the preservative used has no effect on the

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test procedure outcome. Other characteristic criteria are as follows: (Wilson, 1983) (Figure 1).

1. Broad-spectrum activity: The preservative harmful capacity to work against all types of microbes in milk is a vital need. The larger the preservative spectrum, the more useful it is.
2. Minimum inhibitory levels: In order to reduce sample dilution, costs and handling time of the preservative used should be effective at low concentrations in milk.
3. High water solubility: Because the average milk sample contains about 88 % water, the preservative must be able to kill microorganisms in the aqueous phase where high water solubility also ensures easy miscibility without significant agitation.
4. Stability: Under different storage method, the preservative must be stable.
5. Presence of colour: In contrast to cosmetic preservatives, milk preservative that adds colour to the given milk sample is a desired attribute for identification and safety purposes.
6. Compatibility: A good milk preservative should be effective for fresh milk sample from individual cows or other mammals and it should be acceptable for both rich fat and low fat milk samples.
7. Shelf-life activity: The milk preservative activity should last six months to a year in the milk sample.
8. Toxicity and disposal: The preservative must be non-allergenic and non-toxic to handlers or to those who come into contact with it. It should not pose an environmental hazard after disposal despite its necessary biocide property.
9. Cost-effectiveness: The preservative should be inexpensive and easily available.

Preservation of milk

In India, as per the Prevention of Food Adulteration Act (PFA, 1954) and The Food Safety and Standards Act (FSSA, 2006), Formalin (about 40% formaldehyde (CH_2O) in aqueous solution) is the only liquid preservative which is allowed to be used as preservative for the preservation of dairy products for analytical purposes. The amount of formalin preservative is used for analytical purposes in milk and milk product is 0.4 % and for ice cream sample it is 0.6 % (FSSR, 2011). Food Safety Officers collect the samples in varying quantity from the food business operators based on the dairy products as shown in Table 1. In next step, these samples are sent to a Food Analyst (FA) for analysis with the participation of a referral laboratory required when there is any conflict. The standard specifications for formaldehyde (formalin) are shown in Table 2.

In order to avoid polymerization, commercially available formaldehyde is mixed with 10-15 % methanol. Formaldehyde causes damage to nucleic acids in a variety of bacteria by alkylating amino carboxylic or hydroxyl groups. Formalin binds to the adenine, cytosine and guanine amino groups in DNA, denaturing them and inhibiting all forms of bacteria including spore forming. However, formalin's preservative activity in milk and milk products is currently considered undesirable (Subasi 2020). The cross-linking of formalin with protein impeded the repeatability of results from the same sample. Various physiochemical qualities as well as compositional factors (fat, lactose, total solid content, moisture, total solids, specific gravity, pH, viscosity, titratable acidity etc.) have been found to be influenced by the preservative during storage. This has resulted in a conflict between regulatory bodies and businesses. Formaldehyde is a widely used chemical in India was estimated to be over 245 thousand metric tonnes (Globe Newswire 2020). The US Environmental Protection Agency (EPA) in 1987 classified formaldehyde as a potential human carcinogen under the circumstances of a high or chronic exposure. Environmental Protection Agency is evaluating perchloroethylene (PERC) and listed it as a potential human carcinogen and a persistent environmental pollutant (Ceballos et al. 2021).

Formalin is said to interfere with the estimation of numerous elements in milk particularly milk fat through extensive scientific studies. Upon the addition of formalin, formalin cross-linking with protein occurs which causes protein hardening. In the Gerber and Rose-Gottlieb methods, the cross-linked matrix of formalin is not adequately dissolved, resulting in imprisoned milk fat and reduced fat levels. According to the study, after a year of storage at ambient temperature with 0.4 percent formalin, the fat content of milk samples decreased from 6.00 percent to 5.45 percent (Singh et al. 2016). Many researchers have also observed that formalin has an effect on milk protein, lactose, freezing point, acidity and viscosity estimation. Besides milk, the influence of formalin addition and storage on the chemical makeup of ice-cream, *dahi* and *paneer* has been investigated. Some recommendations have been made based on the above investigations either in terms of changing the technique or offering an alternate approach for estimations.

According to Hoffman et al. (2015), adding formalin to a protein causes it to harden due to formalin cross-linking. The anionic form of the amino acid interacts with formaldehyde. Because the amino group of the anionic version of the amino acid has two unpaired electrons and formaldehyde is a dipolar molecule ($+\text{CH}_2\text{O}-$) the two components react as shown in the equation below:

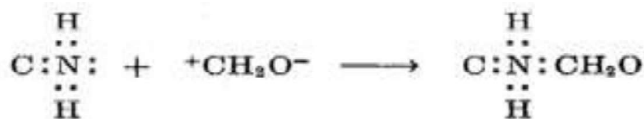
Table 1: Quantity of food sample required to be sent to food laboratory for analysis purpose

Article of food	Quantity of sample
Milk	500ml
Malai/Dahi	200g
Sterilized Milk/UHT Milk	250ml
Ice Cream/ Softy/Kulfi/Ice Candy Ice Lolly	300g
Evaporated Milk/ Condensed Milk	200g
Chhana/ Paneer/ KhoyaShrgand LAF	250g
Infant Food/ Weaning Food	500g
Malt Food/ Malted Milk Food	300g
Butter/ButterOil/Ghee/Margarine	200g
/Cream/BakeryShortening	
Vanaspati, Edible Oils/Fats	250g
Yoghurt/ Sweetened Dahi	300g

(Source: FSSAI,2006)

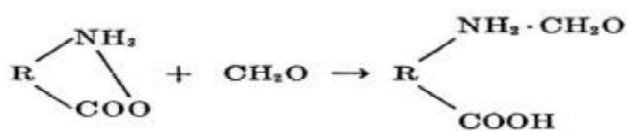
Table 2: Standard specification for formaldehyde

Parameters	Specification
Molecular formula	CH ₂ O
	$\begin{array}{c} \text{O} \\ \parallel \\ \text{H}-\text{C}-\text{H} \end{array}$
Molecular weight	30.026 gram/mol
Density	0.8153 gram/cm ³
Melting point	-92°C
Boling point	-19°C
Solubility in water	400 gram/dm ³



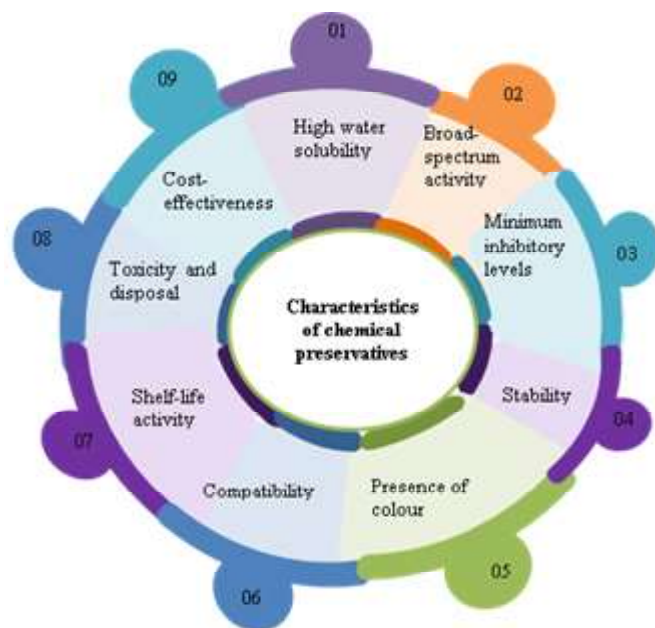
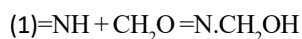
Amino group Formaldehyde

The reaction between protein and formaldehyde is described by Theis (1944) with the aldehyde being held to the amino group by secondary valence.

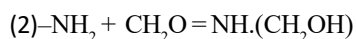


Amino group of protein Formaldehyde Amine Pyrrolidine(N)-methylene acid group

The number of formaldehyde groups that can be added according to Balson and Lawson (1936) correlate to the number of H⁺ atoms linked to the nitrogen atom and have therefore proposed Reactions 1, 2 and 3.

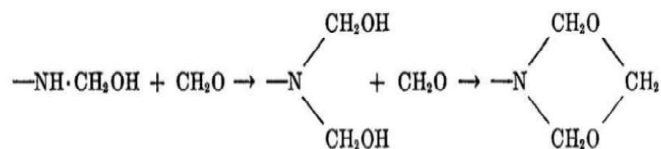
**Fig. 1** Characteristics of chemical preservatives

Imino group Formaldehyde Pyrrolidine(N)-methylene group



Amino group Formaldehyde Amine Pyrrolidine(N)-methylene group

(3)

Amine Pyrrolidine(N)-methylene group Formaldehyde
Ring structure of the type
Trioxymethylene

Other chemical preservatives such as mercuric chloride (HgCl₂), potassium dichromate (K₂Cr₂O₇), hydrogen peroxide (H₂O₂), bronopol (C₃H₆BrNO₄), azidiol and others have been extensively studied. Various studies have found that the official preservatives are mainly used for analytical purposes whereas illegal preservatives are mainly used to remove the defects in the product (Table 3).

However, due to constraints associated with their use, the Food Safety and Standards Authority of India (FSSAI) does not approve them. Some of the chemical preservatives that are typically utilised in the milk system are listed in Table 4.

Preservation of dairy products

Dairy products vary by geographical location in India and play an essential role in the social, economic and nutritional well-being. Approximately half of the total milk consumed in India is used to produce dairy products such as *dahi*, *paneer*, ice cream, *chhena*, *khoa* etc (Kumar S et al. 2014). Traditional Indian dairy products occupy a vital and distinct role in the Indian

market. About half of the milk produced is used to make traditional dairy milk products while the remaining is consumed as liquid milk (USDA 2020). Hence their output has the potential to become a significant profitable market for the organised dairy sector. However, due to a high water activity and easy availability of the nutrients, these items are perishable. Their preservation is also of tremendous relevance in the current context of preservation for analytical purposes. There are a few publications on the use

Table 3: Official and illegal preservatives used in milk system

Common milk preservative	
Official preservative	Illegal preservative
Alcohol (C_2H_5OH)	Sodium Bicarbonate ($NaHCO_3$)
Chloroform ($CHCl_3$)	Sodium Carbonate (Na_2CO_3)
Ether (C_2H_5O)	Hydrogen peroxide (H_2O_2)
Formalin (CH_2O)	Boric Acid (H_3BO_3)
Hydrofluoric Acid (HF)	Benzoic Acid ($C_6H_5O_2$)
Mercuric Chloride ($HgCl_2$)	Salicylic Acid ($C_7H_6O_3$)
Potassium Dichromate ($K_2Cr_2O_7$)	Potassium nitrate (KNO_3)
	Ammonium salt (NH_4^+)

Table 4: Common preservatives used in milk system and its effect on milk composition

Preservatives	Concentration	Findings	References
Methyl Paraben, Propyl Paraben	0.1%	There was no effect on fat, TS or protein while lactose decreased after 21 days.	(Ranvir et al.2015)
Sodium Omatne	0.4%	There was no influence on raw milk stored at 25°C for up to 1 week for fat and protein testing.	(Wilson, 1983)
Sodium Azide, Azidiol,	0.02%-0.04%	At 4°C and 20°C, there is no influence on fat and protein levels.	(Seskana and Jankevica et al. 2007)
Bronopol, Potassium Sorbate	0.4%-0.5%	At 4°C and 20°C, there is no influence on fat and protein levels.	(Seskana and Jankevica et al. 2007)
Hydrogen Peroxide	0.04%	Fat content decreased without affecting protein content at 20°C	(Sanchez et al.2005)
Bronopol	0.04%	Higher fat (4.43%) and protein (3.7%) content observed at 4°C after 42 days	(Sanchez et al.2005)
Formalin	0.2-0.4%	Legally permitted preservative in milk for analysis purpose	(FSSAI, 2006)
Dowicil	0.07%	Suitable for fat determination up to 3 weeks	(Durham et al.1978)
Mercuric chloride ($HgCl_2$)	0.05-0.4%	Satisfactory preservative for a period of month Highly poisonous and having environmental hazard upon disposal	(Grappin et al. 1970)
Potassium dichromate ($K_2Cr_2O_7$)	0.1-0.2%	Gives specific colour to the sample Suitable for analysis by Milkoscan Problematic due to production of allergen —Chromium VI?	
Bronopol and kathon	0.4%-0.5%	There was no influence on fat, moisture for 90 days storage at 37°C	(Chilbule et al. 2018)
Potassium Dichromate	0.08-0.8%	Effective for fat measurement by milko tester for 5 days at 21°C	(Kroger, 1985)

of different preservatives to preserve dairy products. The few studies that have been conducted are outlined in this review as follows:

Chhena and Khoa: Mukherjee and Mathew (1974) treated Chhena and khoa samples with different preservatives and immediately sealed them in dry glass containers kept at room temperature (25-37 °C) at Kolkata Food Laboratory. They analysed the samples for mould growth & colour difference and observed an improvement in the keeping quality and concluded that Formalin (0.1ml in every 25 gram) was the best preservative (Table 5).

Narang et al. (1969) studied the physicochemical parameters including fat content of preserved khoa that had been stored. They treated 10 ml of formalin in 2 kg of khoa and studied the sample both under refrigeration and at room temperature. They discovered that several physicochemical parameters like fat remained unchanged in both the cases for about a week after

which changes followed a similar pattern as in the case of the sample held without formalin. Khoa sample became stale and mold growth was observed indicating that the extra concentration of formalin did not properly preserve the khoa sample.

Dinakar and Sharma (1989) demonstrated that 0.2 ml of formalin for every 25 g sample was effective in preserving khoa sample meant for analytical purposes as compared to the recommended amount of 0.1 ml per 25g as per the FSSAI 2006. There was no discernible effect on moisture, fat or total protein content at this level whereas vitamin A and vitamin C significantly decreased. Upon studying the Khoa sample preserved with optimized formulation of bronopol and kathon kept for chemical analysis for 90 days at 37°C the results indicated that fat, moisture content difference was not significant whereas lactose significantly decreased during 90 days of storage (Chilbule et al. 2019).

Table 5: Preserving the quality of chhena and khoa with various preservatives

Preservative	Maintaining quality (Period)	
	<i>Chhena</i>	<i>Khoa</i>
Formalin CH ₂ O	>10 months	>10 months
Mercuric chloride K ₂ Cr ₂ O ₇ (0.5% alcoholic)	10 days	5 months
Hydrogen peroxide H ₂ O ₂ (3% aqueous)	2 days	3 days

Table 6: The effect of varying formalin concentrations in Dahi (25-30°C)

Parameters (%)	Storage time frame (Months)				Observations
	0	1	2	3	
Total solids	16.6	16.6	16.6	16.2	No effect for the first two months and after two month there was in fat reduction.
Fat	6.9	6.9	6.9	6.6	
Lactose	4.3	4.2	4.1	3.9	Reduced during storage
Titrateable acidity (lactic acid)	0.16	0.22	0.23	0.24	Increased while being stored

Table 7: The effect of varying formalin concentrations in ice cream (-18°C)

Preservative %	Storage (Months)	Observations
Formalin (0.1)	1	There is no moldness or change in composition.
Formalin (0.2)	1	There is no moldness or change in composition
Formalin (0.3)	2	There is no moldness or change in composition
Formalin (0.4)	6	No moldness but change in composition

Table 8: The effect of formalin (0.4%) on Paneer compositional parameters (25-30°C)

Sample	Period	Observations
Control	2-3 day	Mold growth
	Upto 6 day	No mold growth and compositional change
Sample With formalin (0.4%/30°C)	From 6 to 21 day	There is no mold development, but there is a change in composition.
	After 21 day	The sample turned mold and was no longer suitable for analysis.

Dahi: Haridas and Narayanan (1976) investigated the influence of formalin (0.4 %) on the preservation of dahi from buffalo milk and discovered that the addition of formalin at the rate of 0.4 % can preserve the sample of dahi (25-30°C) for two months. Further storage significantly reduced total solids and fat content. Based on the findings, it was suggested that dahi sample maintained with 0.4 % formalin may be analysed within two months to ensure that their compositional standards were met (Table 6). Chilbule et al. (2019) investigated the dahi sample preserved with optimized formulation of bronopol and kathon kept for chemical analysis for 90 Days at 37°C. No significant difference in TS was observed in the study whereas, lactose decreased during 90 days of storage.

Ice Cream: Sharma and Zariwala (1979) studied the effect of different concentrations of formalin (0.1 %, 0.2%, 0.3%, 0.4%) on ice cream sample held at refrigerated temperature (-18°C). They found no significant variation in the total solids (TS) and fat content of ice cream sample regardless of the storage temperature. Ramaiah and Narayanan (1976) studied the impact of different formalin concentrations in frozen ice cream (-18°C) and discovered that adding formalin at a rate of 0.4 % can keep plain ice cream sample (-18°C) for 2 months (Table 7). Further storage significantly reduced TS, fat and sugar levels. As a result, it was suggested that ice cream sample preserved with 0.4 % formalin be tested within two months to confirm that compositional guidelines were met.

Paneer: Singh and Shrivastva (2015) stated that the paneer sample kept with 0.4 % formalin should be analysed within 2 months since the paneer samples were found to be inappropriate for analysis after this time period due to significant changes in compositional and physicochemical parameters. Goyal and Goyal (2016) found that with the use of formalin (0.4 % v/w), paneer could be preserved in sterile and sealed glass bottles for 6 days. The sample showed mold growth and decrease in the fat and total nitrogen content after 21 days storage. However, a rise in the water soluble nitrogen, tyrosine and acidity was observed (Table 8).

Belewu et al. (2005) investigated the shelf life of cheese sample at room temperature using ginger extracts, propionic acid and sodium benzoate. Sodium benzoate and propionic acid were added at the rate of 0.8 %. The shelf life of the sample was extended by sodium benzoate until the ninth day whereas the control sample spoiled on the second day. Titratable acidity in the cheese sample treated with ginger extracts increased from 0.20 % to 0.27 %. The antioxidant qualities of ginger extract may explain why the sample treated with it had a longer shelf life. Similarly Joseph and Akinyosoye (1997) found in a previous investigation that the application of propionic acid and sodium benzoate (0.8%) resulted in the preservation of cheese for 8 days.

Chilbule et al. (2019) investigated paneer samples preserved with optimized formulation of bronopol and kathon kept for chemical

analysis for 90 days at 37°C and found that there wasn't a significant difference in the moisture and fat whereas lactose significantly decreased during 90 days of storage.

Efficiency of combination of preservatives in milk and milk products

There has been little research on the combination of various preservatives and their usage in milk preservation. Upadhyay et al. (2014) discovered that a 1:1 mixture of $K_2Cr_2O_7$ (0.1 %) and $HgCl_2$ (0.1 %) was adequate for measuring density and % fat in analytical milk sample for up to 4 months. Hussain et al. (1984) found that combining formaldehyde and hydrogen peroxide (1:10) did not result in significant variations in SNF whereas combining $HgCl_2$ and $K_2Cr_2O_7$ (1:2) and (1:3) resulted in greater SNF values when analysed after 7 days at ambient temperature. Jandal and Rai (1989) noticed no significant change in milk fat content when 0.4 % and 0.6 % concentrations of $K_2Cr_2O_7$ and $HgCl_2$ (1:1) were employed for 40 days. Jandal and Rai (1989) also investigated the preservation effectiveness of a 1:1 mixture of $HgCl_2$ and $K_2Cr_2O_7$ (0.4 and 0.6 %) in milk held at $30 \pm 10^\circ C$ for three months and found that formation of orange-yellow colour first followed by yellow green hue after 30 days of storage with the intensity of colour increasing with increasing preservative content. The COB test was positive after 90 days of storage and a significant reduction in fat content was observed after 75 days.

Mitul et al. (2021) investigated bronopol and kathon after testing the antibacterial activity of ten different preservatives in milk including sodium azide, Kathon, Sodium omadine, Dowicil, Bronopol, Triclosan, Hydrogen Peroxide, Paraben, Mercuric chloride and Potassium dichromate. It was found that kathon (0.4%) and bronopol (0.10%) were effective against all types of bacteria in milk (Total Plate Count, Lactic Acid Bacteria, coliform and Yeast & Mould Count). There was no significant difference in milk fat, SNF, protein or lactose content when kathon (0.40 %) was added whereas lactose content increased significantly. When bronopol (0.1 %) was added there was no significant impact on the milk fat, SNF, protein or lactose content. Three distinct combinations (A, B and C) were formulated by varying the ratios of kathon and bronopol where the researchers investigated the total plate counts, lactic acid bacteria counts, coliform and yeast & mold counts and concluded that they were all nil in all combinations (A, B and C) at 0.6 % concentration. For 45 days, there was no significant influence of optimised formulation on fat and lactose content estimation, and for 90 days, there was no significant effect on total solid (TS), ash and protein content estimation. The preservative combinations performed well in the milk system without changing its compositional character.

Chilbule et al. (2019) investigated khoya, paneer and dahi added with optimized preservative i.e. bronopol and kathon where researchers found microbiological counts (Total Plate Count, Lactic Acid Bacteria, coliform and Yeast & Mould Count) were

nil in first dilution whereas fat, lactose content, total solid, ash & protein content were no significant effect of optimised formulation of kathon and bronopol. Researcher concluded that these preservative is effective in preservation of paneer and khoa for chemical analysis for 90 day at 30 °C.

Conclusions

When milk and milk products were kept under refrigerated conditions, only 0.4 % formalin with a formaldehyde concentration of 37-41 % is allowed to be used in milk and milk product samples preserved for chemical analysis (FSSR 2011). Formalin is an antibacterial agent that kills all microorganisms including spores. Although formalin preservative action is quite effective but there are inconsistent and contradictory data shown above in this chapter which clearly demarcate the influence of formalin on the estimation of major milk constituents particularly milk fat, protein, lactose etc. which primarily serves as a base for the commerce of milk and milk products. In addition to milk, the effect of addition of formalin on the chemical composition of khoa, paneer, ice cream and other dairy products and its influence on the shelf life has also been studied in this review article. In light of the above discussion, it can be concluded that there is a necessity to re-examine the uses of preservatives other than formalin for the milk and milk product samples intended for analytical purposes.

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RESEARCH ARTICLE

Effect of fortification of glycomacropeptides (GMP) on rheological and sensory attributes of probiotic-yoghurt

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Abstract: The objective of this study was to analyse the rheological and sensory attributes of glycomacropeptides (GMP) fortified probiotic-yoghurt prepared using *Bifidobacterium bifidum* (BB12) and *Lactobacillus acidophilus* (LA) cultures (1:1 ratio @ 4 %) in short set yoghurt. The textural properties of probiotic yoghurt fortified with GMP (0.25, 0.50 and 0.75 %) showed decreased firmness (N) and consistency (g.sec) whereas cohesiveness (N) and index of viscosity (g.sec) increased with increasing levels of GMP addition. The apparent viscosity analysed showed higher viscosity at 0.25 and 0.50 % GMP levels as compared to 0.75 % GMP level and control. The sensory attributes of probiotic-yoghurt on 9 point Hedonic scale as evaluated by a panel of 7 judges, showed maximum scores for all the parameters (body and texture, flavour, sourness and overall acceptability) at 0.25 and 0.50 % levels of GMP. The current study demonstrated that statistically significant positive changes were observed in probiotic-yoghurt by fortification of GMP even at lower levels, with respect to rheological and sensory attributes.

Keywords: Apparent viscosity, Glycomacropeptides (GMP), Probiotic-yoghurt, Rheology, Textural properties

Introduction

Bioactive peptides obtained from different protein sources are physiologically active and have a positive impact on body's functions or conditions, which ultimately influence the health. Similarly, milk derived bioactive peptides may also exhibit a number of physiological effects *in-vivo* on the gastrointestinal, cardiovascular, endocrine, immune, central nervous and other body systems (Lahov and Regelson 1996; Dziuba et al. 1999; Malkoski et al. 2001). Glycomacropeptides (GMP) are one such bioactive peptides derived from casein hydrolysis during the preparation rennet cheese. They are 64 amino acid residues with sugar moiety obtained by hydrolysis of C-terminal part (f 106–169) of κ -casein, released into whey during cheese making by the action of chymosin. GMP constitutes about 20–25 % of the total proteins in whey products like whey powder, whey protein isolates (WPI), whey protein concentrates (WPC), manufactured from cheese whey (Farias et al. 2010; Robitaille et al. 2012).

Incorporation of probiotic bacteria in fermented dairy products enhances their therapeutic value in functional foods. However, insufficient viability and survival of these bacteria remain a problem in commercial food products. Many research work suggests that use of appropriate prebiotics and the optimal combination of probiotics and prebiotics (synbiotics) may be useful in achieving the increased delivery of viable bacteria in fermented products to the consumers (Kailaspathi and chin 2000; Donker 2007). In several studies, GMP also proved as a prebiotic, which was considered to increase the growth and viability of probiotic organisms (Idota et al. 1994; Janer et al. 2004; Robitaille 2013; Pushpa et al. 2020).

The functional properties like emulsification, foaming and gel formation ability have been shown a considerable interest in extending the use of GMP in dairy products, which may in turn affect the structural and rheological properties (Martin-Diana et al. 2004; Thoma-Worringer et al. 2007; Kreub et al. 2009a and 2009b). An interesting component of GMP is sialic acid, has an influence on the biological and functional properties of the foods, into which they are incorporated (Kawasaki et al. 1992, 1993; Nakajima et al. 2005; Fernando and Woonton 2010). However, few more researchers have been linked to peptide part of GMP

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(LaClair et al. 2009; Van calcar and Ney 2012; Robitaille et al. 2012) and still others demonstrated to both peptide as well as glycan part of GMP (Otani et al. 1992; Beucher et al. 1994; Burton-Freenvan 2008; Requena et al. 2010).

Yogurt gels are a type of semi - solid, soft body and are relatively dynamic network systems that are prone to structural rearrangements. Several studies also demonstrated that the microstructure has a major impact on the texture and other physical properties of acid milk gels (Kalab et al. 1983; Lucey et al. 1998b). An understanding of the mechanisms involved in the formation of texture in yogurts and incorporation of food structuring agents such as proteins may influence the texture development and hence influence the food rheology (Lucey 2004; Lee and Lucey 2010).

The composition of GMP (dry basis) isolated by filtration methods and chromatographic techniques in its pure form contain maximum of 0.5 % fat, 1.5 % lactose and 8 % ash with minimum 90 % protein (Laclair 2009). GMP incorporation to yoghurt like gels could alter the structure greatly affecting the various properties including texture/micro-texture, functionality, appearance, sensory properties etc. In this context, the present investigation was aimed at preparation of probiotic-yoghurt as a carrier of GMP for added functional benefits to the consumers with the objectives of studying rheological and sensory attributes.

Materials and Methods

Whole milk

Cow milk was procured from dairy farm maintained by Karnataka Veterinary Animal and Fisheries Sciences University, Bangalore.

Yoghurt Starter cultures

Mixed yoghurt culture of freeze dried - direct vat set (FD-DVS) type containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* (2500 U) were obtained from Chr. Hansen Laboratories, Copenhagen, Denmark.

Probiotic cultures

Probiotic cultures such as *Bifidobacterium bifidum* (Nutrish® BB-12) and *Lactobacillus acidophilus* (Nutrish®) FD-DVS form were obtained from Chr. Hansen Laboratories, Copenhagen, Denmark.

Glycomacropeptide (GMP)

Commercially spray dried GMP (90 % purity) isolated from cheese whey was obtained from AGROPUR Ingredients, Minnesota.

Preparation of GMP fortified short set probiotic-yoghurt

The protocol for preparation of probiotic-yoghurt with the incorporation of GMP powder was adopted as followed by Pushpa et al. (2019). GMP was incorporated at different levels (0.25, 0.50 and 0.75 %) to homogenized whole cow milk (3.5 % fat and 8.5 % SNF) and was heated to 90°C/5 min, cooled to incubation temperature (42°C). Then probiotic-yoghurt cultures viz. *Bifidobacterium bifidum* (BB-12) and *Lactobacillus acidophilus* (1:1; 4 %) were added to milk along with the yoghurt culture (2 % *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. Bulgaricus*; 1:1). The milk mixture was filled into sterilised polypropylene 100 ml cup with air tight lids, incubated at 42°C for 3-4 h (short set) till it sets to curd and then cooled to less than 20°C and stored at refrigerated temperature (5±1°C).

Textural Properties

Textural properties such as firmness, consistency, cohesiveness and index of viscosity parameters were analysed by using the Micro System TA-XT2-Texture analyser (UK) fitted with suitable aluminium probe to analyse force and time taken to penetrate the product. The pattern of these parameters as shown in the Fig. 1, firmness is the maximum peak force (g) required during the penetration and the unit is Newton (N); consistency is the area covered under positive curve and is given by force x time (g. sec); cohesiveness is the maximum negative peak force (N); index of viscosity is the area covered under negative curve and is given by force x time (g. sec). A stainless-steel probe 25 mm diameter with flat end was chosen to study the textural attributes of yoghurt and was inserted to a depth of 1 cm by adjusting following settings: - Test mode to auto; target model to a distance of 5 mm; pre-set speed and post - set test speed of 5 mm / sec. The graph obtained were analysed for firmness, consistency, cohesiveness and index of viscosity using the Expert exceed software supplied along with the instrument.

Apparent viscosity analysis

The apparent viscosities of probiotic-yoghurt samples were determined using a rotational viscometer (Model RVDV-II Pro, Brookfield Engineering Lab., Stoughton, MA) at various spindle speeds, in steps of 10 rpm, in a continuous run mode. The viscosity measurements were carried out at 30°C using RV spindle number 2 was used. The measurement range of torque in the viscometer was maintained between 10 and 100 % by careful selection of the spindle in accordance with the nature of the samples. The start speed for viscosity measurement was 10 rpm and the end speed was fixed at 100 rpm. The viscometer was operated in an external mode using a computer and Rheocalc V. 3.1.1 software. At each rpm, shearing of the sample was done for 30 s before the speed was ramped up to the next level. The torque and apparent viscosity data were collected at 10 s interval for each rpm (3 readings per spindle speed) using the software

supplied by Brookfield Engineering Laboratory. The viscometric data (viscosity vs. torque at various rpm) were converted into shear stress and shear rate and the values of flow behaviour index “n” were calculated.

Sensory evaluation

The GMP fortified probiotic-yoghurt samples were subjected to sensory evaluation by seven experienced and trained panellists to judge sensory attributes viz. colour and appearance, body and texture, flavour, sourness and overall acceptability. The sensory scores were awarded using 9-point Hedonic scale (McEwan and Lyon, 2003) with a maximum score of 9 (‘like extremely’) and least score of 1 (‘dislike extremely’).

Statistical analysis

The results are the average of 3 replications and were statistically analysed by subjecting to R Programme, R - Version 3.4.3.

Results and Discussion

The present investigation focuses on incorporation of GMP at different concentrations into probiotic-yoghurt to study the effect on different functionalities of the product such as rheology and sensory attributes.

Rheological properties of probiotic-yoghurt fortified with GMP

Textural attributes

The results obtained from the penetration test using texture analyser for parameters viz. firmness, consistency, cohesiveness and index of viscosity in GMP fortified probiotic-yoghurt are depicted in Table 1 and Fig. 2.

Firmness and Consistency

It is evident from the values of the Table 1 (Fig.2) that the effect of GMP on firmness (N) at 0.25, 0.50 and 0.75 % levels showed a significant decreasing effect with firmness values of 0.475, 0.472 and 0.451, respectively as against control (0.552). Similarly, the effect of GMP fortification at 0.25, 0.50 and 0.75 % levels on

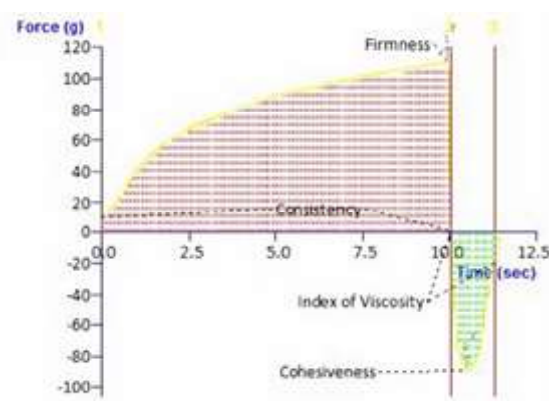


Fig. 1 A typical graph pattern of Texture profile analyser

consistency (g.sec) of the probiotic-yoghurt showed significantly lower values of 373.001, 366.217 and 363.347, respectively as compared to control (416.197). Gel microstructure is related to firmness of gel which depends on the interlinking of caseinate micelles in the structure. GMP being negatively charged even in acidic pH (<4.5) due to the presence of glycan chains which may be influencing the micro-texture of gels (Farias et al. 2010) and is responsible for decreased firmness in the product. On contrary, several workers concluded their results that yoghurt milk bases with increased ratios of denatured whey protein to casein at constant total protein contents seems to yield firmer yoghurts with stronger protein networks (Krzeminski et al. 2011; Jørgensen et al. 2015; Laiho et al. 2017). Since casein micelles are the driving forces in gel formation, the rearrangement of casein particles in presence of GMP and its interference may result in the weaker gel formation.

Cohesiveness and Index of viscosity

In the present study, as could be observed from the table values (Table 1 and Fig. 2) that 0.25, 0.50 and 0.75 % GMP levels exhibited lower values of -0.187, -0.196 and -0.232 of cohesiveness, respectively as compared to control (-0.241). However, increasing GMP concentration increased the cohesiveness. As index of viscosity depicts the area covered under negative peak (cohesiveness), similar results were noticed with the addition of GMP. The values obtained for index of viscosity being -10.126, -

Table 1: Effect of GMP fortification on textural attributes of probiotic-yoghurt

Levels of GMP (%)	Firmness Force (N)	Consistency Area F.T(g.sec)	Cohesiveness Force (N)	Index of viscosity Area F.T(g.sec)
Control*	0.552±0.00 ^a	416.197±0.90 ^a	-0.241±0.00 ^d	-13.501±0.39 ^c
0.25 %	0.475±0.00 ^b	373.001±0.80 ^b	-0.187±0.00 ^a	-10.126±0.09 ^a
0.50%	0.472±0.00 ^c	366.217±0.92 ^c	-0.196±0.00 ^b	-11.269±0.56 ^b
0.75 %	0.451±0.00 ^d	363.347±1.07 ^d	-0.232±0.00 ^c	-13.030±0.06 ^c
CD ($P \leq 0.05$)	0.008	1.44	0.005	0.53

Average of 3 trails

Probiotic-yoghurt with *B.bifidum* and *L.acidophilus* (4 % ;1:1 ratio)

Similar superscripts indicate non-significant at the corresponding CD

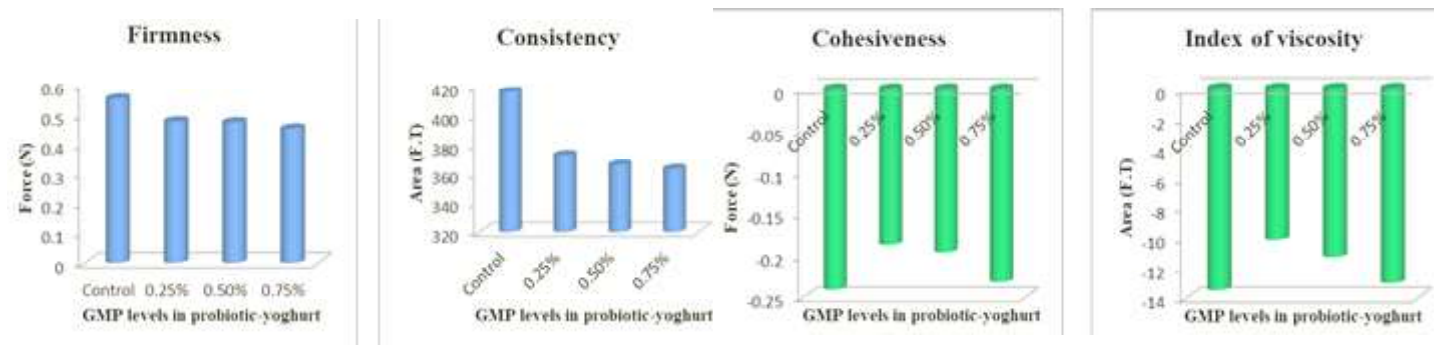


Fig. 2 Graphical representation of textural attributes of GMP fortified probiotic- yoghurt

Table 2: Effect of GMP fortification on apparent viscosity of probiotic -yoghurt

Speed (RPM)	GMP Levels			
	Control*	0.25 %	0.50 %	0.75 %
Apparent viscosity of probiotic-yoghurt				
10	11166.67	8920.00	8480.00	7860.00
20	4433.33	3330.00	3450.00	3260.00
30	2324.44	1942.22	1875.55	1802.22
40	1575.00	1386.66	1333.33	1286.66
50	1221.33	1073.33	1060.00	1065.33
60	1053.33	918.44	893.33	924.44
70	961.90	800.95	722.85	746.67
80	901.66	665.83	594.44	667.50
90	841.48	608.89	541.55	870.37
100	734.66	565.33	495.33	754.66
“n” value	0.142 ^{ab}	0.131 ^b	0.134 ^b	0.147 ^a
CD ($P \leq 0.05$)		0.012		

Average of 3 trails

Probiotic-yoghurt with *B.bifidum* and *L.acidophilus* (4 % ;1:1 ratio)

“n” value: between 0-1

Similar superscripts indicate NS at the corresponding CD

11.269 and -13.030 for 0.25, 0.50 and 0.75 % GMP levels in probiotic-yoghurt as compared to control (-13.501). Statistical analysis has shown a significant difference among treated samples and also with the control ($P < 0.05$). In both the parameters, the treated samples showed increasing values with increase in GMP levels indicated the greater number of internal bonds between similar molecules of GMP and extent of deformation before it ruptures. Presence of hydrocolloids, proteins/ peptide matrix or changing the process variables may change the cohesiveness and viscosity of the product. Higher values of cohesiveness in the treated samples may be related to the interaction of GMP with other protein molecules to give strength to the system or gel matrix. The results obtained in the present study are in correlation with the findings of Mudgil et al. (2012), who reported that an increase in cohesiveness in yoghurt with partially hydrolyzed guar gum (1-5 %).

Apparent viscosity

GMP incorporation into probiotic-yoghurt at 0.25, 0.50 and 0.75 % levels were subjected to rheological studies to know the effect of GMP on apparent viscosity of probiotic-yoghurt. The viscosity analyzed at different RPM was converted to shear stress and shear rate to know the ‘n’ value, are presented in Table 2. The ‘n’ value will be one for Newtonian type of liquids and the value towards zero indicates non-Newtonian behaviour of liquids (more solid like), it shows the viscous nature of the product, lower the value, higher the viscosity.

In the present study, ‘n’ values in the table showed lower values for 0.25 and 0.50 % GMP levels (0.131 and 0.134) when compared to control (0.142) and 0.75 % GMP (0.147). Incorporation of GMP at 0.25 and 0.50 % levels into probiotic-yoghurt showed increased viscosity as compared to 0.75 % and control. Graphs of the entire samples showed shear thinning behaviour i.e. as shear rate increases, viscosity decreased (Fig. 3). The statistical analysis also showed a significant difference among the treated samples

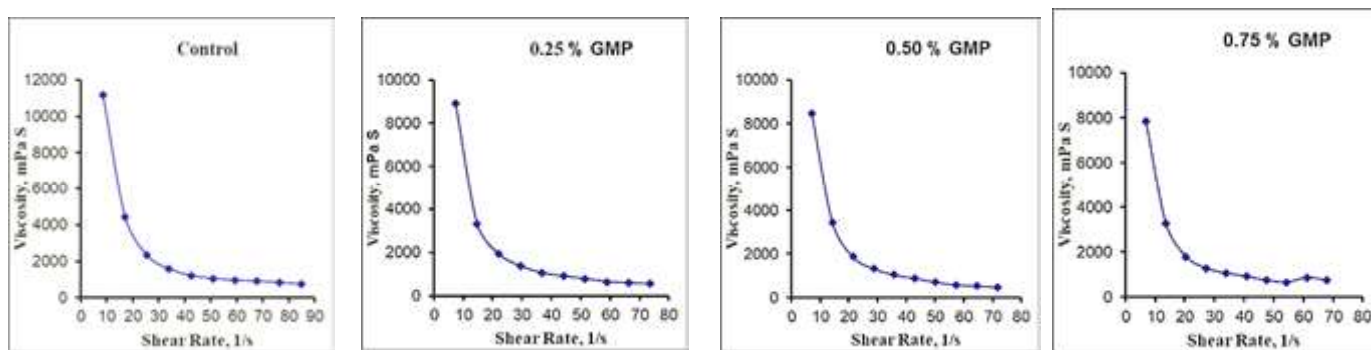


Fig. 3 Effect of GMP fortification on viscosity against shear rate in probiotic-yoghurt

Table 3: Effect of GMP fortification on sensory attributes of probiotic-yoghurt

Levels of GMP (%)	Sensory attributes				
	Colour and Appearance	Body and Texture	Flavour	Sourness	Overall acceptability
	Sensory scores on 9 point Hedonic scale				
Control	8.00±0.65 ^a	8.00±0.34 ^b	7.86±0.70 ^b	7.80±0.61 ^b	8.00±0.68 ^a
0.25	8.00±0.81 ^a	8.50±0.57 ^a	8.25±0.28 ^a	8.25±0.75 ^a	8.12±1.18 ^a
0.50	8.00±0.81 ^a	8.00±1.41 ^b	8.00±0.50 ^a	8.00±0.50 ^a	7.75±1.19 ^a
0.75	8.00±0.81 ^a	7.50±1.00 ^c	7.25±0.81 ^b	7.25±0.81 ^c	7.25±0.50 ^b
CD ($P \leq 0.05$)	0.21	0.43	0.22	0.34	0.29

All the values are average of 5 trials

Control: Probiotic-yoghurt with 4 % *B.bifidum* and *L.acidophilus* (1:1)

Similar superscripts indicate non-significant at the corresponding CD

and control. Probiotic-yoghurt with 0.25 and 0.50 % GMP levels were not significantly different, but 0.75 % concentration showed a decreased viscosity and the values were closer to control. Similar trend was noticed in the Texturo meter readings (Table 2) indicated an increase in the consistency and index of viscosity properties with the addition of GMP. The increased viscosity of GMP fortified samples could be due to the rearrangement of protein and protein-protein interactions and higher water holding capacity. Since yoghurt is a gel matrix of caseinate micelles with entrapped water, the apparent viscosity is affected by the strength and number of bonds between caseinate micelles as well as their structure and spatial distribution. Puvanenthiran et al. (2002) also reported a less viscous set yoghurt containing lower caseinate: WP ratio (0.5:1) and they concluded that the highly soluble proteins like whey proteins and GMP may reduce the viscosity of the fermented product at lower pH (4.6) and at higher concentration. Martin-Diana et al. (2004) concluded that addition of WPC and GMP to fermented goat's milk enhanced elasticity v/s viscosity and reduced the phase angle as compared to fermented cow's milk. They also noted that GMP in fermented milks favoured the formation of a more orderly and structured gel than using WPC, a finding shown by Cryo-SEM. Peng et al. (2009) compared the impact of different types of milk proteins used for fortification on the textural properties of yogurt showed the G2 values at pH 4.6 which, increased in the order of SMP to micellar casein.

Sensory attributes

Probiotic-yoghurt was analysed for influence of fortification of GMP at various levels on sensory attributes of product which is presented in Table 3. GMP fortified probiotic-yoghurt cut smoothly with fine, closed and uniform textured appearance, whereas control had crumbly body and texture when observed the cut portion using spoon. The sensory mean scores also revealed that 0.25 % GMP fortified probiotic-yoghurt had highest scores, 8.00, 8.50, 8.25 and 8.25 with respect to all sensory attributes viz. colour and appearance, body and texture, flavour, sourness and overall acceptability, respectively against control (8.00, 7.75, 7.75 and 7.25, respectively). However, statistical analysis indicated that only 0.25 % GMP fortified sample was significantly different from control sample and 0.75 % GMP level with respect to body and texture, flavour and overall acceptability attributes. Higher level of GMP (0.75 %) was associated with statistically significant lower scores of all sensory attributes. The higher acidity (Pushpa et al. 2018), bland taste of GMP and the textural changes might have appealed the judges that resulted in lesser acceptability of the product at higher levels. Most of the sensory profile scores for 0.50 % GMP level were not statistically significant with 0.25 % level. Hence, lower levels upto 0.50 % GMP fortified probiotic-yoghurt was proved to possess better sensory qualities may be due to improved physico-chemical and textural properties as discussed in this

study w.r.t firmness and viscosity. Pushpa et al. (2018) reported in her studies that the physico-chemical parameters such as acidity, reduced milk setting time, reduced syneresis and higher growth of probiotic bacteria in presence of GMP (0.25 %) helped in securing higher scores. The results of the present study are comparable with Antunes et al. (2005) and Zhao and Zhang (2006) who reported that the supplementation of products with nitrogen source derivatives such as hydrolyzed protein, whey proteins and amino acids had a positive impact on the viability of probiotic strains and on the product quality (syneresis and firmness) in yoghurt. The physico-chemical and textural attributes of yoghurt, including lack of visual whey separation, acidity and perceived viscosity, are crucial aspects of the quality and overall sensory acceptability. However, a very little information is available about the technological functional properties of GMP in food matrices.

Conclusions

The results of the present investigation conclude that incorporation of GMP in probiotic-yoghurt could bring significant effect on the textural, rheological and sensory characteristics upto 0.50 % concentration. Above this concentration (upto 0.75%), the product's viscosity and the textural properties w.r.t. firmness and consistency decreased together with a lower sensory score. For the production of innovative products, the technological functionalities of GMP could be best made use for the product promotion in the market.

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RESEARCH ARTICLE

Effect of alternate culture yoghurt and straining periods on textural and sensory attributes of Greek yoghurts made using buffalo milk

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Abstract: Sensory and textural attributes of probiotic Greek yoghurts prepared using alternate culture yoghurt and a commercial yoghurt culture at different straining periods were studied. Greek yoghurts were prepared from buffalo milk using alternate culture yoghurt C1 (*Streptococcus thermophilus* MTCC 5460 + *Lactobacillus helveticus* MTCC 5463) and C2 (MTCC 5460 + *Lb. rhamnosus* MTCC 5946) and a commercial yoghurt culture C3 (*S. thermophilus* + *Lb. bulgaricus*). Between the two alternate cultures, Greek yoghurt of C1 was found to be superior in sensory attributes. Hence, in the further study, C1 and C3 were used for the preparation of products using different straining periods (30min, 75min, and 105min) to obtain final total solids of 21%, 23% and 25%. Greek yoghurts were evaluated for sensory, physico-chemical, microbiological and textural characteristics. Greek yoghurt prepared using C1 and a straining period of 105 min exhibited superior sensory and textural attributes. The product scored highest for body and texture characteristics and its overall acceptability was judged as the best. It also showed higher values of hardness, cohesiveness in comparison to product of C3. With increasing periods of straining, the hardness, cohesiveness and adhesiveness of the Greek yoghurts increased. The mean culture count of the products varied between 8 to 9 log CFU/g. Results indicated that alternate culture yoghurt comprising of *Streptococcus thermophilus* MTCC 5460 and *Lactobacillus helveticus* MTCC 5463 can be used for the preparation of Greek yoghurts from buffalo milk.

Keywords: Alternate culture yoghurt, Buffalo milk, Greek yoghurt, Sensory, Straining period, Texture

Introduction

Greek yoghurt is a fermented semi-solid product derived from yoghurt by draining away part of its water and water-soluble components (Desai et al. 2013; Gyawali et al. 2022). It is traditionally produced by straining yoghurt in cloth bags until a desired level of total solids is achieved. Greek yoghurt is popular due to its creamy texture, higher protein content and less lactose as compared to regular yoghurt (Gyawali et al. 2022). It is fast gaining popularity among health-conscious consumers due to its proposed health benefits such as weight reduction, managing high blood pressure and in promoting strong and healthy bones (Jyoti et al. 2015). The global Greek yogurt market is projected to grow at a Compound Annual Growth Rate (CAGR) of 10.9 % till 2027 (www.Research and Markets.com, 2020). Rising awareness about the health benefits of fermented dairy products, increasing disposable income levels, growing demand from the middle-class segment, affordable prices, are some of the factors driving the demand for Greek yoghurt globally (www.ResearchandMarkets.com, 2020). Hence the market scenario is apt for development of concentrated fermented milks which can add to the fermented milk portfolio. Greek yoghurt does not have standard of identity in USA and hence various methods are used for its manufacture resulting in variable sensory profile (Desai et al. 2013; Jørgensen et al. 2019).

The type of starter culture and manufacturing process used for the preparation of Greek yoghurts have huge impact on its sensory and textural properties. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are the typical strains used as starter cultures in yogurt manufacturing. As per Codex standards for fermented milks, Alternate Culture Yoghurt comprising of cultures of *Streptococcus thermophilus* and any *Lactobacillus* species can also be used for preparation of yoghurt. Alternate culture yoghurt can be useful in terms of getting additional benefits without altering manufacturing process. For example, some bacterial exopolysaccharides (EPS) have been reported to influence yogurt texture, and specific EPS producing *Lactobacillus* strains could be useful as thickeners

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and fat replacers (Gawai et al. 2017; Gyawali et al. 2022). Further, use of a probiotic *Lactobacillus* strain as one of the partner in yoghurt starter culture can impart additional specific health benefits to the product.

Greek yogurt can be made by concentrating curd before or after fermentation. One of the crucial steps in the preparation of Greek yoghurt is the straining of curd to remove the whey to obtain a thicker and creamier consistency in the product. This straining step improves the texture of the yoghurt and reduces whey syneresis which is often found in regular yoghurt (Desai et al. 2013; Jørgensen et al. 2019). Traditionally, the cloth bag method is used to remove whey from plain yoghurt until the desired total solids level has been reached and usually it is about 23 to 25% (Al-Kadamany et al. 2002) and may take about 15-20 hours at $<10^{\circ}\text{C}$ (Chandan and Kilara, 2013). According to the Codex Standard for Fermented Milk, the strained yogurt should have increased protein content before or after fermentation to a minimum of 5.6% (compared with regular yogurt, which has a protein content of minimum 2.7%) (Codex Alimentarius, 2003). The removal of whey and concentration of curd affect compositional, sensory and textural quality and impart variations in Greek yogurt. The product obtained from this draining action has a better keeping quality than normal yogurt, mainly as a result of the higher concentration of lactic acid (Tamime and Robinson, 2007; Lange et al. 2020). To make concentrated yogurt, other methods including the use of centrifugation and ultrafiltration (Nsabimana et al. 2005; Desai et al. 2013; Jørgensen et al. 2019) or addition of dried ingredients such as dairy protein ingredients or other hydrocolloids are also being tried (Desai et al. 2013; Jørgensen et al. 2019; Gyawali et al. 2022).

Different combinations of starter cultures can have a direct influence on the sensory properties of Greek yogurt. Greek set type yoghurt produced using *L. paracasei* subsp. *tolerans* ACA-DC 4037 exhibited the best sensory properties with a rich, traditional smooth taste in comparison to the yoghurt prepared using *L. plantarum* ACA-DC 146 when the strains were tried as starters or starter adjuncts (Gyawali et al. 2022). However, there is a dearth of information about the systematic studies related to the effect of alternate culture yoghurt and straining periods on the quality attributes of buffalo milk Greek yoghurts. This study aimed to understand the effect of using alternate culture yoghurt and straining periods on the sensory and textural attributes of Greek yoghurts prepared from buffalo milk.

Materials and Methods

Microbial cultures and their maintenance

Two alternate culture yoghurts (C1 and C2) and one commercial yoghurt culture (C3) were used in the study. Alternate cultures included: C1 (comprising of *Streptococcus thermophilus* MTCC 5460 and *Lactobacillus helveticus* MTCC 5463) and C2 (comprising of *Streptococcus thermophilus* MTCC 5460 and *L.*

rhamnosus MTCC 5946). C3 comprised of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in DVS form. The strains *Lactobacillus helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5460 and *L. rhamnosus* MTCC 5946 were obtained from the culture collection of Dairy Microbiology department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India. *Lb. helveticus* MTCC 5463 is a potential probiotic culture (Prajapati et al. 2011). All cultures were propagated in sterilized reconstituted skim milk (11% T.S.) medium by incubation at $37\pm1^{\circ}\text{C}$ for 6 h and stored at $5\pm2^{\circ}\text{C}$. Three successive transfers of cultures were given in the same medium prior to their use to ensure the activity of cultures during the course of study. C3 culture in DVS form was stored at -20°C and used as per manufacturer's instructions.

Experimental design

First phase of study was aimed to select the best alternate culture yoghurt out of C1 and C2. For the said purpose Greek yogurts were prepared (as explained below) using two alternate cultures (C1 and C2) and one commercial culture (C3). Straining period of 30 min was used in this phase. Between C1 and C2, the best one was selected on the basis of acidity, sensory evaluation. In the second phase of study, the alternate culture selected from first phase and C3 were used to prepare the Greek yoghurt. Three different straining periods viz., 30 min (P1), 75 min (P2) and 105 min (P3) to obtain final total solids of 21%, 23% and 25% were used in the study. The products were evaluated for sensory, physico-chemical, microbiological and textural characteristics to assess the desirability of the products. Each treatment was replicated four times.

Preparation of Greek yogurt

Fresh Buffalo milk of Surti breed was collected from Reproductive Biology Research Unit, Anand Agricultural University, Anand, Gujarat, India, and chilled immediately. Raw buffalo milk (Fat 6.8%, SNF 9.6%) was preheated to 40°C and subjected to cream separation in a cream separator (Suryoday Engineering Co., India). Buffalo milk was standardized using cream (65-70% Fat), skim milk and skim milk powder (SAGAR brand) to obtain standardized milk having 3% Fat and 11% SNF. Standardized milk was then subjected to a heat treatment of $90^{\circ}\text{C}/10$ min and subsequently cooled to $42\pm1^{\circ}\text{C}$. Starter culture was inoculated @ 2% and incubated at $37\pm1^{\circ}\text{C}$ for alternate cultures (C1 and C2) and at $42\pm1^{\circ}\text{C}$ for commercial yoghurt culture, until acidity reached to 0.6 % LA. It was then subjected to straining using muslin cloth for different periods to obtain different total solid levels. The curd mass obtained after straining was stirred to a smooth consistency and subsequently stored in polystyrene cups with lids at $5\pm2^{\circ}\text{C}$.

Sensory evaluation

Sensory evaluation of Greek yoghurts was carried out by an expert panel of judges (n=9) using the 9-point Hedonic Scale. The samples were evaluated for sensory attributes such as flavor, color and appearance (C&A), body and texture (B&T), acidity and overall acceptability.

Texture Profile Analysis (TPA) of Greek yoghurts

Textural properties of Greek yoghurts were measured using a texture analyzer (Stable Micro Systems, TAHD Plus) fitted with a 5-g trigger force. Measurements were performed at refrigerated temperature ($5 \pm 2^\circ\text{C}$). The measurement parameters were: P/36 probe, trigger type, auto-10 g; Pre-test speed 1.0 mm/sec and post-test speed at 2.0 mm/s; test speed, 1.0 mm/s; strain, 70%; interval between two compressions, 6 s. Measurement depth 25 mm. The textural parameters of hardness, cohesiveness, adhesiveness, springiness, gumminess, chewiness and resilience were calculated automatically using TPA.

Titratable acidity and Total solids

Titratable acidity of Greek yoghurts was estimated by the procedure described in FSSAI (2015). Total solids were determined according to the procedure described in Bureau of Indian Standards handbook (BIS, 1981).

Microbiological analysis of Greek yoghurts

Eleven grams of product was aseptically weighed and transferred to 99ml sterile phosphate buffer to obtain 1:10 dilution. Subsequently, 1 ml of above dilution was used for making further dilutions in 9 ml phosphate buffer tubes. Suitable dilutions were prepared and poured in a set of sterile Petri plates in duplicates. For the enumeration of probiotic count, 1.0 ml from selected dilutions were pour plated by mixing with 10-15ml of sterile cooled MRS agar. After the solidification of agar, a second layer of the same medium (5-7ml) was poured. The plates were then incubated at $37 \pm 1^\circ\text{C}$ for 72 h. After incubation, the typical lactobacilli colonies in the plates were counted and the count was expressed as log CFU/g. For the enumeration of *Streptococcus* count, in place of MRS agar, M17 agar was used.

Statistical Analysis

Data obtained from textural, sensory, physico-chemical and microbiological properties were analyzed by Completely Randomized Design (CRD) and Factorial Completely Randomized Design (FCRD) as per the methods described by Steel and Torrie (1980).

Results and Discussion

Effect of cultures (C1, C2, C3) on pH, titratable acidity and sensory attributes of the Greek yogurts

Culture C1 took 5 h to reach up to titratable acidity of 0.6% LA, while cultures C2 and C3 took 6 h for reaching the same level of titratable acidity. pH of the Greek yoghurts ranged from 4.56 to 4.50 while the titratable acidity ranged from 0.84 to 0.87 % LA. Greek yogurts prepared using alternate cultures C1 and C2 as well as commercial culture C3 were compared for sensory attributes. Average *Lactobacillus* count of Greek yoghurts from C1, C2 and C3 were found to be 9.77 ± 0.02 , 9.70 ± 0.9 and 9.67 ± 0.02 log CFU/g respectively and *Streptococcus* count were found to be 9.76 ± 0.01 , 9.70 ± 0.05 and 9.67 ± 0.01 log CFU/g respectively.

Figure 1 shows the effect of starter cultures on the sensory attributes of the products. Greek yoghurts prepared using all the three cultures (C1, C2 and C3) had a pleasant, sweet-sour taste and an overall acceptable score of above 7.5. The scores for flavor, B&T, C&A and overall acceptability of products made using alternate culture C1 were significantly ($P < 0.05$) higher than the scores of products made using cultures C2 and C3. The average flavor score of products prepared using cultures C1, C2 and C3 were found to be 8.28 ± 0.18 , 7.95 ± 0.18 and 7.96 ± 0.06 , respectively. Yoghurt flavor is determined by a unique combination of lactic acid and volatile organic compounds like acetaldehyde, diacetyl and other flavor compounds with acetaldehyde being the prominent flavor compound. These compounds are mainly formed during the first couple of hours in the fermentation process (Routray and Mishra, 2011). The difference in the flavor score of experimental Greek yoghurt samples could be attributed to the production of varied flavoring components as a result of the inherent metabolic activity of starter cultures. C1 scored significantly ($P < 0.05$) higher B&T score than C3. This may be because the product of C1 exhibited a uniform, firm, shiny, homogenous texture which might be due to the exopolysaccharide production by *L. helveticus* MTCC 5463. Senan et al. 2015 reported that *L. helveticus* MTCC 5463 is capable of producing exopolysaccharide. We could not observe any significant difference in the acidity score (as perceived by the judges during sensory evaluation) of products prepared using alternate cultures and commercial yoghurt culture (Figure 1). However, the acidity score of products prepared using culture C1 was found to be relatively higher than that of products of C2 and C3. It is a well-known fact that the inherent ability of LAB strains to produce lactic acid from lactose can vary from strain to strain (Patel, 2016). Hence the use of different starter strains tends to produce fermented dairy products having varying levels of acidity, even change in the proportion of component starters can affect the final acidity of the product. The C&A score of Greek yoghurts prepared using C1 (8.62 ± 0.37) was significantly ($P < 0.05$) higher than the scores of products made using C2 and C3 cultures. This may be because the Greek yoghurt prepared using C1 had a uniform and homogeneous body compared to products of C2 and C3 which exhibited syneresis and loose body. The overall acceptability score was found to be higher (8.24 ± 0.21) for product of C1. Results of physico-chemical, sensory and microbiological analysis of Greek yoghurts revealed that, between

Fig.1 Mean score (n=4) for effect of starter cultures on sensory attributes of the Greek yoghurts prepared using starter cultures alternate culture yoghurt C1, C2 and commercial yoghurt culture C3

B&T = Body and Texture; C&A= Color and Appearance; OA= Overall Acceptability

C1 (*Streptococcus thermophilus* MTCC 5460 and *Lactobacillus helveticus* MTCC 5463); C2 (*Streptococcus thermophilus* MTCC 5460 and *L. rhamnosus* MTCC 5946) and C3 (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*)

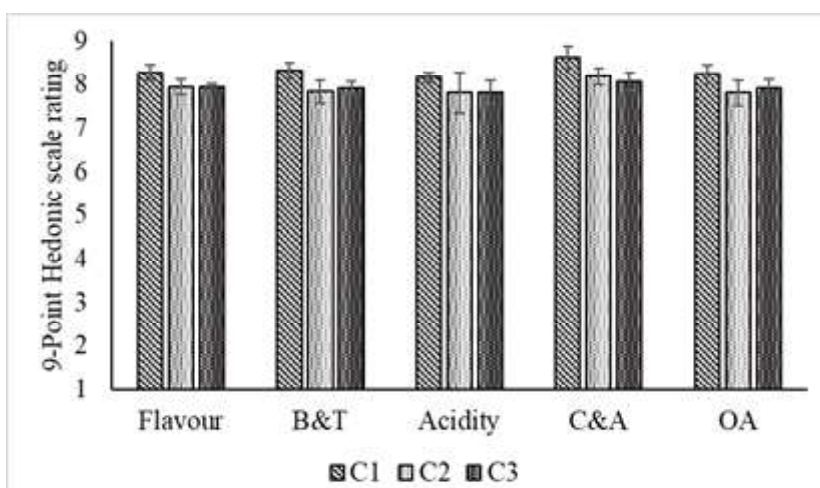


Table 1: Effect of straining periods on total solids and titratable acidity of the Greek yoghurts prepared using alternate culture yoghurt C1 and commercial yoghurt culture C3

Cultures	Straining periods		
	P1 (30 min)	P2 (75 min)	P3 (105 min)
	Total solids (%)		
C1	21.34 ± 0.21	23.48 ± 0.30	25.30 ± 0.36
C3	21.45 ± 0.32	23.47 ± 0.30	25.32 ± 0.35
	CD(0.05): C=NS, P=0.33, C*P=NS		
	Titratable acidity (%LA)		
C1	0.90 ± 0.01	1.08 ± 0.05	1.27 ± 0.06
C3	0.86 ± 0.05	1.12 ± 0.05	1.29 ± 0.03
	CD(0.05): C=NS, P=0.05, C*P=NS		

Each observation is a mean ± SD of four replicate experiments (n=4), C1 (*Streptococcus thermophilus* MTCC 5460 and *Lactobacillus helveticus* MTCC 5463); and C3 (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). P1, P2, P3 are straining periods of 30 min, 75 min and 105 min respectively.

the two alternate cultures, product of C1 was found to be superior compared to product prepared using culture C2. Hence it was decided to use alternate culture yoghurt C1 (*Streptococcus thermophilus* MTCC 5460 + *Lactobacillus helveticus* MTCC 5463) in the further study and C3 (Commercially used Yoghurt culture) was used as control.

Effect of cultures C1 and C3 and straining periods on quality attributes of Greek yoghurts

Effect on total solids and titratable acidity of the Greek yoghurts

Straining periods had a significant ($P < 0.05$) effect on the total solids of the products. Straining periods of 30 min (P1), 75 min (P2) and 105 min (P3) resulted in Greek yoghurts having average total solids of 21.39%, 23.47% and 25.31% respectively (Table 1). However, cultures did not have any significant effect on total solids of the products. Increased length of straining period resulted in an increase in titratable acidity of products due to continued production of lactic acid by starter cultures. Average values of titratable acidity of the products were 0.88, 1.1 and 1.28% LA for P1, P2 and P3 respectively. Development of acidity is an essential requirement for good flavor in yoghurt. According

to CODEX standards, concentrated fermented milks should have titratable acidity more than 0.3% lactic acid.

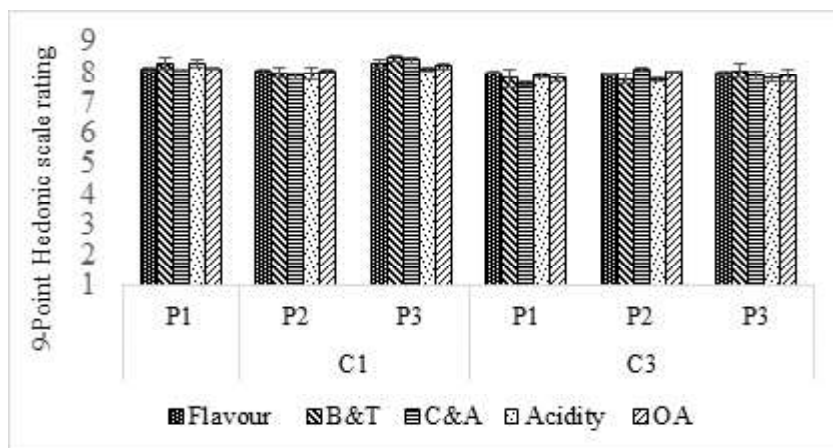
Effect on sensory attributes of the Greek yoghurts

Effect of starter cultures and straining periods on the sensory attributes of the products are shown in Figure 2. Cultures had a significant ($P < 0.05$) effect on all the sensory attributes whereas straining periods showed significant effect on B&T and acidity score. Between the two cultures, Greek yoghurts of C1 scored significantly higher for all sensory attributes compared to products of C3. Mean flavor scores of products prepared using C1 and C3 were 8.09 and 7.97 respectively. Product of C1P3 scored significantly ($P < 0.05$) higher (8.28) followed by C1P1 (8.08) and C1P2 (8.02) which did not differ significantly. Cultures and straining periods significantly ($P < 0.05$) affected the B&T of the products. C1P3 scored significantly higher (8.45) for B&T in comparison to C3P3 (8.05). Likewise, a significantly better score was seen in case of C1P2 compared to C3P2 and C1P1 scored higher than C3P1. Increased straining period had a positive influence on the B&T characteristics of Greek yoghurts.

Fig. 2 Mean score (n=4) for effect of starter cultures and straining periods on sensory attributes of the Greek yoghurts prepared using alternate culture yoghurt C1 and commercial yoghurt culture C3 at straining periods P1, P2 and P3.

C1 (*Streptococcus thermophilus* MTCC 5460 and *Lactobacillus helveticus* MTCC 5463); and C3 (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). P1, P2, P3 are straining periods of 30 min, 75 min and 105 min respectively.

B&T = Body and Texture; C&A= Color and Appearance; OA= Overall Acceptability



Cultures and straining periods, both had a significant ($P < 0.05$) effect on the acidity score of the products (Figure 2). Perception of acidity during sensory evaluation has a correlation with the titratable acidity of the products. Titratable acidity of the products was found to be increasing with increased straining period. Organoleptic assessment of acidity in different samples showed variations and the judges' score ranged from 7.78 to 8.27. As shown in Figure 2, highest acidity scores were seen for Greek yoghurts of C1 in comparison to culture C3. Among the products of C1, straining periods had a significant effect on acidity score and the product of C1P1 scored significantly higher (8.27) in comparison to C1P2 (7.95) and C1P3 (8.08). Between C1P2 and C1P3 no significant difference was seen. Generally, acidity in the range of 0.85 to 1.2 % LA is appropriate for good quality yoghurt (FSSAI, 2011). Right balance of the acidity is essential for good taste of Greek yogurt. Excessive acidity imparts too much sourness and sharp astringent taste to the product, whereas low acidity in yoghurt leads to a bland product.

A significant ($P < 0.05$) difference was found in the C&A scores of products made using cultures C1 and C3 (Figure 2). Greek yoghurts of C1 showed higher values at all the straining periods in comparison to products of C3. Among all, product of C1P3 scored maximum (8.39). This may be because the products made using C1 appeared shinier, whitish and uniform. Mean C&A scores for products prepared using C1 and C3 were 8.24 and 7.97 respectively. However, within the culture straining periods do not had any significant effect on C&A scores.

Overall acceptability of a food product is largely governed by sensory parameters such as flavor, C&A and B&T. Highest overall acceptability score was found for product of C1P3 (8.19) followed by C1P1 and C1P2 (Figure 2). The mean overall acceptability score of products made using cultures C1 and C3 were found to be 8.10 and 7.88 respectively and the mean overall acceptability scores of products of P1, P2 and P3 were found to be 8.00, 8.01 and 7.99 respectively. The interaction effect was not significant ($P > 0.05$). Previous research studies reported that increasing the

TS of concentrated milk beyond 23.00%, led to a decreased flavor score of the product (Mahdian and Tehrani, 2007; Yeganehzad et al. 2007). According to our study results, the product prepared using culture C1P3 having TS of 25.30% scored highest for overall acceptability.

Effect on texture of the Greek yogurts

Texture is an important parameter contributing to the sensory properties of food. For food products, according to the International Standard Organization (ISO), texture represents all the rheological and structural attributes perceptible by means of mechanical, tactile, and, when appropriate, visual and auditory receptors. Rheology and structure of a product evaluated by instrumental methods also give relevant information on its textural properties. Textural properties of yoghurt gels are important factors in establishing consumer preference and manufacturing strategies for yoghurt (Tunick, 2000). Textural characteristics of yoghurts have been defined by hardness, adhesiveness, cohesiveness, fracturability and springiness (Megenis et al. 2006) and were measured by TPA.

TPA of Greek yoghurts revealed a significant ($P < 0.05$) effect of straining periods on textural attributes of the products (Table 2). Hardness, cohesiveness and adhesiveness of the products increased significantly ($P < 0.05$) with increased period of straining.

Hardness is a commonly evaluated parameter when determining yoghurt texture. It is defined as the force necessary to attain a given deformation. It has been reported that the hardness of yoghurt increases with increase in fermentation time, increase in total solids content and straining of whey (Walia et al. 2013). The results depicted in Table 2 clearly showed a significant ($P < 0.05$) difference between the hardness values of products made using C1 and C3 cultures at different periods of straining. Values of hardness were higher for Greek yoghurts made using C1 at all straining periods (533.99g, 862.69g and 1097.95g respectively) in comparison to product of C3 (488.12g, 756.17g and 917.29g respectively). The mean hardness values of the products for

straining periods P1, P2 and P3 were 511.05g, 809.43g and 1007.62g, respectively, whereas for cultures, it was 831.54g and 720.52g. Product of C1P3 had exhibited the highest hardness. A linear relation has been reported between TS and hardness of yoghurt (Yeganehzad et al. 2007) and the same was evident in our results also. Mahdian and Tehrani (2007) reported that the use of high TS milk leads to yoghurt having higher acidity and greater hardness, but with reduced pH and syneresis.

Cohesiveness is defined as the extent to which a material can be deformed before it ruptures. Cohesiveness depends upon the strength of the internal bonds (Uprit and Mishra, 2004). Results shown in Table 2 indicate that the type of cultures and straining periods had a significant ($P<0.05$) effect on the cohesiveness of the products. Cohesiveness of products increased with longer straining periods and the mean cohesiveness value ranged from 0.45 to 0.70. Product made using culture C1 showed significantly ($P<0.05$) higher cohesiveness (0.59) than C3 (0.53) at all periods of straining. Highest cohesiveness was seen in the product of C1P3. Springiness is the rate at which the sample returns to its original shape when the deforming force is removed (Kahyaoglu et al. 2005). As shown in Table 2, there was significant ($P<0.05$)

difference found between springiness values of products prepared using cultures C1 (1.53 mm) and C3 (1.80 mm). Highest springiness (1.97 ± 0.06 mm) was found in product made using culture C3 and straining period 30 min and the lowest (1.33 ± 0.01 mm) was in a product made using C1 and straining period of 105 min. Significant difference was not observed in the mean values of P1 and P2. Greek yoghurt of C1P3 exhibited significantly lesser springiness than that of C3P1. Greater cohesiveness and springiness may be related to stronger gel structures, indicating greater structural integrity; perhaps due to increased charged groups on the amino acid groups, which is considered a function of whey protein denaturation (Megenis et al. 2006).

Adhesiveness is the force necessary to remove the material that adheres to the mouth during eating (Uprit and Mishra, 2004). As shown in Table 2, a significant ($P<0.05$) difference was found between adhesiveness of products prepared using cultures C1 and C3 as well as using different straining periods. Greek yoghurts prepared using C3 showed a higher mean adhesiveness value (1584.77g sec) than the products prepared using C1 (1561.91g sec). The mean adhesiveness of the products increased with increase in straining period. Product of C1P3 showed highest

Table 2: Effect of straining periods on textural attributes of the Greek yoghurts prepared using alternate culture yoghurt C1 and commercial yogurt culture C3

Cultures used in the study	Straining periods			Mean
	P1(30 min)	P2(75 min)	P3 (105 min)	
		Hardness (g)		
C1	533.99±9.54	862.69±16.79	1097.95±17.73	831.54 ^a
C3	488.12±9.89	756.17±11.83	917.29±9.59	720.52 ^b
Mean	511.05 ^C	809.43 ^B	1007.62 ^A	
	CD(0.05): C=11.17, P=13.68, CxP=19.34			
		Cohesiveness		
C1	0.47 ± 0.01	0.55 ± 0.01	0.75 ± 0.006	0.59 ^a
C3	0.43 ± 0.01	0.52 ± 0.01	0.65 ± 0.02	0.53 ^b
Mean	0.45 ^C	0.54 ^B	0.70 ^A	
	CD(0.05): C=0.014, P=0.017, CxP=0.024			
		Springiness (mm)		
C1	1.49 ± 0.05	1.76 ± 0.02	1.33 ± 0.01	1.53 ^b
C3	1.97 ± 0.06	1.73 ± 0.03	1.71 ± 0.03	1.80 ^a
Mean	1.73 ^A	1.74 ^A	1.52 ^B	
	CD(0.05):C=0.03, P=0.04, CxP=0.05			
		Adhesiveness (g sec)		
C1	1434.87±10.52	1557.92±21.72	1692.95±8.40	1561.91 ^b
C3	1521.38±9.98	1612.95±11.75	1619.99±12.70	1584.77 ^a
Period Mean	1478.12 ^C	1585.44 ^B	1656.47 ^A	
	C=11.36P=13.91, CxP=19.67			

Each observation is a mean $\pm$ SD of four replicate experiments (n=4). ^{a-b} Different superscripts in the column indicate significant ($p<0.05$) difference between cultures for each attribute.

^{A-C} Different superscripts in the row indicate significant ($p<0.05$) difference between straining periods for each attribute

Table 3: Effect of straining periods on culture count of the Greek yoghurts prepared using alternate culture yoghurt C1 and commercial yogurt culture C3

Cultures	Straining periods		
	P1 (30 min)	P2 (75 min)	P3 (105 min)
	<i>Lactobacillus</i> count (log CFU/g)		
C1	8.92 ± 0.61	9.39 ± 0.31	8.61 ± 0.50
C3	9.12 ± 0.44	8.87 ± 0.53	9.15 ± 0.45
	CD(0.05): C=NS, P=NS, C*P=NS		
	<i>Streptococcus</i> count (log CFU/g)		
C1	8.95 ± 0.41	9.20 ± 0.54	8.78 ± 0.67
C3	9.51 ± 0.32	9.22 ± 0.47	9.04 ± 0.42
	CD(0.05): C=NS, P=NS, C*P=NS		

Each observation is a mean ± SD of four replicate experiments (n=4), C1 (*Streptococcus thermophilus* MTCC 5460 and *Lactobacillus helveticus* MTCC 5463); and C3 (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). P1, P2, P3 are straining periods of 30 min, 75 min and 105 min respectively.

adhesiveness followed by C3P2 and C3P3 which were found to be at par with each other. Highest mean value for adhesiveness was observed in case of P3. This difference may be because of the difference in the inherent metabolic activities of the cultures and its effect on B&T characteristics of the products. Further the protein content has been reported to be a dominant factor influencing adhesiveness of protein rich products with varying composition (Kumar and Mishra, 2004). The higher the value, the more energy required to break the probe/sample contact as the probe withdraws from the sample.

Textual properties of yoghurt gels are important with regard to its overall quality including sensory perception. Such properties play a significant role in establishing consumer preference and manufacturing strategies for yoghurt. Along with the composition and preparation of milk base, the starter cultures and processing steps are important factors affecting the rheological and textural properties of yoghurt (Jaros and Rohm, 2003; Lange et al. 2020). Strained Greek yoghurts are generally characterized by the denseness before and after straining, firmness before and after straining, cohesiveness, and viscosity. Texture properties are further related to overall sensory quality. Like any other food product, for the Greek yoghurt, its textural and sensory attributes are important factors for quality and consumer acceptance and the type of culture used has a great influence on the textural and sensory attributes (Jaworska et al, 2005; Gyawali et al. 2022). The study by Desai et al. (2013) on the sensory properties and drivers of liking for Greek yoghurts reported that people liked Greek yoghurts having denseness, firmness and cohesiveness. Factors such as processing conditions, starter culture, ingredients used, all can significantly affect the flavor and texture of yoghurts (Ayub, 2004; Sodini and Tong, 2006; Lange et al. 2020). Previous research studies have shown that milk solids not fat (Hai-Yan et al. 2016), protein content (Kumar and Mishra, 2004) and EPS (Han et al. 2016; Gyawali et al. 2022) affect yoghurt texture. It had also been reported that exopolysaccharide

produced by yoghurt starter cultures could affect the texture of yoghurt and improve sensory characteristics such as mouthful, shininess, clean cut, and creaminess. (Han et al, 2016; Gyawali et al. 2022). In our study also, the increased total solids resulted in increased strength of fermented gel which was obvious in the TPA results. Additionally, the strain *L. helveticus* MTCC 5463, we have used is capable of producing EPS also, which might have contributed to the sensory as well as texture characteristics of the Greek yoghurt. Even though lot many studies are available on the textural attributes of yoghurts, regarding Greek yoghurt, very few studies have been published.

Effect on probiotic culture count of the Greek yoghurt

We could not find any significant difference in the lactobacilli and streptococci count (Table 3) of Greek yoghurts. The mean culture counts in the products varied between 8 log CFU/g to 9 log CFU/g which was in agreement with the legal requirements that the viable count of culture should be in abundance. Further one of the strains we have used in the study is a potential probiotic. Hence the product fulfills the requirement of a probiotic food. Researchers have also reported that the probiotic count available to the consumers for therapeutic benefits should be in the range of 10^6 to 10^9 in the product (Kurmman and Robinson, 1991; Shah, 2000).

According to the results of this study, use of alternate culture yoghurt had a positive influence on the sensory and textural characteristics of Greek yoghurt. Product made using alternate culture appeared more shiny, whitish and uniform. It has exhibited better denseness, firmness and cohesiveness.

Conclusions

The results of the study indicated that the type of cultures and straining period have a profound effect on the quality attributes of Greek yoghurt. The results of the physico-chemical, sensory

evaluation and TPA of products indicated that Greek yoghurt made using alternate culture (comprising of *Streptococcus thermophilus* MTCC 5460 and *Lactobacillus helveticus* MTCC 5463) was found to be superior than the commercial yoghurt culture. The overall acceptability of the products obtained using different straining periods did not show significant differences, and the product obtained using a straining period of 105 min scored better in comparison to other straining periods. Hence this research findings on the effect of straining periods and cultures on the quality of Greek yoghurts will provide basic research data for the industrial application and development of Greek yoghurt from buffalo milk using alternate culture yoghurt.

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A comprehensive study on processing parameters, yield, quality characteristics and shelf life of *malai* – a heat desiccated indigenous dairy product

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Abstract: *Malai* is an indigenous fat rich dairy product which is used in the manufacture of various products as well as for direct consumption. The present study was conducted with the aim to standardize the manufacturing process of *malai*, study the effect of various processing parameters and evaluate the shelf life of *malai* packed in glass and polypropylene containers by recording changes occurring during the storage period. The production process was standardized based on the quality and yield obtained; the maximum yield of *malai* was obtained at 90°C temperature of heating the milk, holding at that temperature for 50 minutes and allowing *malai* formation till a cooling temperature of 30°C was attained. The *malai* produced was evaluated for sensory and textural attributes. Based on the observations of the sensory panel members, it is stated that *malai* should have a typical yellowish colour, a rich nutty aroma and a soft, granular texture which imparts a flaky mouthfeel. The *malai* prepared using the standardized conditions obtained highest sensory acceptance scores. The effect of fat % and SNF content of milk on the yield of *malai* was also studied. It was observed that as the surface area of the milk exposed, the yield obtained also increased. The average composition of optimised *malai* samples was moisture, 49.6; fat, 38.2; and protein, 9.01%. It was observed that the *malai* could be stored at 5±1°C for 18 days with satisfactory acceptance. No significant differences were observed between the packaging materials during the storage period.

Keywords: *Malai*, Physico-chemical properties, Sensory evaluation, Textural attributes, Shelf life

Introduction

It is a common observation that milk when heated and held for a while at cooler temperatures forms milk skin on surface which is considered as fat rich portion of the milk. Heating of milk denatures whey proteins which along with casein and some air become lighter and ascend to the surface. The fat globules also rise to the surface and get entrapped in the denatured proteins. The accumulated surface layer gets solidified on coming in contact with cooler air at the surface, thus forming *malai* layer. *Malai* is a common term used in India which denotes not only the product *malai* but also as an adjective meaning ‘rich in fat and taste’. Though in India not much scientific work has been carried out, in other countries research work has been carried out on a similar product called clotted cream. Clotted cream is a fat rich dairy product which is widely consumed in the European countries, mostly with baked products such as scones and breads. There are several variations of clotted cream viz: Devonshire clotted cream or Cornwall clotted cream based on the regional differences, but name and production process vary according to the regional preferences. In Turkey, it is known as *kaymak*, in the Balkan region it is called *kajmak*, in Iraq the variant is known as *gaimar*, *geymar* or *qaimar* and when it comes to India the name changes to *malai*. *Malai* is used in various household culinary dishes and also in the preparation of traditional sweets, especially Bengali sweets. *Malai* is an essential ingredient in the production of various traditional Indian products such as *basundi*, *rabri* and *khurchan*. *Malai* should have a typical golden yellowish colour with rich nutty flavour. It should also have a typical granular texture. *Malai* is used either for direct consumption along with sugar or used in other sweet preparations as stated above. It is also used by some consumers added to tea. It is liked not only for rich taste but also for its firm, but soft and chewy body.

FSSAI rules and regulations defined *malai* as the product rich in butter fat prepared by boiling and cooling cow or buffalo milk or a combination thereof and stipulated that it shall contain not less than 25.0% milk fat. However, later in October 2017, in a substitute by Notification dated 12th Oct 2017, it was clubbed with category

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of cream and defined as follows: *malai* means the product rich in milk fat prepared by boiling and cooling of cow milk, buffalo milk or milk of any other species as defined under regulations or a mixture thereof (FSSAI, 2018). It is characterised by presence of insoluble mass, principally fat and denatured protein, formed on heating and cooling of milk. Compositionally, *malai* should contain milk fat not less than 10.0%; the same standard holds true for cream also, however, unlike *malai*, different categories of cream have been further elaborated.

The manufacture of *malai* is limited to unorganized sector namely 'halwais'. No particular significance is given to hygienic production and for the proper packaging of the product. Low shelf life of the product is another challenge faced when selling the product on a wider scale. No scientific data is still available on the manufacturing process and textural attributes of *malai*. Further, no study has been conducted on the preservation and modern packaging material of *malai*. At the same time, several scientific data are available on the western counterpart of *malai* viz clotted cream and *kaymak* (Sadleir, 1917; Chapman, 1953; Dozet and Stanisic, 1983; Akalin et al. 2006; Cakmakci and Hayaloglu, 2011;).

Thus, due to lack of scientific data a lot of pitfalls are encountered in developing production process of *malai*. It is the need of the hour to standardize the manufacturing process and to collect relevant scientific data so that future upgradation in the production of *malai* by dairies would be possible.

Thus, a systematic technical study is needed to be carried out on this traditional product. Hence, this study has been taken up to standardise the manufacturing process, to study the physico-chemical composition of *malai* and to study the effect of different packaging materials on the shelf life of the product.

Materials and Methods

Materials

Fresh cow milk was obtained from Livestock Research Centre of ICAR-National Dairy Research Institute, Southern Regional Station, Bengaluru. Stainless steel open pan (Milkmax Engineers, Pune) provided with steam line and steam control valve was used for the preparation of *malai*. Packaging materials viz. polystyrene and glass containers were obtained from local market in Bengaluru. Analytical grade (AR) chemicals procured from firms like RANKEM, SD Fine Chemicals Ltd and NICE chemicals Pvt Ltd were used in the physico-chemical analysis.

Methods

Process of malai preparation

One lit of milk standardised to 3% fat and 8.5% SNF was taken in steam kettle, heated to desired temperature and held for desired

time. The heated milk was taken out from the kettle into a SS vessel and allowed to cool spontaneously without closing the vessel to desired temperature. During this period, milk skin accumulated at the surface. When the temperature of milk reached desired temperature, the milk skin was carefully scooped out with a ladle into a separate container and the weight of the collected *malai* was recorded.

Processing variables and experimental details

Temperature of heating, duration of heating and cooling temperature of milk are the important considerations in *malai* layer formation. The temperature of heating should be such that it should induce irreversible denaturation of proteins. deWit and Klarenbeek (1984) observed that whey protein denaturation is reversible at about 60°C, and between 60–100°C, the denaturation is irreversible. Hence, temperatures of 70°C and above were chosen in this study. Further, it is understood that the higher the exposure to the heating temperature more would be the denaturation. Further, the amount of *malai* collected at the surface depends on the temperature to which milk is cooled for *malai* accumulation. Milk was heated separately to 70°C, 80°C and 90°C and held for a constant duration of 30 minutes and then it was allowed to cool to 30°C. The yield of *malai* obtained at each of these temperatures was calculated and the temperature with maximum yield was selected. Then, by keeping the heating temperature constant as chosen above, duration of heating was varied at three levels viz. 30, 45 and 50 min, and allowed to spontaneously cool to 30°C. The yield of *malai* obtained at each of these durations was calculated and the duration with maximum yield was selected. For optimising the temperatures of cooling, temperature of heating and holding time were kept constant and the cooling temperature varied at levels of 30°C, 45°C and 50°C. The yield of *malai* obtained at each of these cooling temperatures was calculated and the temperature with maximum yield was selected. In these experiments milk fat and SNF levels were kept constant at 3% and 8.5%, respectively.

Effect of fat, SNF content and surface area of milk exposed on the yield of malai

Using the above optimised heating temperature, holding time and cooling temperature, fat levels in milk were varied as 3, 4.5 and 6% at a constant SNF of 8.5% and *malai* was prepared. The yield of *malai* obtained from different fat contents was determined. Similarly, *malai* was also prepared using milk standardised to 8.5 and 9% SNF and optimised fat level, and the yield obtained was calculated. By keeping all the above optimised conditions constant, *malai* was prepared by exposing heated milk to different surface area (346.36 cm², 490.87 cm² and 706.85 cm²) in SS vessels and the variation in the yield obtained was calculated. The volume of the milk (1 litre) was kept same in all the trials.

Sensory evaluation

Malai samples were tempered to 30°C before evaluation. The *malai* samples were evaluated by a panel of 6-8 semi-trained judges from Dairy Technology Section of the institute. The panellists were requested to assess the samples for colour and appearance, mouthfeel, flavour and overall acceptability on 9 point hedonic scale (1 dislike extremely, 9 like extremely).

Textural analysis

Firmness and consistency of *malai* samples were determined using Texture Analyser (TA-XT plus, Stable Micro Systems, Surrey, UK). The textural parameters were worked out from force-time curve for each sample, with force experienced by probe on Y-axis and time on X-axis. The probe used was aluminium P/25. The *malai* sample tempered at 20°C was filled in a 50 ml beaker, kept positioned centrally over the platform and the probe penetrated the sample to a specified distance of 10 mm and then returned to original position generating a "Force- Time curve". The height of the force peak was the value of firmness measured in Newton. The area of the peak measured under the curve in Newton-seconds was expressed as consistency.

Shelf life studies of malai

Two different kinds of packaging materials viz. glass container and polypropylene (PP) cups were used for the packaging of *malai*. These two containers were used because these are the most commonly used low cost containers in food industry. *Malai* prepared from optimised method was packed in these two packaging containers (100 g each) which were tightly closed and stored at 5±1°C in a refrigerator. The product was analysed for changes in sensory, physico-chemical, colour, textural and microbiological properties during the storage.

The total solid content of *malai* was analysed using the method detailed in BIS (1981). Fat and ash contents were determined as per AOAC methods (2005). Total protein content of samples was determined by micro Kjeldahl method (BIS, 1981) with slight modifications. Lactose was estimated by difference method. The free fatty acid content was analysed by the method of Deeth and Fitz-Gerald (1976). Titratable acidity of *malai* was estimated by BIS (1981) method. Microbial analysis was conducted by the procedure detailed in BIS (1981). The colour attributes of *malai* were analysed by scanning method using Adobe photoshop Version 8.0 (Vyawahare and Rao, 2011). Formulae for estimation of colour parameter values were (Yam and Papadakis, 2004):

$$L^* = (100 * L) / 255; a^* = ((240 * a) / 255) - 120; b^* = ((240 * b) / 255) - 120$$

Whiteness indices (WI) are widely used to measure consumers' preferences for white colours. It mathematically

combines lightness and yellow-blue into a single term (Pathare et al. 2013). In the present study, whiteness index (WI) was determined by the equation (Bernat Perez et al. 2015):

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$$

In the same manner, yellowness index (YI) was also determined by the equation (Rhim et al. 1999):

$$YI = 142.86 b^* / L^*$$

Acidity

Titrateable acidity of *malai* was estimated by BIS (1981) method. 10 g of sample was weighed into 250 ml beaker and 100 ml of hot distilled water was added to it followed by addition of few drops of 0.5% phenolphthalein indicator. The contents were titrated against 0.1 N NaOH with continuous stirring till a faint pink colour persisted for 30 sec.

$$\text{Acidity} = v/w \times 0.9$$

Where, v = volume of 0.1 N NaOH required for titration w = weight of the sample (g)

FFA

Method described by Deeth and Fitz-Gerald (1976) was used. Five g of finely dispersed *malai* sample was mixed with 37.5 ml of 2% sodium citrate in a hundred ml conical flask and kept in the water bath at 50-60°C for 5 min to mix it properly. Bureau of Dairy Industry (BDI) reagent of 10 ml was added into the flask and was kept in gentle boiling water bath for 20 min (BDI reagent: Triton X-100 of 30 g of and 70 g of sodium hexametaphosphate was dissolved distilled water and volume made up to 1 litre). Sample was shaken intermittently till a clear fat separation was obtained. Contents were transferred to a butter butyrometer and centrifuged for 1 minute. Enough methyl alcohol was added to bring the fat column well within the graduated portion and further centrifuged for 1 min. The butyrometer was then placed in a water bath (57±3°C) for 5 min. About 0.2-0.4g fat was transferred at 57°C from the butyrometer to previously weighed 100 ml Erlenmeyer flask using 1 ml micropipette. Fat was dissolved in 5 ml of fat solvent and 5 drops of phenolphthalein was added as indicator. The mix was titrated with standardized alcoholic potassium hydroxide solution to faint pink colour. Blank titration was performed on fat solvent (in absence of fat) using 5 drops of phenolphthalein indicator.

Calculation:

$$FFA = \frac{(ml \text{ of KOH solution for sample} - ml \text{ of KOH solution for blank}) \times N \times 100}{\text{Weight of fat taken for titration, g}}$$

where, N= normality of alcoholic KOH solution (0.02)

In this study, FFA was expressed as m.eq of KOH per 100 g fat

Microbiological analysis

All the *malai* samples were analysed for the standard plate count (SPC) and yeast and mould count (YMC) by the methods described in BIS (1981). For SPC, eleven g of *malai* sample was diluted in 99 ml of sterile 2% (w/v) tri-sodium citrate. Further dilutions were prepared using sterile citrate buffer blanks. Suitable dilutions of each sample were transferred into sterile petri plates and molten nutrient agar was added. The plates were incubated at $37 \pm 0.5^\circ\text{C}$ for 48 h and the number of colony forming units (cfu) were noted. For YMC estimation, suitable dilutions of each sample were transferred into sterile petri plates and molten potato dextrose agar was added. The plates were incubated at $27 \pm 0.5^\circ\text{C}$ for 3-5 days and the number of colony forming units (cfu) were noted. The counts were expressed as $\log_{10}$ counts.

Statistical analysis

Statistical analysis of data for effects of the various factors on physico-chemical and sensory properties was performed by single factor completely randomized design using SPSS software (version 23.0) in three replications and analysis of variance was done with Duncan's multiple range test at $p < 0.05$.

Results and Discussion

Effect of process variables on the sensory acceptance of *malai*

Malai should have a firm body and slightly grainy texture so that it may have a relishing mouthfeel. The quality of *malai* accumulated at the surface of milk depends on the various factors such as temperature and time to which milk has been heated, and

the temperature at which *malai* is allowed to be collected at the top. These are the important factors to be optimised for getting *malai* of desirable qualities.

Temperature of heating milk

For different temperatures of heating, it is evident from Table 1 there was no significant ($p > 0.05$) difference on the colour and appearance, mouthfeel and flavour. But with 90°C of temperature of heating, the overall acceptability of *malai* has significantly ($p < 0.05$) increased. This may be attributed to the fact that at 90°C of heating, there was an increased likeability to the product due to the slight cooked flavour imparted at this elevated temperature. It is known that heating at higher temperatures causes denaturation of whey proteins and release of sulfhydryl compounds (Webb and Johnson, 1965). These compounds are responsible for cooked flavour which is desirable attribute of *malai*. Also, more granular layer formation has occurred and this in turn increased the mouthfeel of the product.

Duration of heating milk

The mouthfeel and flavour scores of *malai* prepared at 30 minutes duration of heating was significantly ($p < 0.05$) less (Table 1) compared to 45 and 50 minutes of heating. It was observed that there was no significant ($p > 0.05$) difference between the colour and appearance and flavour scores of *malai* prepared at 45 minutes and 50 minutes of heating. However, the mouthfeel score at 50 minutes of heating was significantly higher, this may be due to the increased granularity of layers of *malai* formed. The overall acceptability score of *malai* produced at 50 minutes of heating was significantly ($p < 0.05$) higher compared to other two durations of heating. This may be due to the reason that flavour and mouthfeel were not fully developed at 30 minutes of heating.

Table1 Effect of processing variables on the sensory acceptance and yield of *malai*

Processing variable	Colour and appearance score	Mouthfeel score	Flavour score	Overall acceptability score
Temperature of heating of milk				
70°C	7.96±0.05 ^a	8.02±0.13 ^a	7.87±0.25 ^a	7.75±0.05 ^a
80°C	7.96±0.57 ^a	8.16±0.12 ^a	8.06±0.05 ^a	8.16±0.12 ^a
90°C	8.00±0.00 ^a	8.18±0.07 ^a	8.30±0.17 ^a	8.33±0.67 ^b
Duration of heating of milk				
30 min	8.00±0.00 ^a	8.18±0.07 ^a	8.30±0.17 ^a	8.33±0.06 ^a
45 min	8.03±0.01 ^b	8.39±0.10 ^b	8.51±0.16 ^b	8.42±0.13 ^a
50 min	8.06±0.01 ^b	8.57±0.15 ^c	8.58±0.16 ^b	8.57±0.13 ^b
Temperature of cooling of milk				
30°C	8.00±0.00 ^b	8.18±0.07 ^b	8.30±0.17 ^b	8.33±0.06 ^b
40°C	7.84±0.05 ^a	7.69±0.17 ^a	7.97±0.06 ^a	7.84±0.15 ^a
50°C	7.81±0.07 ^a	7.57±0.28 ^a	7.85±0.03 ^a	7.69±0.03 ^a

Note: Results are expressed as Mean ± S.D; values with different superscripts in a column within a processing variable are significantly different ($p < 0.05$)

Whey proteins are completely denatured only by heating their solutions at 90°C for 10 min (Fox, 2003). When globular proteins are heated, they unfold completely to random coil conformation or to a conformation close to random coil. The unfolded conformation exposes side chain groups that were buried in the native structure, and the process thus causes an increased reactivity of such groups. Especially important is the increased reactivity of thiol groups of cysteine, resulting in cooked flavour of *malai*. In addition, the unfolded protein is more susceptible to protein to protein interaction via calcium bridging and hydrophobic bonding (Paulsson and Dejmek, 1990). It is logical to expect that these reactions are involved in *malai* formation. The insoluble whey proteins, which are smooth by nature, might have also enhanced the mouthfeel of *malai*.

Temperature of cooling of milk

The colour and appearance, mouthfeel, flavour and overall acceptability scores of *malai* prepared by cooling the heated milk to 30°C was significantly ($p<0.05$) higher compared to those of *malai* prepared by cooling the milk to 40°C and 50°C (Table 1). The sensory attributes like mouthfeel and flavour were more notably perceived at 30°C than at other two temperatures. It can be noted that the mouthfeel score at 40°C was statistically similar to that of mouthfeel score at 50°C. Hence, it can be inferred that at this temperature the 'mouthfeel' was in the developing stage and it was significantly developed at 30°C. This is attributed to more solidification of fat at 30°C than at other temperatures. It is well known that the solidification and crystallization of fat increases when the temperature is reduced to below melting point (Jennes and Patton, 1959a). Hence, at lower the temperatures, better mouthfeel may be obtained. However, below 30°C was not tried, because it requires refrigeration.

Effect of processing variables on textural attributes of *malai*

It is evident from Table 2 that as the temperature of heating increased, the firmness value of *malai* also increased. *Malai* had significantly ($p<0.05$) higher firmness when it was prepared with 90°C as temperature of heating compared to other temperatures. This may be due to more evaporation of moisture at 90°C thereby more concentrated layers of product were formed. The consistency was significantly ($p<0.05$) higher for *malai* prepared at 80°C. *Malai* prepared at 90°C had the least consistency value compared to consistency of *malai* prepared at other two temperatures. This is probably because of more air incorporation during coagulation and skin formation.

The firmness of *malai* prepared by heating for 50 minutes was significantly ($p<0.05$) higher when compared to other two durations of heating. It can be implied that as the duration of heating increased more moisture evaporated and because of which the firmness of *malai* layers also increased. The same trend can be observed in the case of consistency also; as the durations of heating increased the consistency of *malai* prepared also increased. The consistency was significantly ($p<0.05$) higher when milk was heated for 50 minutes. This is because heat denaturation leads to a complex mixture of whey protein aggregates and whey protein coated casein micelles (de Kruif and Vasilbinder, 2003).

It is evident that the firmness value of *malai* increased as the temperature of cooling also increased. It was observed that the firmness of *malai* separated at 50°C was significantly ($p<0.05$) higher than that of *malai* separated at other two temperatures (Table 2). This may be due to the less amount of moisture in the *malai* layers at this temperature. The *malai* separated at 50°C had the significantly higher ($p<0.05$) consistency compared to other two temperatures. At 30°C, solidification of fat accumulated

Table 2 Effect of processing variables on textural attributes of *malai*

Processing variable	Firmness, N	Consistency, N s
Temperature of heating milk		
70°C	0.80± 0.00 ^a	5.63± 0.05 ^a
80°C	0.88± 0.03 ^b	6.45± 0.25 ^b
90°C	0.96± 0.05 ^c	5.47± 0.20 ^a
Duration of heating of milk		
30 min	0.96± 0.05 ^a	5.47± 0.2 ^a
45 min	1.56± 0.1 ^b	8.52± 0.69 ^b
50 min	1.76± 0.05 ^c	9.48± 0.38 ^c
Temperature of cooling of milk		
30°C	0.96± 0.05 ^a	5.47± 0.20 ^a
40°C	1.39± 0.15 ^b	7.56± 0.54 ^b
50°C	1.67± 0.04 ^c	9.45± 0.58 ^c

Note: Results are expressed as Mean ± S.D; values with different superscripts in a column within a processing variable are significantly different ($p<0.05$)

at the surface layer is higher and in this solidified fat some of the denatured proteins also were encompassed. But it was observed that even though firmness and consistency of *malai* were higher at 50°C of cooling, it was difficult to separate *malai* at this temperature. The *malai* layer is easily cracked or torn apart when separated at this temperature. *Malai* layer can be separated without any difficulties at 30°C of cooling. It may be due to the fact that at 30°C most of the fat present in the *malai* layer would be in solid state so the layer did not crack or tear.

Factors affecting yield of *malai*

Temperature of heating milk

It was observed that maximum yield was obtained at 90°C and least yield was obtained at 70°C of heat treatment (Fig. 1a). The yield values obtained at different temperatures of heating milk were significantly different ($p < 0.05$). At 90°C of heating, 49.5g of *malai* was obtained and it was reduced to 35.8g when temperature of heating was reduced to 80°C. The yield was reduced to 31.5 g when *malai* was prepared by heating milk to 70°C. This can be explained by the observation of Short (1956) that milk when heated at 90- 95°C, its density decreased due to denaturation of soluble protein. Further, this decrease in density enabled unobstructed rising and accumulation of fat at the surface which in turn led to the higher yield at 90°C.

Duration of heating milk

Maximum yield of *malai* was obtained at 50 minutes duration of heating and least yield was obtained at 30 minutes duration of heating (Fig. 1b). There were significant differences ($p < 0.05$) in the yield obtained during different durations of heating milk. When milk was heated at 90°C for 50 min the average yield of *malai* obtained was 71.5g. The average yield obtained when heated for 45 min was 62.1g. The yield obtained was reduced to 49.5g when the duration of heating was reduced to 30 minutes. Qian et al. (2017) reported that the denaturation of whey protein increased as the duration of heating is increased at 85 to 95°C. Further, on heating of milk, calcium from soluble state is transferred to colloidal state (Anema, 2009), thus into *malai*. This might have contributed to the firm body of *malai*, formed at high heat treated milk.

Temperature of cooling of milk

When the temperature of cooling the milk was 30°C, 49.5g of *malai* was obtained, and the yield was reduced to 43.9g when the temperature of cooling of milk increased to 40°C (Fig. 1c). The least yield of 41.3g was achieved at 50°C. Significant difference ($p < 0.05$) was observed in the yield obtained at different temperatures of cooling. The fat during solidification also encompassed some of the denatured proteins, thus enhancing the yield. The melting point of milk fat is 37°C and as it is cooled

progressive solidification and crystallization of fat would occur (Jennes and Patton, 1959a), facilitating *malai* formation.

Fat and SNF content of milk

It was observed that the *malai* yield increased as the fat content in the milk increased. There were significant differences ($p < 0.05$) in the yield obtained at the three fat levels. Maximum yield 77.43g was obtained when *malai* was prepared from milk containing 6% fat (Fig. 1d). The yield reduced to 74.83g when the milk fat content was reduced to 4.5 % and least yield of 71.53g was obtained when milk containing 3% fat was used. The reason for higher yield of *malai* may be attributed to more amount of fat accumulated at the surface because of less density of fat. According to Jennes and Patton (1959b), as the fat content increased there was a subsequent decrease in the specific gravity of the milk skin (Jennes and Patton, 1959b).

The yield of *malai* increased as the SNF content of milk increased. The *malai* yield obtained was higher from milk with 9% SNF (72.15g) compared to milk containing 8.5% SNF (71.53g). However, this difference was statistically not significant ($p > 0.05$). This is because the *malai* collection at the surface of milk is mainly because of fat content rather than proteins.

Surface area of milk

It was observed that as the exposed surface area increased, the yield of *malai* obtained also increased. The maximum yield of 61.8 g was obtained at exposed surface area of 706.86 cm² which was significantly higher than ($P < 0.05$) the yield obtained at other two surface areas (Fig. 1e). The yield was reduced to 51.36g when the surface area was reduced to 490.87cm². The least yield of 33.53g was obtained when surface area was reduced to 346.36 cm². This is because as contact surface increases, more accumulation of *malai* occurred, thus yield increased.

Compositional analysis of *malai*

The composition of *malai* prepared by optimised parameters viz. heating toned milk at 90°C for 50 min and allowing spontaneous cooling to 30°C for *malai* formation was analysed. The total solid content of such *malai* was 49.64%. As per the study conducted by Dozet and Stanisic (1983) on *clotted cream*, the total solids content was higher. The fat content of *malai* was found to be 38.16%. As per Sadlep (1917) the fat content in *clotted cream* and *kaymak* (Cakmakci and Hayaloglu, 2011) was higher than that of *malai*. The variation in the fat content of *malai* when compared to *clotted cream* and *kaymak* is due to the difference in the production process and also due to the regional variation in the fat content of milk used. Protein content in the *malai* was found to be 9.011%. The high protein content in *malai* is due to the prolonged heating process. Similar observation was reported by Dozet and Stanisic (1983) and Sadlep (1917) in case of *clotted cream*. The lactose content of *malai* was found to be 1.24% and

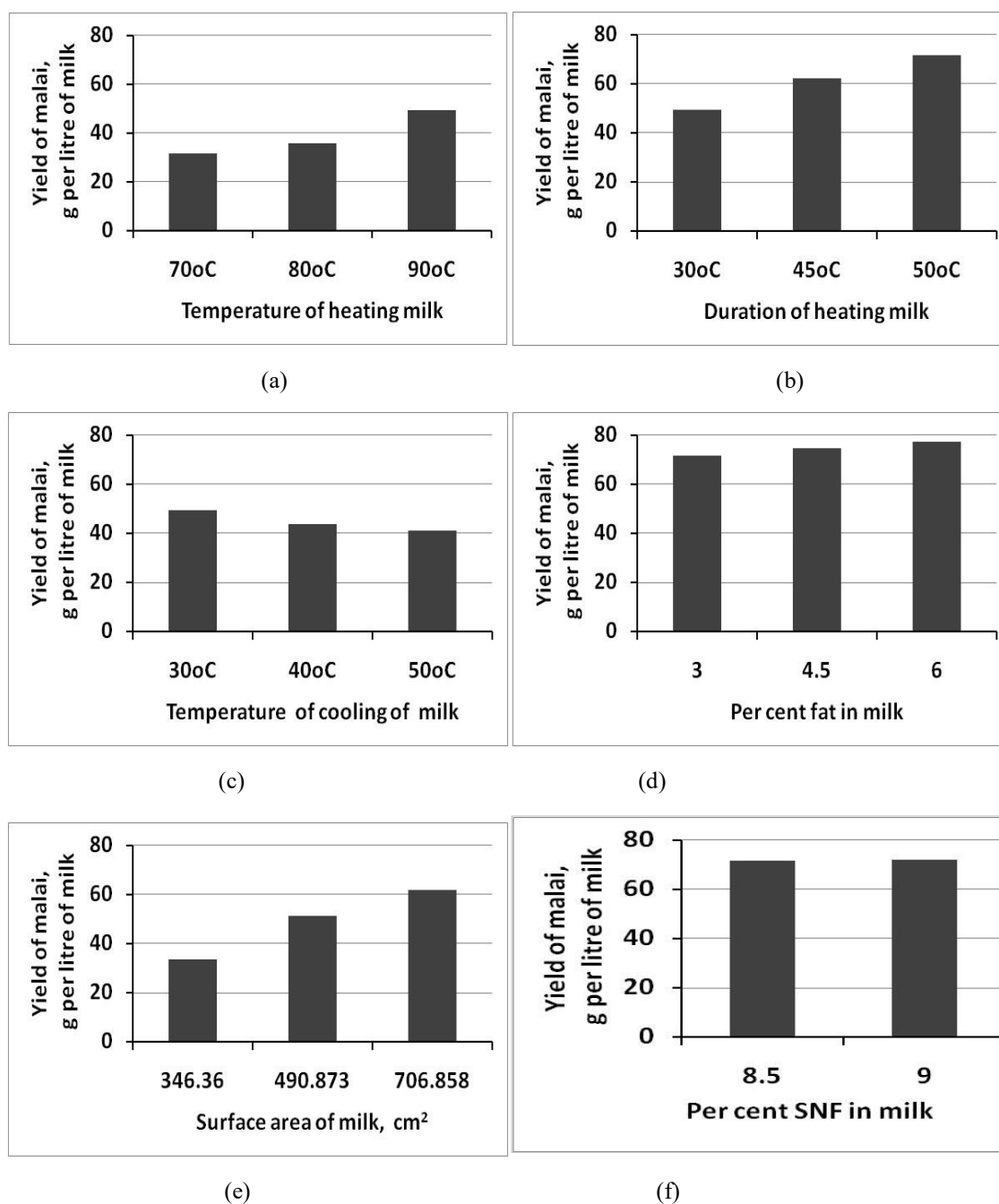


Fig.1 Effect of various factors on yield of *malai* (a) temperature of heating of milk (b) duration of heating of milk (c) temperature of cooling of milk (d) per cent fat in milk (e) surface area of milk and (f) per cent SNF in milk

ash content also 1.24%. Dozet and Stanisic (1983) reported similar values for clotted cream and Cakmakci and Hayaloglu (2011) for kaymak.

Effect of storage period and packaging materials on the quality of *malai*

Sensory attributes

In this study, two different packaging containers, glass and polypropylene, were used. *Malai* was prepared as per the optimised production procedure and was packed in these two different containers and was stored at $5 \pm 1^\circ\text{C}$.

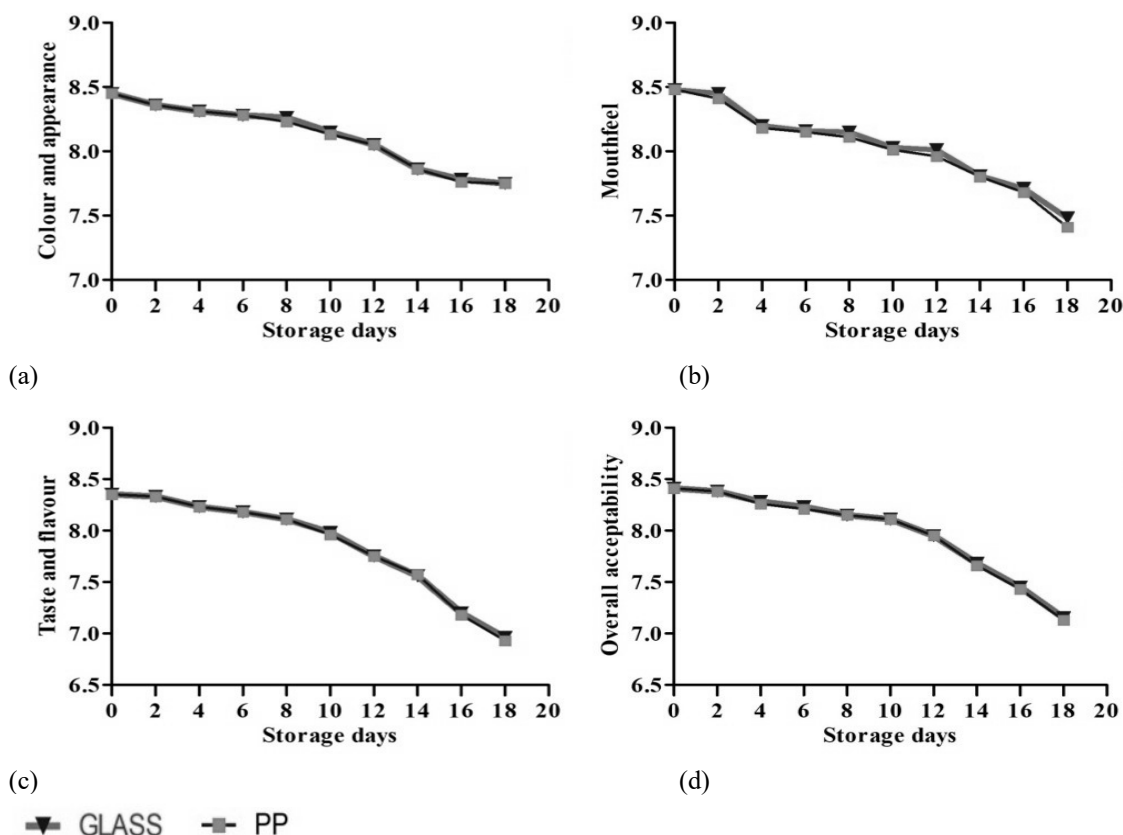


Fig.2 Changes in sensory properties of *malai* during storage at 5°C (a) colour and appearance (b) mouthfeel (c) taste and flavour and (d) overall acceptability

The initial colour and appearance score of *malai* was 8.45. There was significant ($p < 0.05$) decrease in the colour and appearance score during the storage period. The least score was obtained during the 18th day, in both glass and polypropylene container and the score was 7.75 (Fig.2a). There was no significant ($p > 0.05$) difference in the colour and appearance score of *malai* between the packaging materials. The reduction in colour and appearance score during the storage period can be attributed to the change in appearance of the product due to the increase in acidity and release of free fat from the product which made the product look duller. Regarding mouthfeel, fresh product had an average score of 8.48 which gradually, but significantly ($p < 0.05$) declined during the storage period. The least score was observed during the 18th day (Fig.2b). The mouthfeel score on the 18th day was 7.48 and 7.41 in glass and polypropylene containers, respectively. The decline in the mouthfeel score of *malai* during the storage period may be due to the loss of granular and grainy texture due to the increase in acidity which had influence on the texture of *malai*. As for flavour, the fresh product was liked very much by the judges on the 0th day the average score being 8.35 and it was reduced to 6.96 in glass container and 6.93 in polypropylene container (Fig.2c). There was significant ($p < 0.05$) reduction in the flavour scores over the storage period in both the packaging

materials, which is because the samples gradually turned sour and lost freshness. All these changes reflected in overall acceptability scores as well.

The initial mean overall acceptability score of *malai* during the 0th day was 8.41. It was reduced to 7.15 and 7.13 in glass container and polypropylene on the 18th day (Fig.2d). It was observed that there was no significant ($p > 0.05$) difference in the overall acceptability scores between the packaging materials. The rate of decline of the overall acceptability was gradual and similar in both the packaging materials over the storage period. The reduction in the overall acceptability score of *malai* can be attributed to the reduction of freshness and pleasant aroma, loss of grainy texture and reduction in flavour due to acidity development and release of free fat. As a result of all these changes, overall acceptability scores decreased and the product was not in a consumable stage on the 20th day.

The observations recorded for changes in *malai* are commonly observed in any food product because during storage, the food products undergo physico-chemical changes which significantly influence sensory acceptance. These changes occur faster in high a_w products and slower in low a_w products. *Malai* may be

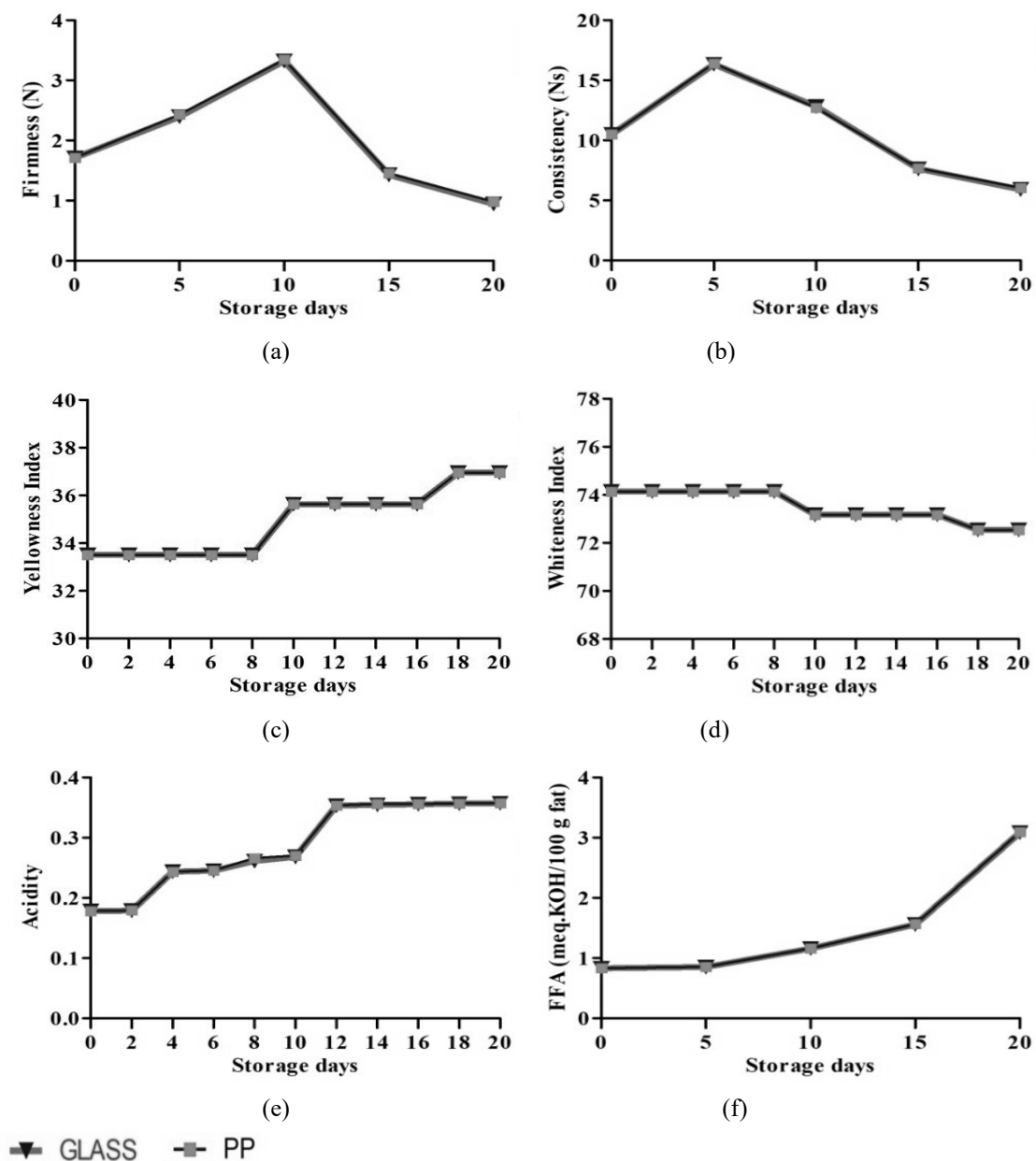


Fig.3 Changes in physico-chemical properties of *malai* during storage at 5°C (a) firmness (b) consistency (c) yellowness index (d) whiteness (e) acidity and (f) free fatty acids (FFA)

classified as high moisture product which showed faster physico-chemical changes like softening, stickiness, and free fat release. These changes adversely affected sensory acceptance during storage. The sensory acceptance gradually declined, and ultimately, after 18th day it was no longer acceptable. The decline in sensory score may be attributed to microbial growth. Firstly, lactic acid bacteria multiplied and developed acidity using lactose, that is why *malai* turned gradually sour and rancid during storage. The enhanced acidity resulted in concomitant changes in mouthfeel and appearance. These were recorded in products which are similar to *malai*, like *basundi*, *rabri* and *khurchan*

(Parmar et al.2018; Gayen and Pal 1991; Yadav2016). These products have similarity with *malai* as far as heat coagulation is concerned, but they contained added sugar which was absent in *malai*.

Physico-chemical attributes

The initial mean of firmness of *malai* on 0th day was 1.71N which increased to 2.4 and 2.43N on 5th day in glass and polypropylene

container, respectively (Fig.3a). The maximum firmness was seen in *malai* stored at both the packaging material on the 10th day. The firmness value was 3.32N in glass container during 10th day and 3.35N in polypropylene container. From the 15th day onwards decline in the firmness value was observed, firmness value being 0.95 N in glass container and 0.98 N in polypropylene container on the 20th day. The rate of increase in firmness value from 0 to 10th day was gradual and decline in the firmness value from 10 to 20th day also followed a similar trend. There were no significant differences ($p>0.05$) in firmness value between the packaging materials. On the 0th day the mean value of consistency of *malai* was 10.49Ns. There was significant ($p<0.05$) increase in consistency of *malai* during the 5th day of storage (Fig.3b). The value increased to 16.33Ns and 16.42Ns in glass and polypropylene container, respectively. From the 10th day onwards there was a decline in the consistency value; in glass container it got reduced from 12.80Ns to 5.91Ns on the 20th day and in polypropylene container it was reduced from 12.69Ns to 6.04Ns. These changes in firmness and consistency can be attributed to the acidity development in the product and continued changes in the proteins as a result of heat coagulation. There was no significant ($p>0.05$) difference in the consistency value of *malai* packed in glass and polypropylene during the storage period. Apart from the textural changes, colour variations were also observed during storage.

Colour of *malai* is important because of consumer appeal. It should look fresh and rich without any paleness. Generally, it is rich yellowish in appearance because of concentration of fat. Yellowness is because of natural colour imparted by use of cow milk whereas buffalo milk *malai* is expected to be white in colour. Hence, both yellowness index and whiteness index have been measured. The initial mean yellowness index at the 0th day was 33.50. No change in yellowness index was observed in both the packaging materials up to 8th day (Fig.3c). Yellowness index significantly ($p<0.05$) increased to 35.63 on the 10th day and it remained same from 10 to 16th day. The maximum value of yellowness index (36.957) was observed on 18th day in both the packaging materials. In fresh *malai*, light penetrates the superficial layers and get scattered by fat globules and moisture droplets imparting white appearance to surface of *malai*. But yellowness index increased during storage, because hydration of proteins increased during storage, thereby causing decrease in number of free moisture droplets and thus reduced degree of light scattering exposing the natural yellow colour of fat globules. Between both the packaging materials, there were no significant ($p>0.05$) differences. Whiteness index (WI) of *malai* was also measured during storage, and its changes followed a similar trend as yellowness index, but in an opposite way (Fig.3d). This is on expected lines because whiteness is a measure of total reflected light; as yellowness increases, the total reflected light reduced. Similar significant changes in yellowness index were observed in *basundi* due to increase in the formation of HMF compounds by Maillard reactions (Parmar et al. 2018). However, since *malai* was

prepared from cow milk the product also got yellow colour due to the carotenoids present in cow milk, and carotenoids are fat soluble pigments. Singh et al. (2018) reported WI values of more than 80 for rice kheer made from buffalo milk, and Indrasinh et al. (2014) reported L* values of 74 – 80 for dietetic peda, and for sandesh the values were up to 78 (Nalwade et al 2014). For yoghurt, WI values up to 76 were recorded (Akeem et al. 2018). In the present study, WI up to 74 was observed indicating that the WI values were comparable with other dairy products reported as above. But, for almond milk, WI was 89.1 (Bernat Pérez et al. 2015) nearing to the value of cow milk i.e. 91 reported by Teichert et al. (2020).

On the 0th day, the acidity of *malai* was 0.17% LA. There was gradual significant ($p<0.05$) increase in the acidity as the storage period progressed. There was gradual increase in the acidity of product from 4th day to 10th day (Fig.3e). The maximum acidity was observed during the 20th day. The acidity was 0.35% in both glass and polypropylene container, respectively. Liquid milk has 4.5% lactose and *malai*, has 1.24% lactose. Lactic acid bacteria can grow utilising lactose producing lactic acid. This has led to the increase in acidity of *malai* during storage. Though fat rich products like cream and butter are acceptable as sour products, sour *malai*, is not accepted for consumption. Generally, *malai* is consumed before it turns sour and bitter.

Since *malai* is rich in fat content in comparison to liquid milk, fat break down is certain to take place. Break down of fat can be measured by measuring fat break down products like free fatty acids. The increase in FFA signifies increasing fat break down which can be attributed to growth of microorganisms as well as fat hydrolysis by enzymes (Deeth et al. 1979). The initial mean FFA content of *malai* was 0.833 meq.KOH/100 g fat. There was slight increase in the FFA content of *malai* stored in both the packaging materials during 5th day. The lipolytic activity was seen increasing on the 10th day also (Fig.3f). Peak value of FFA content was observed on the 20th day. In *malai* stored in glass container, the FFA content was 3.085 meq.KOH/100 g fat and in polypropylene container the FFA content was 3.092 meq.KOH/100 g fat on 20th day of storage. The rate of increase up to 15th day was gradual. However, from the 15th day to 20th day the rate was more. This can be attributed to the increase in the yeast and mould of the product during that period. No significant ($p>0.05$) difference was observed between the packaging materials. In fat rich dairy products like butter, lipolysis is a common spoilage reaction occurring during storage that is measured by measuring free fatty acids (Raza et al 2009). Though the free fatty acids content is in small amounts, they can contribute significantly to the flavour as their threshold value is low (Belitz et al. 2009).

Microbial attributes

On the 0th day, standard plate count was 1.079 log cfu/g. This is a very low count because the product has been obtained from

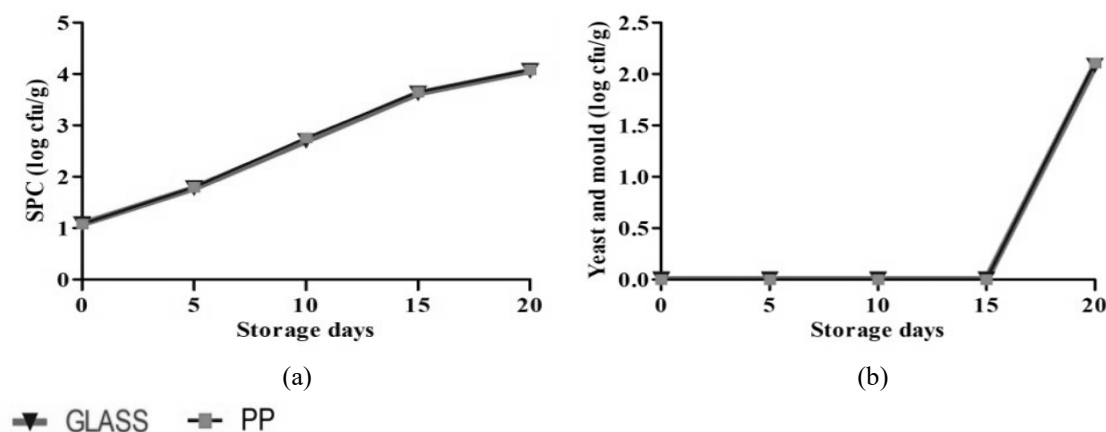


Fig.4 Changes in microbial count of *malai* during storage at 5°C (a) SPC and (b) yeast and mold count

heat treated milk. However, the standard plate count gradually increased during the storage period which can be attributed to the growth of surviving organisms as well as contaminants gained entry during cooling and packaging steps (post production contamination). On the 20th day the standard plate count of *malai* stored in glass container was 4.06 log cfu/g and in sample stored in polypropylene container the count was 4.08 log cfu/g (Fig. 4a). Similar observations in *rabri* were reported by Gayen and Pal (1991); in the fresh product, the total viable count was 34,800 per g which increased to 67400 per g within 18 hours when product was stored at 30°C. In case of product stored at 5°C the counts remained relatively same up to 20 days. After 20 days of storage, total viable count was 38600/g. Parmar et al. (2018) reported that the initial total plate count of *basundi* samples stored at 4±1°C increased significantly from 3.8 log CFU/g to 5.10 log CFU/g at the 16th day. A rapid increase in total plate count was observed after 4th day of storage. In *malai* samples, initially during the 0th day, there was no yeast mould count observed in the product. From the 0th day up to 15th day also no yeast and mould count was observed in the sample. Growth was observed on the sample during the 20th day in both the packaging materials. The count was 2.08 log cfu/g and 2.10 log cfu/g in glass container and polypropylene container, respectively (Fig. 4b).

In case of *basundi* samples significant rise in yeast and mould count was reported by Parmar et al. (2018). Similar observation was reported by Gayen and Pal (1991) in case of *rabri*, in which the initial yeast and mould count was 70/g. The count increased to 5 times to a count of 320 per g within 18 hours at 30°C storage. In case of product stored at 5°C the counts remained relatively same as initial counts up to 20 days. The count increased to 100/g after storage of 20 days. Yadav (2016) has reported significant increase in yeast and mould count of *khurchan* samples during the storage period. In case of the yeast and mould count, initially for the fresh sample it was 41.67/ g and it got multiplied three

times to 140.67/g at the end of 12th day. The growth of organisms in *malai* is because of the presence of nutrients in it, however the growth rate of organisms is not as prolific as in liquid milk and similar to other products such as *rabri* and *basundi*. This is because *malai* is fat rich portion of milk, rich in fat and less in other nutrients. Further, the product turned not only acidic, but also bitter as the storage progressed because of breakdown of fats by microorganisms as in case of fat rich products like cream and butter (Alvarez, 2016).

Conclusion

The production process of *malai* was standardised on the basis of processing variables like temperature of heating milk, duration of heating milk and temperature of cooling the milk. In the standardised process, the temperature of heating milk was 90°C, duration of heating was 50 minutes and temperature of cooling was 30°C. The sensory scores obtained were also highest at these parameters. Yields depending on fat and SNF of milk and surface area, ranged from 61.8 – 77.43 g per litre of milk. As the fat content of milk increased, the yield of *malai* also increased. However, the effect of SNF of content of milk was not significant. The yield obtained also increased as the surface area of the milk exposed increased. Optimised product had a shelf life of 18 days in glass and polypropylene containers at 5±1°C. These results will be helpful for commercial production of *malai* by organised sector.

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RESEARCH ARTICLE

Evaluation of short chain fatty acids production by potent *Lactobacillus* cultures: an *in vitro* study

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Abstract: Production of short chain fatty acids (SCFAs) is one of the mechanisms thought to be involved in cure of many GI tract disorders. Present study was undertaken to evaluate the probiotics for its SCFAs production capacity. In the study, we selected several potential probiotics cultures and planned this project to screen the Lactic Acid Bacteria (LAB) based on short-chain fatty acids (SCFAs) production mainly Butyric acid, Propionic acid and Acetic acid at different time intervals (0, 12 and 24 h) of incubation during fermentation in reconstituted skim milk (RSM) media (*in vitro study*). SCFAs (acetate, propionate, butyrate) production ability was checked by *Lactobacillus* cultures. The average content of acetic acid was 1.20 µg/ml at 0 h, which increased to 1.53 and 1.93 µg/ml, respectively after 12 and 24 h of incubation. Cultures M5 and NK9 showed relatively higher amount of acetic acid after 24 h. Overall propionic acid production ranged from 1.44 µg/ml (M10 at 0 h) to 3.42 µg/ml (V3 at 24 h). M5 and M31 produced significantly higher propionic acid than the rest of the cultures. It was observed that butyric acid produced by the cultures M5 (2.09 µg/ml) was highest followed by 13 (2.08 µg/ml) and M31 (2 µg/ml). Out of ten *Lactobacillus* cultures, five cultures viz. V3 (*L. helveticus*), M5 (*L. fermentum*), NK9 (*L. casei*), M31 (*L. rhamnosus*) and 13 (*L. acidophilus*) were selected on the basis of higher SCFAs production for further phase of study.

Keywords: Acetic acid, Butyric acid, Lactic acid bacteria, Probiotics, Propionic acid

Introduction

In 2008, prebiotics were defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as “a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (Hill et al. 2014), a definition that is currently being further revised by ISAPP. Prebiotics are fermented by the gastrointestinal microbiota and contribute to healthy modulation of the gut (Di Bartolomeo et al. 2013). Synbiotics are a relatively new area that involve a combination of probiotic and prebiotic in one product; the prebiotic is intended to improve the survival/growth/performance of the probiotic or other beneficial bacteria in the colon, which in turn has beneficial health effects on the host (Pranckute et al. 2014). Functional foods are foods or dietary components that can provide a health benefit beyond basic nutrition. The use of the functional oligosaccharides in diet is a part of the management of dyslipidemia (Perry et al. 2007). Optimal intake of the functional oligosaccharides reduces the risk of obesity, blood pressure and many other cardiovascular diseases. Fibers lead to high amounts of SCFAs, lower the pH in the colon, which in turn affects the composition of the colonic microbiota and thereby the SCFA production.

Probiotics and prebiotics have been used for several health benefits and are acting as supplementary therapy in many ailments. Short-chain fatty acids (SCFAs), the end products of fermentation of dietary fibers by the anaerobic intestinal microbiota, have been shown to exert multiple beneficial effects on mammalian energy metabolism. SCFAs are saturated aliphatic organic acids that consist of one to six carbons of which acetate (C₂), propionate (C₃), and butyrate (C₄) are the most abundant (c. 95%) (Cook and Sellin, 1998). Acetate, propionate, and butyrate are present in an approximate molar ratio of 60:20:20 in the colon and stool (Hijova and Chmelarova, 2007). Depending on the diet, the total concentration of SCFAs decreases from 70 to 140 mM in the proximal colon to 20 to 70 mM in the distal colon (Topping and Clifton, 2001). The

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acetate:propionate:butyrate ratio in humans was similar in the proximal and distal regions of the large intestine (Cummings et al. 1987). In the cecum and large intestine, 95% of the produced SCFAs are rapidly absorbed by the colonocytes while the remaining 5% are excreted in the feces (Topping and Clifton, 2001). Several *in vitro* studies as well as experiments in different laboratory and production animals have demonstrated the impact of SCFAs on mammalian physiology. In addition, it has become evident that each of the individual SCFAs affects health differently (Wolever et al. 1991).

SCFA enhance mucosal barrier function, reduce the levels of pro-inflammatory mediators and stimulate the production of immunosuppressive cytokines (Wasilewski et al. 2015). Lipolysis in adipose tissue is strongly reduced by SCFAs (Lin et al. 2012). Some of the beneficial metabolic effects induced by propionate and butyrate are mediated by de-novo-synthesized glucose from the gut epithelium, which is sensed in the portal vein and signals through a gut-brain neural circuit to increase insulin sensitivity and glucose tolerance (Vadde et al. 2014). acetate can cross the blood-brain barrier and reduce appetite via a central homeostatic mechanism (Frost et al. 2014). SCFAs, especially acetic, propionic and butyric acid, are vital to maintain the normal function of intestine and human body. Acetate is absorbed and transported to the liver and peripheral tissues, less metabolized in the colon and acts as substrate for cholesterol synthesis and lipogenesis (Zambell et al. 2003). Propionate is a primary precursor for gluconeogenesis and it reduces the synthesis of hepatic cholesterol (Cheng and Lai, 2000). Butyrate is considered one of the most important colon metabolites, as it serves as the majorly preferred energy source for the colonocytes, has anti-inflammatory properties and regulates gene expression, differentiation, cellular proliferation and apoptosis in host cells, resulting in reduced risk of colon cancer (Hamer et al. 2008; Canani et al. 2011).

Thus, owing to the benefits received due to SCFAs, this study was aimed to evaluate the production of SCFAs by potent lactic acid bacteria.

Materials and Methods

Preparation of Standard Solutions and Plotting of Calibration Curve

The stock standard solution of Acetic acid, Propionic acid and Butyric acid (5 M) were prepared by dissolving 30.02, 37.04 and 44.05 ml of each respectively in 100 ml HPLC grade water. Desired concentrations of acid were prepared from the stock solution. Five-point calibration curves were prepared by injecting 0.5 M, 1 M, 1.5 M, 2 M and 2.5 M for each individual acid. Calibration curves were then drawn by plotting concentration against peak area and correlation coefficients (R^2) were determined. Linear

regression equation was determined for acetate, propionate and butyrate standards to evaluate the linearity of the system.

Determination of pH, Acidity and *Lactobacillus* Counts of Fermented Skim Milk

All the cultures were activated by growing in sterilized skim milk. The activated cultures were added to 100 ml of skim milk flasks at the rate of 2%. After mixing them thoroughly, the culture tubes were incubated at 37 °C and at different intervals of 12 and 24 h-samples were taken out for determination of pH, titratable acidity and viable counts.

Determination of pH

pH of fermented skim milk samples was measured using digital pH meter (OAKTON pH700, India). Well mixed 10 ml of fermented skim milk sample was put into a beaker and then pH was measured by immersing the pH meter electrode into the milk sample. Standard buffer solution of pH 4, 7 and 9 were used to calibrate the pH meter before analysis.

Determination of titratable acidity

The titratable acidity was estimated by the procedure described in (AOAC, 1995). Ten ml sample was taken after each interval of 12 and 24 h into porcelain dish and an equal volume of lukewarm distilled water was added to it. Then 1.0 ml of phenolphthalein indicator was added and the contents of dish were titrated against 0.1 [N] NaOH till the appearance of light pink colour, which persisted for 30 seconds in the solution. Titratable acidity was calculated by the following formula:

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

Where, V = Volume (ml) of 0.1 [N] NaOH required for the titration

N = Normality of NaOH solution, and

X = Volume of milk (ml) taken for the titration

Determination of Lactobacilli counts

Lactobacilli counts of fermented skim milk samples were determined as per the method described by IDF standards (146:2003). One ml sample was taken out from the tubes and added to 9 ml phosphate buffer tubes. Similarly, required number of serial dilutions were prepared. One ml diluted sample from appropriate diluted tubes was transferred to labelled petri plates (performed in duplicates). Then 15-20 ml of melted and cooled (45 °C) MRS agar was poured -into 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 completely over the solidified medium. Again, -medium was allowed to solidify, then incubated at 37 °C

for 24h in inverted position. Typical colonies were calculated and the counts were expressed as log cfu/ml.

Determination of SCFAs Production Through HPLC

Sample preparation

All the cultures were activated by growing in MRS broth tubes. The activated cultures were added to 100 ml of skim milk flask at the rate of 2%. After mixing them thoroughly, the culture flasks were incubated at 37 °C and at different intervals of 0, 12 and 24 h, samples were taken out for estimating SCFAs production using HPLC.

Estimation of SCFAs

Analysis of SCFAs in the fermented milk was performed by slight modification of the method of Roopashri and Varadaraj (2014). Aliquot of 2 ml of homogenized fermented milk sample was taken in a 15 ml centrifuge tube, and 7 ml of 10 mM NaOH containing 0.1 mM crotonic acid was added. The mixture was kept in a shaker incubator for 6 h at 30 °C. One ml chloroform was then mixed with the sample to remove fat soluble substances. This mixture was centrifuged at 10,000 rpm for 15 min at 4 °C in a refrigerated centrifuge. The supernatant was isolated and filtered through 0.22 µm membrane filter. Using Hamilton syringe, 20 microlitre samples were injected in a Shimadzu HPLC model LC-20 (Shimadzu, Japan) to perform the HPLC analysis. An analytical column [C 18] was used for this purpose. 0.1 % (v/v) phosphoric acid isocratic mixture was used as the solvent for elution at 30 °C and at a flow rate of 0.6 ml/min, using an UV detector at 210 nm.

Results and Discussion

Screening of Different Isolates of LAB on the Basis of Short-Chain Fatty Acids (SCFAs) Production

With an objective to screen different cultures of LAB on the basis of short-chain fatty acids production, a total of ten pure *Lactobacillus* cultures were collected from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science,

Anand Agricultural University, Anand. The LAB cultures were propagated in sterilized reconstituted skim milk (11% TS) and stored at 5 ± 1 °C. The transfer was -done every week during the course of the study. The detailed information of all *Lactobacillus* cultures is given in Table 1.

Growth behavior of *Lactobacillus* cultures

The *Lactobacillus* cultures were inoculated at the rate of 2% in sterilized reconstituted skim milk. Then titratable acidity (% Lactic acid), pH and *Lactobacillus* count were estimated after 0, 12, 24 h of incubation and depicted in as well as Fig. 1, 2 and 3, respectively.

During the growth in sterilized reconstituted skim milk, culture V3 showed the highest reduction in pH (4.53), followed by M5 (4.85), NK2 (5.11), M31(5.12), M38 & 13 (5.13), NK10 (5.17), NK9 (5.19), M16 (5.36) and M10 (5.44) after incubation of 24 h at 37 °C.

It was observed from Fig. 5 that the titratable acidity was significantly higher at 24 h of incubation. During the growth in sterilized reconstituted skim milk, V3 produced highest titratable acidity (0.752 % LA), followed by M31 (0.732 %), M5 (0.681 %), NK9 (0.654%), NK10 (0.634 %), M10 (0.567%), M38 (0.566%), 13 (0.556 %), M16 (0.531 %), and NK2 (0.474 %) after 12 h incubation at 37 °C.

Lactobacillus counts (log cfu/ml) of all the cultures were measured at 12 and 24 h of incubation at 37 °C. From the Fig. 6, it was observed that the *Lactobacillus* counts (log cfu/ml) of the cultures significantly ($P < 0.05$) increased up to 24 h of incubation period. Overall *Lactobacillus* counts (log cfu/ml) ranged from 8.12 log cfu/ml (12 h) to 9.706 log cfu/ml after 24 h incubation at 37 °C.

During the growth of *Lactobacillus* cultures in sterilized reconstituted skim milk, V3 showed maximum *Lactobacillus* counts (9.66 log cfu/ml), followed by M5 (9.62), M31 (9.54), 13 (9.53), M38 (9.41), M16 (9.32), NK9 (9.29), NK10 (9.25), NK2 (9.23), and M10 (8.52) after 24 h at 37 °C.

Determination of SCFAs Production through HPLC

Table 1 *Lactobacillus* cultures used for the study

S. No.	Culture Name	Growth Conditions	Gene bank accession no.
1	<i>L. helveticus</i> MTCC 5463 (V3)	37°C/24h	GQ253960
2	<i>L. rhamnosus</i> NK2	37°C/24h	KR080695
3	<i>L. casei</i> NK9	37°C/24h	KR732325
4	<i>L. rhamnosus</i> MTCC 25062 NK10	37°C/24h	KR732326
5	<i>L. fermentum</i> M5	37°C/24h	KU366365
6	<i>L. paracasei</i> M16	25°C/24h	KU366368
7	<i>L. rhamnosus</i> M31	37°C/24h	KU366370
8	<i>L. plantarum</i> M38	37°C/24h	KU366371
9	<i>L. plantarum</i> M10	37°C/24h	KU366366
10	<i>L. acidophilus</i> NCDC 13	37°C/24h	NCDC 13

Fig 1. Changes in pH of Reconstituted Skim Milk at 37 °C by *Lactobacillus* cultures

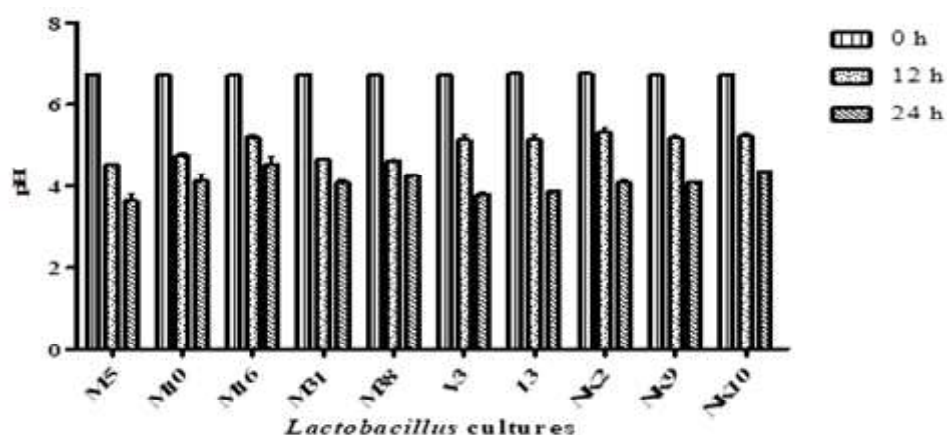


Fig 2. Lactic acid production by *Lactobacillus* cultures incubated at 37 °C in Reconstituted Skim Milk

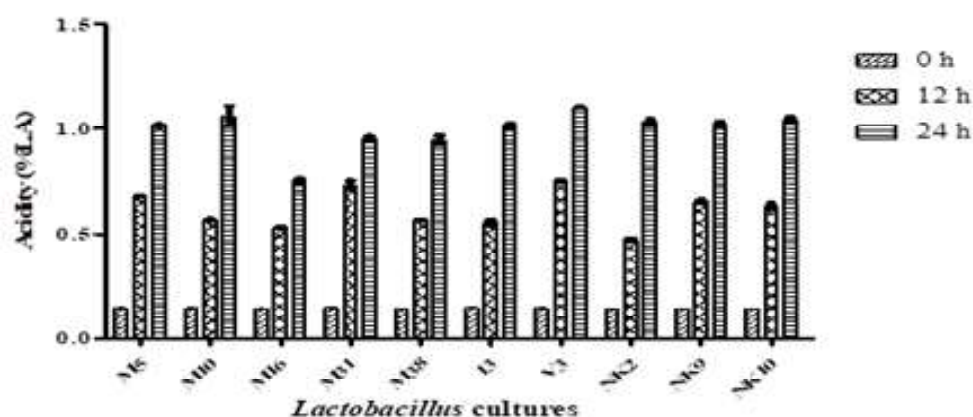
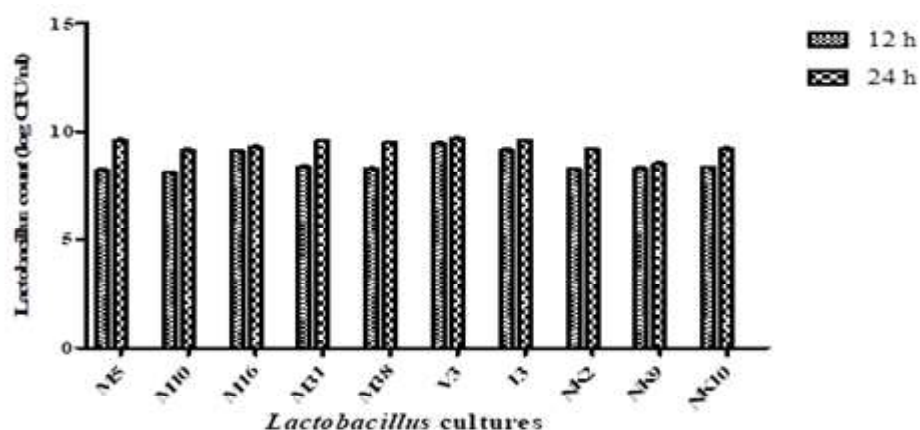
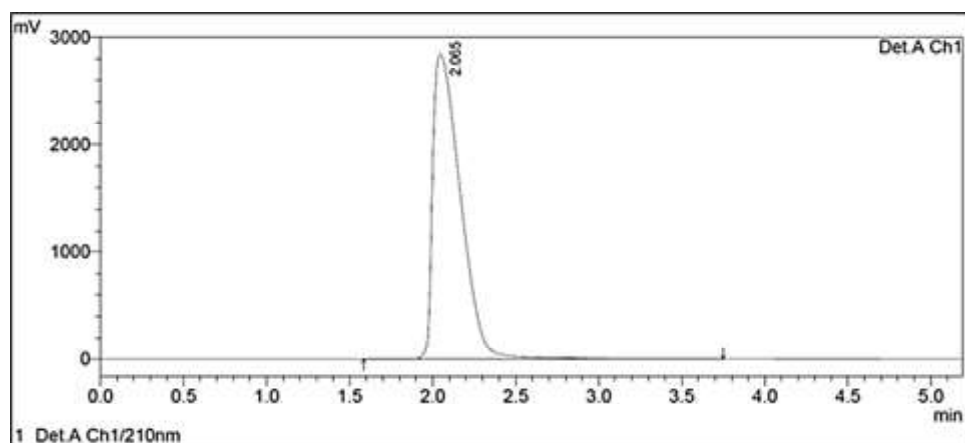
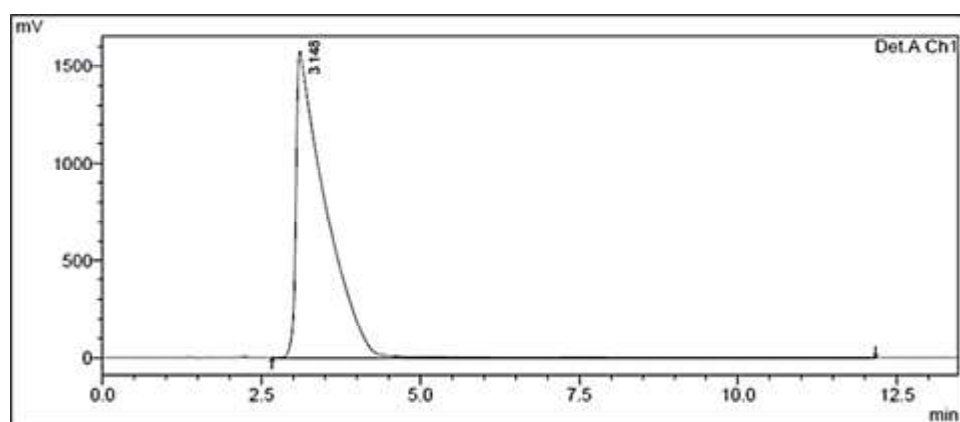
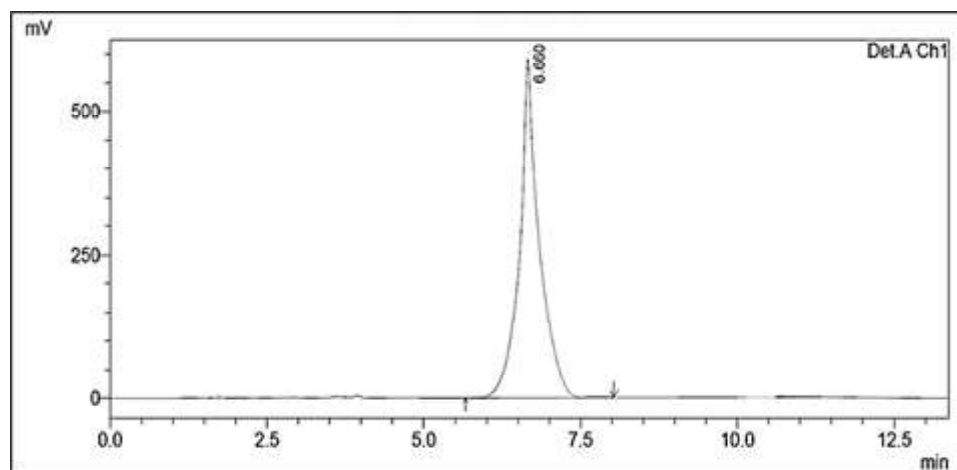


Fig 3. Counts of *Lactobacillus* cultures incubated at 37 °C in Reconstituted Skim Milk



Lactobacillus cultures were inoculated in reconstituted skim milk medium @ 2% and incubated at 37 °C. The samples taken at 0, 12 and 24 h of incubation were subjected to analysis of SCFAs production using HPLC. Individually each *Lactobacillus* culture was statistically analyzed for the production of each of the three SCFAs, namely Acetic acid (AA), Propionic acid (PA) and Butyric acid (BA). SCFAs production by *Lactobacillus* cultures is presented in Table 2, 3 and 4 respectively for AA, PA and BA.

In the current method, the retention times (Rt) were used as the way of identifying target compounds; thus, individual standard acids were prepared and chromatographed separately to determine the retention times, maximize the resolution of peaks and establish the chromatographic conditions. The retention times of SCFAs in chromatographs of the tested samples were matched with retention times of standards in the samples (± 0.05) as described earlier in 2.1. The retention times for standards of acetate, propionate and butyrate were 2.065, 3.148 and 6.660 minutes, respectively (Fig. 4, 5 and 6).

Fig 4. HPLC chromatogram of standard for AA ($R_t=2.065$)**Fig5.** HPLC chromatogram of standard for PA ($R_t=3.148$)**Fig 6.** HPLC chromatogram of standard for BA ($R_t=6.660$)

It was observed from the Table 2, that production of acetic acid increased significantly ($P<0.05$) with the increase in incubation period, irrespective of the type of culture. The average content of acetic acid was $1.20 \mu\text{g/ml}$ at 0 h, which increased to 1.53 and $1.93 \mu\text{g/ml}$, respectively after 12 and 24 h of incubation. Even though, cultures i.e. M5 and NK9 showed relatively higher amount of acetic acid after 24 h, -still it was at par with other cultures.

From Table 3, it was observed that the production of propionic acid was significantly ($P<0.05$) increased up to 24 h of incubation period for all the *Lactobacillus* cultures individually. Overall propionic acid production ranged from $1.44 \pm 0.62 \mu\text{g/ml}$ (M10 at 0 h) to $3.42 \pm 1.23 \mu\text{g/ml}$ (V3 at 24 h). It was also observed that propionic acid produced by the cultures M5 and M31 was significantly higher than the rest of the cultures.

From Table 4, it could be seen that the production of butyric acid significantly ($P<0.05$) increased up to 24 h of incubation period

Table 2 Acetic acid production by different *Lactobacillus* cultures at different incubation periods

<i>Lactobacillus</i> cultures (C)	Incubation Period (P)			Mean
	0 h	12 h	24 h	
	Acetic acid production (µg/ml)			
M5	1.48±0.1	1.78±0.2	2.58±0.1	1.95 ^{bc}
M10	1.23±0.1	1.28±0.1	1.19±0.1	1.23 ^a
M16	1.02±0.002	1.13±0.2	1.5±0.4	2.00 ^c
M31	1.22±0.03	1.5±0.1	2.18±0.07	1.64 ^b
M38	1.02±0.003	1.13±0.2	1.5±0.4	1.22 ^a
NK2	1.19±0.1	1.29±0.3	1.36±0.2	1.28 ^a
NK9	1.33±0.1	2.16±0.2	2.39±0.1	1.96 ^{bc}
NK10	1.25±0.1	1.25±0.03	1.29±0.1	1.26 ^a
V3	1.14±0.1	1.94±0.6	3±0.2	1.95 ^{bc}
I3	1.19±0.03	1.89±0.8	2.32±0.1	1.80 ^{bc}
Mean	1.20 ^a	1.53 ^b	1.93 ^c	
Source	Sem	CD _(0.05)	CV%	
C	0.11	0.32		
P	0.06	0.17	1.618	
C*P	0.19	0.56		

Each observation is a mean ± SD of three replicate experiments (n=3)

Table 3 Propionic acid production by different *Lactobacillus* cultures at different incubation periods

<i>Lactobacillus</i> cultures (C)	Incubation Period (P)			Mean
	0 h	12 h	24 h	
	Propionic acid production (µg/ml)			
M5	1.41±0.2	1.51±0.1	1.67±0.2	1.53 ^c
M10	1.08±0.02	1.16±0.1	1.18±0.1	1.14 ^a
M16	1.10±0.03	1.27±0.1	1.40±0.1	1.26 ^{ab}
M31	1.11±0.03	1.23±0.2	1.67±0.1	1.34 ^b
M38	1.15±0.1	1.18±0.1	1.18±0.1	1.17 ^a
NK2	1.18±0.2	1.19±0.07	1.19±0.04	1.19 ^a
NK9	1.06±0.02	1.15±0.09	1.48±0.1	1.23 ^{ab}
NK10	1.09±0.03	1.18±0.02	1.21±0.1	1.16 ^a
V3	1.07±0.03	1.22±0.1	1.50±0.4	1.27 ^{ab}
I3	1.08±0.6	1.19±0.9	1.37±0.1	1.21 ^{ab}
Mean	1.13 ^c	1.23 ^c	1.40 ^c	
Source	SEm	CD _(0.05)	CV%	
C	0.05	0.14		
P	0.03	0.07	12.1	
C*P	0.08	NS		

Each observation is a mean ± SD at the level of P < 0.05 of three replicate experiments (n=3), values with different superscripts indicate that they are significant at P < 0.05

for all the *Lactobacillus* cultures individually. It was also observed that butyric acid produced by the cultures M5 (2.09 µg/ml), 1 (2.08 µg/ml) and M31 (2 µg/ml) was significantly higher than the rest of the cultures. Culture M38 was found to be poorest (0.99 µg/ml) in terms of butyric acid production.

Hati et al. (2015) studied the growth performance of *Lactobacillus helveticus* MTCC 5463 (V3), *Lactobacillus rhamnosus* (NS4 and NS6), *Enterococcus faecalis* (ND3), *Lactobacillus rhamnosus* (SH8) and *Enterococcus faecalis* (ND11) by determining viable

counts (log cfu/ml) and production of lactic acid measured by decline in pH in skim milk inoculated at the rate of 1 % and incubated at 37 °C for 12 h. It was observed that M5 lowered down the pH at a maximum level compared to other cultures. However, it was observed that 13 produced maximum acidity compared to other cultures. Viable counts of all the cultures were measured after 12 h of incubation at 37 °C. From the study; it was found that V3 gives highest viable cell counts 9.66 log cfu/ml than other cultures. It was also concluded that, pH, acidity and

Table 4 Butyric acid production by different *Lactobacillus* cultures at different incubation periods

<i>Lactobacillus</i> cultures (C)	Incubation Period (P)			Mean
	0 h	12 h	24 h	
		Butyric acid production ($\mu\text{g/ml}$)		
M5	1.79 $\pm$ 0.6	2.04 $\pm$ 0.4	2.43 $\pm$ 0.6	2.09 ^b
M10	1.05 $\pm$ 0.02	1.09 $\pm$ 0.2	1.13 $\pm$ 0.2	1.09 ^a
M16	0.98 $\pm$ 0.7	1.99 $\pm$ 0.8	1.84 $\pm$ 1	1.60 ^{ab}
M31	1.58 $\pm$ 0.4	2.02 $\pm$ 0.3	2.39 $\pm$ 0.10	2.00 ^b
M38	0.89 $\pm$ 0.5	1.01 $\pm$ 0.8	1.07 $\pm$ 0.8	0.99 ^a
NK2	0.68 $\pm$ 0.2	1.15 $\pm$ 1	1.21 $\pm$ 1	1.01 ^a
NK9	1.36 $\pm$ 0.8	1.43 $\pm$ 0.7	1.58 $\pm$ 0.8	1.46 ^{ab}
NK10	1.05 $\pm$ 0.8	1.17 $\pm$ 0.3	1.32 $\pm$ 0.8	1.18 ^a
V3	1.38 $\pm$ 0.7	2.09 $\pm$ 0.4	2.39 $\pm$ 0.03	1.95 ^b
I3	1.94 $\pm$ 0.1	2.11 $\pm$ 0.1	2.19 $\pm$ 0.2	2.08 ^b
Mean	1.27 ^a	1.61 ^b	1.76 ^b	
Source	SEm	CD _(0.05)	CV%	
C	0.21	0.61		
P	0.12	0.33	2.6	
C*P	0.37	NS		

Each observation is a mean $\pm$ SD at the level of $P < 0.05$ of three replicate experiments ($n=3$), values with different superscripts indicate that they are significant at $P < 0.05$.

viable cell counts varies with the type of cultures and period of incubation.

As a function of heat treatment, long-chain fatty acids may be converted into short-chain and medium-chain fatty acids (McSweeney and Fox, 2003). Wang et al. (2006) reported that concentration of short-chain and medium chain fatty acids increased after pasteurization of goat milk. Yang and Choong (2001) tested five fermented milk market samples for acetic acid using GC method and they found that the amount ranged from 1.08 mg/ml to 2.83 mg/ml. Also, they checked for acetic acid in three yogurt market samples and they found 2.46 mg/ml, 6.25 mg/ml and 6.89 mg/ml acetic acid, which is contradictory to our results as they have detected higher amount of acetic acid as compared to our results which may be due to difference in strains used for fermentation of milk. Adhikari et al. (2002) checked for acetic acid content in stirred yogurt made using *Bifidobacterium* ATCC 15708 through HPLC. The amount detected on 0 day (Approx. 6 h) was 6.2 mg/100g, which is almost comparable to our results. Fernandez-garcia & McGregor (1994) tested yogurt concentrated frozen starter culture [consisting of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*, (1:1)] for acetic acid content determination using HPLC and found 61.9 and 63.3 $\mu\text{g/g}$ respectively for two different solvents. Our results differ from them which may be due to use of different strains of starter cultures and growth conditions.

Yang and Choong (2001), evaluated for propionic acid using GC for five fermented milk market samples and they found 0.47 mg/ml in one sample only. Also, they checked for propionic acid in three yogurt market samples and they found 0.06 mg/ml and 0.09 mg/ml propionic acid in two samples. Adhikari et al. (2002)

checked for propionic acid content in stirred yogurt made using *Bifidobacterium* ATCC 15708 through HPLC. The amount detected on 0 day (Approx. 6 h) was 31.8 mg/100g, which is contradictory to our results which may be due to different strain of culture used. Fernandez-garcia & McGregor (1994), tested yogurt (concentrated frozen starter culture [consisting of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*, (1:1)] for propionic acid content determination using HPLC and found 48.8 and 67.8 $\mu\text{g/g}$ respectively for two different solvents. Our results differ from them which may be due to use of different strains of starter cultures and growth conditions.

Khan et al. (2017), reported 3.98, 4.11 and 4.33 g/100g butyrate amount in raw, pasteurized and boiled buffalo milk on 0 day. Yang and Choong (2001), tested for butyric acid using GC method in five fermented milk market samples and three yogurt market samples and they could not detect butyric acid, which is contradictory to our results, which may be due to difference in strains used for fermentation of milk. Adhikari et al. (2002) checked for butyric acid content in stirred yogurt made using *Bifidobacterium* ATCC 15708 through HPLC. The amount detected on 0 day (Approx. 6 h) was 212.2 mg/100g. Fernandez-garcia & McGregor (1994), tested yogurt (concentrated frozen starter culture [consisting of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*, (1:1)] for butyric acid content determination using HPLC and could not detect it. Our results differ from them which may be due to use of different strains of starter cultures.

Conclusions

During the growth in reconstituted skim milk, all the ten cultures grew well and they reduced pH in the range of 4.53 to 5.44, increased acidity in the range of 0.75 to 1.10 % L. A. and the Lactobacilli count varied from 8.52 to 9.66 log CFU/ml. SCFAs (acetate, propionate, butyrate) production ability was checked during reconstituted skim milk (RSM) fermentation by *Lactobacillus* cultures. The average content of acetic acid was 1.20 µg/ml at 0 h, which increased to 1.53 and 1.93 µg/ml, respectively after 12 and 24 h of incubation. Cultures M5 and NK9 showed relatively higher amount of acetic acid after 24 h. Overall propionic acid production ranged from 1.44 µg/ml (M10 at 0 h) to 3.42 µg/ml (V3 at 24 h). M5 and M31 produced significantly higher propionic acid than the rest of the cultures. It was observed that butyric acid produced by the cultures M5 (2.09 µg/ml) was highest followed by 13 (2.08 µg/ml) and M31 (2 µg/ml). Therefore, from this study we can conclude that the probiotics can be used as a potential SCFAs producer and can benefit our GI tract system with its applicable therapeutic attributes. Further research in this field is required for introducing a commendable therapeutic product.

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RESEARCH ARTICLE

Comparative study on cost effectiveness and quality characteristics of ghee prepared from different animals milk

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Abstract: The present study was conducted to compare the cost effectiveness and physico-chemical properties of ghee prepared from different animals milk. In first experiment, ghee was prepared from Sahiwal, cross breed and Murrah buffalo milk separately from two different methods *i.e.* traditional method and creamery butter method. Cost of formulation per liter ghee was lowest in Murrah buffalo ghee (MG) per kg was less than Sahiwal ghee (SG) and crossbred ghee (CG) for both methods, again creamery butter method was found cost effective than traditional method for ghee preparation on the basis of raw material and other factors. In next experiment, MG, SG and CG were prepared with butter creamy method and compared for various physico-chemical properties and sensory evaluation. Among the physico-chemical properties, BR, iodine, polenske values and cholesterol content of SG and CG were significantly ($P<0.05$) higher than MG. Fat content, saponification and Reichert values of MG were significantly ($P<0.05$) higher than SG and CG. There was no significant difference between SG and CG for any parameter. Peroxide value of freshly prepared ghee samples was negligible (almost near zero). For sensory evaluation, colour scores of SG and CG were significantly ($P<0.05$) higher than M, however there was no significant difference in texture and ghee residues scores. Flavour and total scores of MG were significantly ($P<0.05$) higher than SG and CG. Therefore, it was concluded that creamery butter method was more cost effective for ghee preparation than traditional method and cost of formulation was less in Murrah

buffalo ghee than Sahiwal ghee and crossbred ghee. All ghee samples were very well acceptable by sensory panelists, however sensory scores of Murrah buffalo ghee were comparatively higher than Sahiwal and cross breed ghee in flavor terms and overall acceptability.

Keywords: Ghee, Sahiwal, Crossbred, Murrah buffalo, Cost effectiveness and cholesterol content

Introduction

Dairy sector forms an essential component of the rural Indian economy, serving as an important source of employment engaging about 80 million rural households with a high proportion comprising landless, small and marginal farmers. India's dairy industry is worth Rs 5.4 trillion by value, having grown at an average of 15 per cent CAGR (Compound Annual Growth Rate) during the present decade. India is leading milk producer in the world with 187.7 million tonnes of milk (NDDB, 2019) due to advancement of technology, proper nutrition and appropriate managerial practices. The country has experienced noteworthy improvement in the per capita availability of milk has reached to 394 grams milk per day in 2018-19. Livestock contributes about 9.2 % in gross value added (GVA) and 26.2 % in agriculture sector in India (DAHD, 2019). The milk processing industry in India is expected to expand at a compound annual growth rate (CAGR) of ~14.8% between FY 2018 and FY 2023, and will reach Rs. 2,458.7 billion in FY 2023. Milk processing capacity in the country has also increased over the years due to increase in demand of good quality, hygienic and packaged milk and milk products. Out of total milk produced in India, 65% to 70% is sold as raw milk and the rest is processed into dairy products like cheese, butter, ghee, ice cream, curd etc.

Ghee, widely considered as the Indian name for clarified butterfat. The word ghee comes from Sanskrit word (*gh/ta*), is usually prepared from cow milk or buffalo milk or combination thereof. Ghee is by far the most ubiquitous indigenous milk product and prominent in hierarchy of Indian dietary, being a rich source energy, fat soluble vitamin, essential fatty acid and pleasing flavour it enjoys supreme status. In India ghee is considered as an excellent cooking material and used in religious rites by hindu

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and also used as many medicinal uses (Rajorhia, 2003). About 30-35% of produced milk is converted to ghee in India every year (Varkey, 2010). It is the most widely used milk product in Indian sub-continent. Ghee production forms the largest segment of the milk consumption and utilization in India. Ghee production was estimated in year 2020 to exceed 170 thousand metric tons and its demand is increasing with increase in purchasing capacity of Indian consumer ([www. https://www.statista.com](http://www.statista.com)). It is considered as the supreme cooking and frying medium. In its table use, ghee is served in hot melted form and used for garnishing rice or spreading lightly on chapattis. The flavour of ghee cannot be duplicated by any other fat. Sweets and meals cooked in ghee enjoy special status and are recognized for their distinguished flavour attributes derived from ghee. Ghee contributes significantly towards nourishment of people of all age groups. It is a good source of fat-soluble vitamins (A, D, E and K) and essential fatty acids (Rangappa and Achaya, 1974; Chand et al. 1986). Milk fat is most easily digestible than other oils and fats. Milk fat contains a number of components like sphingomyelin, conjugated linoleic acid, carotene, vitamin D etc, which show anticarcinogenic activity (Parodi, 1996). Although medical criticism has been directed at milk fat on the basis of its saturated nature, it is more accurate to consider it as lacking in polyunsaturates because of its high 18:1 content. Debate on the role of milk fat in human health is still continuing. Conjugated linoleic acid (CLA) is a type of fatty acid that occurs in high concentrations in ghee and recognized as an anticarcinogen (Chin et al. 1992). Ghee contains about 0.25 to 0.4% cholesterol (Nath et al. 1996) and is present in both free as well as in esterified form (Bindal and Jain, 1972). Both forms are important structural components of cell membranes. Cholesterol is also a precursor for the synthesis of steroid hormones such as progesterone and estrogen. It is also converted to vitamin D by ultraviolet radiation in skin (Mayant, 1981; Thomas and Holub, 1994). Like any clarified butter, ghee is composed almost entirely of fat, 62% of which consists of saturated fats and other 38% unsaturated fats. The shelf-life of ghee is considerably longer as compared to other dairy products. It undergoes oxidative degradation during storage resulting in an alteration of major quality parameters such as colour, flavour, aroma and the nutritive value (Pawar et al. 2012).

The yield percentage of ghee depend upon milk fat which in turn influenced by a number of factors which could be genetic, physiological and environmental. The yield of ghee also depends upon the method of ghee preparation like traditional, direct cream, creamery butter and stratification method. Nutritional levels, feed, stage of lactation, time of milking, and other animal husbandry practices also effect milk composition as well as yield of ghee. The milk fat is the most variable component of milk (Sahu et al. 2021). The reported fat percentage in Jersey is 5.2%, Zebu 4.7%, Brown Swiss 4.0%, Holstein-Friesian 3.6% and Murrah buffalo 6.7%. The variation in cow and buffalo ghee colour is due to beta-carotene present in cow milk providing yellowish tinge to

the product. The brown colour comes from milk-solids on heating during ghee production, again Beta-carotene, a precursor of vitamin-A from feed of animals may also provide yellowish colour to ghee. Ghee prepared from milk of different indigenous (Sahiwal, Murrah buffalo) and cross breed milch animals may have quite variations in fat and cholesterol content as well as other quality characteristics. Therefore, present study was carried out to compare the cost effectiveness, physico-chemical properties and sensory attributes of ghee prepared from different animals milk.

Materials and Methods

The experiments were conducted in Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, 281001 (UP), India. Fresh clean wholesome milk of Sahiwal, Crossbreed and Murrah buffalo was procured from Livestock Farm Complex, College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura. All the chemicals used in the study were of analytical grade and procured from Hi Media laboratories (P) Ltd, Mumbai. Clear glass bottles with lid were sourced from local market and sterilized by exposing to U.V. light for 30 minutes before use.

Preparation of Ghee

The ghee was prepared by using two different methods *i.e.* traditional and creamery butter method for Sahiwal, Crossbreed and Murrah buffalo milk separately as per method described by De (2011). Flow diagram of ghee preparation from traditional and creamery butter method are shown in flow diagram 1 and flow diagram 2 respectively.

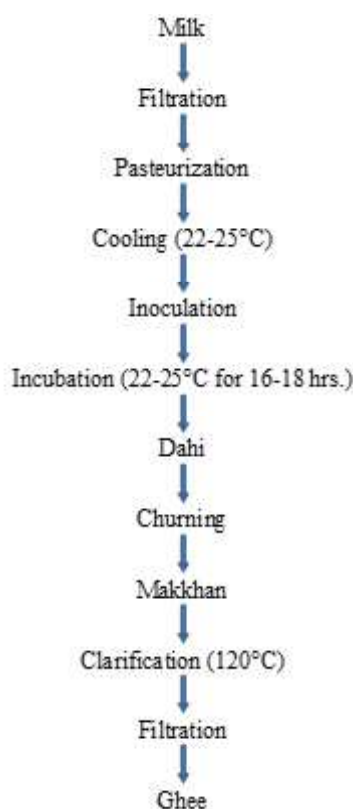
Analytical methods

Physico-chemical properties

The Butyro refractometer value of ghee was determined by using digital Butyro refractometer meter (WTW, Germany, model pH 330i) as per method described by SP: 18 (part XI)- 1981. Moisture and fat content were estimated as per AOAC (2000) and Rose-Gottlieb method respectively. The cholesterol content in ghee samples was determined by direct colorimetric method (Bindal and Jain, 1972). Iodine value of control ghee samples were determined by the Wij's method whereas other parameters viz. saponification value, free fatty acid, Reichert-Meissl (RM value) and Polenske value were estimated as per method described by SP: 18 (part XI)- 1981.

Sensory evaluation

Sensory evaluation was conducted by seven experienced panelist using score card method as described in (IS 7770 (1975) Method



Flow diagram 1. Ghee preparation from traditional Method

for Sensory Evaluation of Ghee (Clarified Butterfat) by Bureau of Indian Standards.

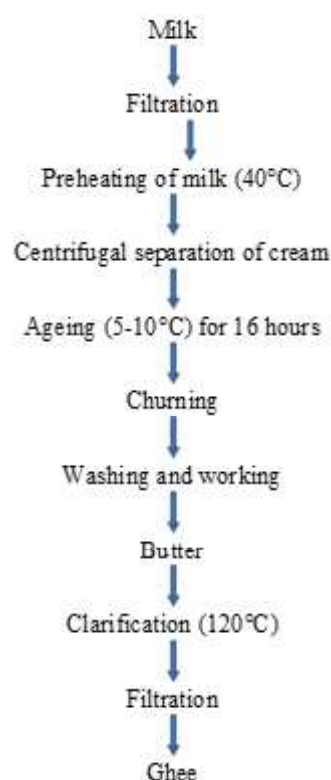
Statistical analysis

The data obtained in the study on various parameters were statistically analyzed on 'SPSS-16.0' software package as per standard methods of Snedecor and Cochran (1995). Duplicate samples were drawn for each parameter and the experiment was replicated thrice ($n=6$). Sensory evaluation was performed by a panel of seven member judges three times, so total observations being 21 ($n=21$). Data were subjected to one way analysis of variance, homogeneity test and Duncan's Multiple Range Test (DMRT) for comparing the means to find the effects between samples.

Results and Discussion

Standardization of cost effective technology for ghee making

Based on available literature, several preliminary trials were conducted to standardize the processing technology of ghee production. The final formulation of ghee was optimized following the method prescribed by De (2011) with slight modifications. The production cost of formulation of ghee using traditional method and creamery butter method were calculated and presented in Table 1 and table 2 respectively. Economics is an important criterion to determine marketability along with nutritive



Flow diagram 2. Ghee preparation from creamery butter method

value and sensory acceptability of a food product. Total cost of ghee production was assessed on the basis of price of raw milk, electricity, labour charge, water, LPG, refrigeration, sundries, packaging material, administrative and depreciative cost. Creamery butter method of ghee production was found more cost effective than traditional method as more fat recovery was observed in creamery method as compared to traditional method. Lower cost in creamery butter method than traditional method for ghee preparation might be due to higher recovery of fat in former method, again cost difference in Sahiwal, Cross breed and Murrah buffalo ghee within the same method was obviously due to difference in fat content and price of raw milk. Similar findings were also reported by Rajorhia (1993) and Aneja et al. (2002). Although cost of production as presented for industrial preparation of ghee by Rangasamy and Dhaka (2008) and Doni and Chauhan (2018) were comparatively lower than our findings and this difference is mainly due to lower scale of production for our study and inflation in prices.

Cost of formulation for preparation of ghee using Traditional method

Raw material cost: Raw material required for preparation of ghee was taken from Livestock farm complex (LFC) however the cost of raw ingredient varied among ghee prepared from three different milch animals because of different in price per litre of Sahiwal,

Cross breed and Murrah buffalo milk and amount of milk required to produce 1 litre of ghee also varied depending upon the fat present in the milk. Price of milk was Rs.34/litre for Sahiwal and Cross breed milk whereas Rs.42/litre for buffalo milk. Since 22 litre of Sahiwal, 21.5 litre of Cross breed and 15.4 litre of Murrah milk was required to get one litre of ghee. Raw material cost for per litre of ghee preparation was Rs.748.00 for Sahiwal ghee, Rs.731.00 for Cross breed ghee and Rs.684.60 for Murrah buffalo ghee.

Labour cost: The labour cost of skilled person is presently Rs.483/8 hr work

And only 30 minutes of labour was required for production of 1 litre of ghee using creamery butter method. So the labour cost would be Rs.30.18.

Electricity: For making of 1 litre of ghee by creamery butter method about 0.37KWh of electricity required and the price of electricity is Rs.5.5/KWh thus cost of electricity would be Rs.2.01.

LPG: Prices of LPG cylinder are highly variable in Indian markets so Rs 800 per cylinder is fixed as average cost and minute amount (52 gm) of LPG is required for making 1 litre of ghee which would cost Rs.2.85.

Packaging material: Glass bottle with lid were purchased from local market for keeping the ghee material and the cost of these glass bottle were Rs.40/pc.

Depreciation: Depreciation cost was calculated @ 1.08 % of total other component costs and this rate of depreciation was taken as reported by Doni and Chauhan, (2018) during their research on economics of manufacturing of different milk products. The depreciation cost for Sahiwal, Cross breed and Murrah buffalo ghee was 8.81, 8.63 and 8.13 respectively.

Total cost: Total cost of formulation of Sahiwal, Cross breed and Murrah buffalo ghee was Rs. 835.06, 817.88 and 770.98/- respectively.

Cost of formulation for preparation of ghee using Creamery butter method

Raw material cost: Raw material required for preparation of ghee was taken from Livestock farm complex (LFC) however the cost of raw ingredient varied among ghee prepared from three different milch animals because of different in price per liter of Sahiwal, Cross breed and Murrah buffalo milk and amount of milk required to produce 1 liter of ghee also varied depending upon the fat present in the milk. Price of milk was Rs.34/liter for Sahiwal and Cross breed milk whereas Rs.42/liter for buffalo milk. Since 20 liter of Sahiwal, 19.5 liter of Cross breed and 15.5 liter of Murrah milk was required to get one liter of ghee. Raw material cost for per liter of ghee preparation was Rs.680.00 for Sahiwal ghee, Rs.663.00 for Cross breed ghee and Rs.651.00 for Murrah buffalo ghee.

Labour cost: The labour cost of skilled person is presently Rs.483/8 hr work and only 20 minutes of labour was required for

Table 1: Cost of formulation of Sahiwal, Crossbred and Murrah buffalo ghee using traditional method

Component of cost	Sahiwal Ghee Unit Cost (Rs./Litre)	Cross breed Ghee Unit Cost (Rs./Litre)	Murrah Ghee Unit Cost (Rs./Litre)
Raw material	748.00	731.00	684.60
Labour	30.18	30.18	30.18
Electricity	2.01	2.01	2.01
Water	0.07	0.07	0.07
LPG	2.85	2.85	2.85
Refrigeration	1.93	1.93	1.93
Sundries	1.21	1.21	1.21
Packaging material	40.00	40.00	40.00

Table 2: Cost of formulation of Sahiwal, Cross breed and Murrah buffalo ghee using Creamery butter method

Component	Sahiwal Ghee Unit Cost (Rs./L)	Cross breed Ghee Unit Cost (Rs./L)	Murrah Ghee Unit Cost (Rs./L)
Raw material	680.00	663.00	651.00
Labour	20.12	20.12	20.12
Electricity	1.36	1.36	1.36
Water	0.07	0.07	0.07
LPG	2.71	2.71	2.71
Refrigeration	1.83	1.83	1.83
Sundries	1.15	1.15	1.15
Packaging material	40.00	40.00	40.00

production of 1 liter of ghee using creamery butter method. So the labour cost would be Rs.20.12/-.

Electricity: For making of 1 liter of ghee by creamery butter method about 0.25KWh of electricity required and the price of electricity is Rs.5.5/KWh thus cost of electricity would be Rs.1.36.

LPG: Prices of LPG cylinder are highly variable in Indian markets so Rs 800 per cylinder is fixed as average cost and minute amount (50 gm) of LPG is required for making 1 liter of ghee which would cost Rs.2.71.

Packaging material: Glass bottle with lid were purchased from local market for keeping the ghee material and the cost of these glass bottle were Rs.40/pc.

Depreciation: Depreciation cost was calculated @ 1.08 % of total other component costs and this rate of depreciation was taken as reported by Doni and Chauhan (2018) during their research on economics of manufacturing of different milk products. The depreciation cost for Sahiwal, Cross breed and Murrah buffalo ghee was 7.96, 7.78 and 7.64 respectively.

Total cost: Total cost of formulation of Sahiwal, Cross breed and Murrah buffalo ghee was Rs. 755.2, 738.02, 725.88/- respectively.

Comparative quality evaluation of Sahiwal ghee, Cross breed ghee and Murrah buffalo ghee

Physico-chemical properties

The physico-chemical properties of Sahiwal ghee, Cross breed ghee and Murrah buffalo ghee are presented in table 3. BR and iodine values of Sahiwal (SG) and Cross breed ghee (CG) were significantly ($P<0.05$) higher than that of ghee prepared from Murrah buffalo and this difference was due to high unsaturated fatty acid content in cattle ghee and similar findings were also

reported by Kumar et al. (2010). Fat content, saponification value and Richert values of MG were significantly ($P<0.05$) higher than SG and CG, however there was no significant difference between SG and CG. Lower saponification values of SG and CG indicated higher molecular weight fatty acid present in cow ghee than buffalo ghee. Cholesterol content and Polenske values of SG and CG were significantly ($P<0.05$) higher than MG due to higher amount of $C_{18:3\ n3a}$, $C_{20:1\ n9}$ and $C_{24:1n9}$ content of cow ghee as compared to buffalo ghee (Sharma et al. 2009). Cow ghee contains about 0.32% cholesterol while that of buffalo has around 0.27%. Around 10% of the cholesterol in milk fat is esterified, remaining is in free form (Kumar et al. 2010). $C_{16:0}$ and *cis*- $C_{18:1}$ were the most predominant fatty acids in both the samples, representing around 57% of the total fatty acids.

These fatty acid contributed one fourth and three fourth to the polenske value of ghee and significant ($P<0.05$) higher level of caprylic acid and capric acid were reported in ghee of cow ghee as compared to buffalo ghee by Pena-Serna and Restrepo-Betancur (2020). The higher cholesterol content of Cross breed and Sahiwal ghee was due to higher cholesterol per 100 grams of fat in milk cow as compared to buffalo milk and values of cholesterol for three different ghee sample are in range as described for species by Kumar et al. (2010). Higher RM value and saponification value while lower Polenske value, iodine value, BR reading (at 40°C) reading for Murrah buffalo ghee was observed as compared to cow ghee, as observed in the present study, may be ascribed to the species characteristics, since the study was carried out under identical condition of feeding and management. Similar type of relationship in physico-chemical properties of a cow ghee as well as buffalo ghee have also been reported by earlier workers (Kumar et al. 2002, Kumar et al. 2013, Gandhi et al. 2014). All parameters of ghee prepared from Murrah buffalo, Sahiwal and Cross breed cow milk are within the limit of Indian standards prescribed for ghee (AGMARK 1988; PFA 2009). Ramya et al. (2019) developed flavored special Uthukuli ghee

Table 3: Physico-chemical properties of Sahiwal, Crossbreed and Murrah buffalo ghee (Mean±SE) (n=6)

Parameters	SG	CG	MG	TreatmentMean
BR value	43.60 ^a ±0.26	42.34 ^a ±0.11	41.99 ^b ±0.46	42.64±0.26
Moisture (%)	0.21±0.04	0.19±0.06	0.15±0.03	0.18±0.01
Fat (%)	99.60 ^b ±0.05	99.67 ^b ±0.04	99.97 ^a ±0.06	99.67±0.02
Saponification value	223.95 ^b ±1.93	225.65 ^b ±1.57	235.23 ^a ±1.38	228.28±1.93
Iodine value	36.01 ^a ±1.26	34.25 ^a ±0.13	31.36 ^b ±0.37	33.87±0.77
Free fatty acid (% oleic acid)	0.23±0.01	0.23±0.01	0.21±0.01	0.22±0.01
Cholesterol (%)	0.32 ^a ±0.01	0.33 ^a ±0.01	0.24 ^b ±0.01	0.30±0.01
Reichert value	28.90 ^b ±0.32	28.44 ^b ±0.86	30.66 ^a ±0.96	29.01±0.74
Polenske value	1.57 ^a ±0.02	1.59 ^a ±0.02	1.38 ^b ±0.04	1.56±0.03

- Mean bearing different superscripts (a, b, c,) within row differ significantly ($P<0.05$)
- n=6
- SG-Sahiwal cow ghee
- CG-Cross breed cow ghee
- MG-Murrah buffalo ghee

Table 4: Sensory evaluation of Sahiwal, Cross breed and Murrah ghee (Mean±SE) (n=21)

Characteristics	SG	CG	MG	Treatment	Mean
Flavour	48.28 ^b ±0.17	48.14 ^b ±0.15	49.85 ^a ±0.13	48.75±0.11	
Texture	27.88±0.21	27.76±0.20	28.28±0.31	27.97±0.13	
Colour	9.57 ^a ±0.12	9.42 ^a ±0.09	9.06 ^b ±0.11	9.39±0.11	
Freedom from suspended impurity (Ghee Residue)	9.42±0.20	9.48±0.20	9.57±0.20	9.49±0.11	
Total Score	95.15 ^b ±0.28	94.80 ^b ±0.24	96.76 ^a ±0.26	95.60±0.17	

- Mean bearing different superscripts (a, b, c,) within row differ significantly (P<0.05)
- n=21
- SG-Sahiwal cow ghee
- CG-Cross breed cow ghee
- MG-Murrah buffalo ghee

from full fat cow and buffalo ghee and reported that melting point, RM value, polenske value, saponification value and iodine value of cow and buffalo ghee were 31.3±0.43, 28±0.24, 0.5±0.04, 221±0.04, 32.2±0.91 and 33.5±0.61, 33±0.56, 0.63±0.05, 227±0.12, 27.1±0.74 respectively.

Sensory evaluation

The sensory scores of Sahiwal ghee, Cross breed ghee and Murrah buffalo ghee are presented in table 4. Flavour scores of MG were significantly (P<0.05) higher than SG and CG, however there was no significant difference between SG and CG. Parmar et al. (2018) also reported significantly (P<0.05) higher flavour scores in buffalo ghee than cow ghee due to higher concentration of butyric acid in buffalo ghee than cow ghee. As per Wadhwa and Jain (1990) key ghee flavouring compounds were carbonyls, lactones and short chain fatty acids fatty acids which were greatly influenced by different factors like fermentation of the cream or butter and the heat treatments used. Colour scores of SG and CG were significantly (P<0.05) higher than MG, which might be due to presence of β -carotene in cow milk which imparted yellow colour to cow ghee. Achaya (1997) and Bharwade et al. (2017) also observed deep yellow to straw yellow colour of cow ghee while that of buffalo ghee was white in colour. There was no significant difference in texture and ghee residue scores, however total sensory scores of MG were significantly (P<0.05) higher than SG and CG. Pena-Serna and Restrepo-Betancur (2020) evaluated sensory qualities of cow and buffalo ghee and reported that different ghee samples were characterized by an odor described as predominantly lactic, cooked and fatty, while the taste was defined mainly as fatty, lactic, sweet and cooked. They also observed that buffalo ghee had significantly (P<0.05) higher sensory scores than cow ghee in terms of flavour, texture and aroma.

Conclusion

Ghee is one of the most acceptable fat rich dairy products in India. It is actually clarified milk fat prepared from different methods, where creamery butter method was found to be more

cost effective than traditional method in terms of raw material cost and yield per liter. On comparison of Sahiwal, cross breed and Murrah buffalo ghee prepared from cost effective creamery butter method, physico-chemical properties showed significant difference among the treatments except for moisture and free fatty acid content. There was no significant difference between Sahiwal and cross breed ghee for any parameter. Freshly prepared ghee samples had 0 peroxide values and all treatments were very well acceptable by sensory panelists. Colour scores of cow ghee were significantly (P<0.05) higher than buffalo ghee, however flavour and total scores of Murrah buffalo ghee were significantly (P<0.05) higher than Sahiwal and cross breed ghee. The study may be further recommended for complete product profile in terms of fatty acids and amino acids analysis.

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RESEARCH ARTICLE

Effect of different concentrations of Taurine on certain Physico-morphological attributes during cryopreservation of Gir bull semen

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Abstract: The study was carried out on four Gir bull (4-6 years) located at Cattle Breeding Farm J.A.U. Junagadh for a period of 6 weeks. Semen was collected using artificial vagina method once weekly for six weeks from each bull. The semen was evaluated for individual motility, sperm viability and sperm abnormality at pre-freeze and post-thaw stages of cryopreservation using different Taurine concentrations viz. 25 mM taurine, 50 mM taurine, 75 mM taurine and control (without taurine). At pre-freeze stages of cryopreservation, 50 mM taurine group had value of 70.20 ± 1.22 and 69.87 ± 3.09 percent for individual motility and sperm viability respectively, values were significantly ($P < 0.05$) higher as compared to that of the 25 mM taurine, 75 mM taurine and control groups. Mean per cent sperm abnormality was 15.37 ± 0.89 in 50mM taurine group at pre-freeze stages of cryopreservation, which were significantly ($P < 0.05$) lower as compared to that of the other groups. At post-thaw stages of cryopreservation, 50 mM taurine group had values of 62.70 ± 0.42 and 65.66 ± 0.58 percent for individual motility and sperm viability respectively, the values were significantly ($P < 0.05$) higher as compared to that of the 25 mM Taurine, 75 mM Taurine and control groups. Mean per cent sperm abnormality was 20.29

± 0.73 in 50mM Taurine group at post-thaw stages of cryopreservation, which were significantly ($P < 0.05$) lower as compared to that of the other groups. Individual motility and sperm viability was found to be significantly better in 50 mM taurine group at pre-freeze and post-thaw stages of cryopreservation of Gir bull semen.

Keywords: Gir bull, Individual motility, Semen, Taurine, Sperm viability

Introduction

Gir internationally well recognized milch breed of India, It is well known for its milk production, docility and emotional temperament. This breed is found in and around districts like Junagadh, Amreli, Bhavnagar, Gir Somnath, Rajkot, Porbandar and some parts of Jamnagar, Morabi, Surendranagar districts of Saurashtra. This breed is also known as Bhodali, Kathiyawari, and Sorathi. Pure Gir herd is well maintained at Cattle Breeding Farm Junagadh, JAU.

Frozen bull semen has been widely used for artificial insemination which is the most inexpensive and the quickest mode of genetic improvement. The freezing and thawing process lead to development of reactive oxygen substances that impair the post thaw motility. One of the most important factors contributing to poor quality semen has been reported to be oxidative stress. (Bucak et al. 2010) Moreover, bovine spermatozoa are prone to membrane damage due to high content of polyunsaturated fatty acids in the sperm membrane and lack of a significant antioxidant system in the cytoplasm (Bailey et al. 2000)

Taurine (2-aminoethanesulfonic acid; $MW = 125.15 \text{ g mol}^{-1}$), is an organic acid widely distributed in animal tissues. It maintains the stability of bio membranes, minimizes the end products of lipid peroxidation (Huxtable, 1992), modulates Ca^{2+} uptake (Singh et al. 2012) and inhibits protein phosphorylation (Kumar and Atreja, 2012). It protects sperm against the harmful effects of ROS and improve sperm motility and membrane integrity during sperm storage. In addition, supplementation of the taurine in the extender significantly ($P < 0.05$) increases the sperm viability, host reactive spermatozoa and the glutathione reductase whereas,

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decreases the sperm abnormality, acrosomal abnormality and the MDA production (Perumal et al. 2013).

Materials and Methods

A total of four sexually mature pedigreed Gir bulls aged between 4 to 6 years maintained at Cattle Breeding Farm, Junagadh. were used for the study. All bulls had normal libido were maintained under same housing and feeding conditions and were properly examined and screened for their normal reproductive health. All bulls had normal libido and sexual behaviour.

The semen was collected by Artificial Vagina (Danish model) once a week using male dummy. A total of 24 ejaculates (six from each bull) were collected for assessment and preservation. Immediately after collection, semen collection tubes were placed in water bath at 35°C. All the chemicals used in the present study were of analytical grade and purchased from Himedia chemicals. The collected semen was examined microscopically for routine seminal traits viz volume, color sperm concentration, mass activity, individual motility, sperm viability, morphological abnormality were recorded. Ejaculate volume was recorded visually with the help of graduated semen collection tube in millilitres. The mass motility of semen was observed by placing a small drop of freshly collected neat semen on clean, grease free warm glass slide and examined without coverslip under low magnification (10 X). The mass motility of semen was graded from 0 to 5 grades (Tomar, 1984). Sperm concentration of million per ml of semen was estimated using photometer (Accucel, IMV, France) against 530 nm wavelength. The Individual motility was subjectively evaluated using the standard method described by Serpil et al. (2009). The sperm viability and sperm abnormality were calculated using Eosin-Nigrosin stain as per the method described by Evans and Maxwell (1987).

After initial dilution with AndroMed® extender, semen ejaculates with more than 70 per cent initial progressive motility, were divided in four equal aliquots. Subsequently, Aliquot-1 was diluted with 25 mM Taurine (25 mM Taurine group), Aliquot-2 was diluted with 50 mM Taurine (50 mM Taurine group), Aliquot-3 was diluted with 75 mM Taurine (75 mM Taurine group) and Aliquot-4 was kept control (only semen sample and AndroMed® extender). The dilution rate was calculated automatically by the photometer (Accucel, IMV, France) against 530nm wavelength. After dilution, each semen aliquots were filled and sealed in French Medium transparent Straw (0.5 ml capacity, TBS™, IMV, France) by Automatic Filling and Sealing Machine (MRS1 Dual, IMV, France) and printed using straw printer (IMV, France). Straws were then arranged in Freezing Rack and were transferred to Cold Handling Cabinet (Macro scientific Pvt. Ltd, New Delhi.) already maintained at 4°C kept for 4 hours of equilibration period. After equilibration, half of the straws were collected and semen was evaluated for individual motility, sperm viability, sperm abnormality. Remaining straws were frozen by

Programmable Bio freezer (Micro-Digitcool, IMV, France) in which temperature of straws was achieved -140°C from 4°C in 7 minutes at the rate of 5°C, 40°C and 20°C per minute from 4°C to -10°C, -10°C to -100°C and -100°C to -140°C, respectively. After achieving -140°C temperature, all the straws were submerged in Liquid Nitrogen at -196°C for storage.

After cryopreservation, straws were thawed at 37°C for 30 seconds in a water bath and were evaluated for post-thaw individual motility, sperm viability and sperm abnormality after twenty four hours of cryopreservation.

Statistical analysis the data were analysed statistically using ANOVA Duncan's new multiple range test by employing IBM SPSS Statistics version 20.00(online) to know the variation between different levels of additives

Results and Discussion

Individual motility (%)

Individual sperm progressive motility is an important criterion of semen quality (Lasley, 1951) and is an important determinant of success rate of the fertilization. The mean individual motility percent using different concentrations of Taurine in different stages have been presented in Table 1. The mean individual motility percent in 50 mM Taurine group was significantly ($P < 0.05$) higher at pre-freeze and post-thaw stages of cryopreservation as compared to that of the 25 mM Taurine, 75 mM Taurine and control groups (Table 1).

Whereas, individual motility percent was found to be significantly ($P < 0.05$) higher in all the groups at pre-freeze as compared to post thaw stage in different concentrations of taurine as well as control group (Table 1).

These findings are in accordance with Kumar and Atreja (2012) and Kumar et al. (2013) in buffalo bulls, Chhillar et al. (2012) and Kumar et al. (2013) in Karan-Fries bulls who also have reported significantly higher individual motility at post-thaw stage with 50mM Taurine. Similarly, Beheshti et al. (2013) have also reported a significantly higher individual motility at post-thaw stage but at lower concentration of Taurine. in buffalo bull sperm quality. Contrary to present findings, Serpil et al. (2009) has reported a significant lower percent individual motility at 2 mM Taurine concentration in bull. The results may be due to different concentrations of taurine, and the composition of the semen extender used in the study. Several workers have also reported a non-significant effect on individual motility percent using Taurine in semen extender in cattle bull (Uysal et al. 2007), in buffalo bull (Mughal et al. 2013), in Angora goat (Atessahin et al. 2008) and in ram (Bucak et al. 2007). Variable effect of the Taurine observed in the literature, might be due to species variation,

technique used for semen collection, freezing extender composition, preservation protocol and concentration of Taurine used, thawing duration, time taken by the observer, disordered functioning of axonemal proteins as per Mughal et al. (2013).

The axosome and associated dense fibers of the middle pieces in sperm are covered by mitochondria that generate energy from intracellular stores of ATP. These are responsible for sperm motility. The Taurine might have displayed cryoprotective effect on the functional integrity of mitochondria that is responsible for the generation of energy from intracellular stores of ATP led to improved post-thaw sperm motility (Reddy et al. 2010). Taurine, in sperm cells, is able to react with many reactive oxygen species directly for protecting mammalian cells against oxidative stress, and hence maintaining sperm motility (Bucak et al. 2007).

Sperm viability (%)

The overall mean sperm viability percent, using different concentrations of Taurine in different stage were presented in Table 2. The overall mean sperm viability percent in 50 mM Taurine group was significantly ($P<0.05$) higher as compared to 25mM Taurine; 75 mM Taurine and control group at pre-freeze stage and post-thaw stage of cryopreservation, respectively (Table 2) Whereas, sperm viability percent was found to be significantly higher ($P<0.05$) in all the groups at pre-freeze stage(different concentrartions) as compared to different groups in post-thaw stage of cryopreservation (Table 2). These findings are in accordance with Kumar and Atreja (2012), Beheshti et al. (2013) and Kumar et al. (2013) in buffalo bulls and Chhillar et al. (2012) and Kumar et al. (2013) in Karan-Fries bulls who also have reported significantly higher sperm

viability at post-thaw stage with 50 mM Taurine. Similarly, Kishore et al. (2011) in cattle bulls, also reported a significantly higher sperm viability at post-thaw stage but at lower concentration of Taurine. Whereas, a non-significant effect on percent sperm viability using Taurine, in buffalo bull (Mughal et al. 2013), in Angora Goat (Atessahin et al. 2008) and in ram (Bucak et al. 2007) has been reported. Variable effect of the Taurine observed in the literature, might be due to species variation, freezing extender composition, preservation protocol and concentration of Taurine used.

Taurine is a permeating cryoprotectant and act as an antioxidant and cause membrane lipid and protein rearrangement, which results in increased membrane fluidity, greater dehydration at lower temperatures and therefore increased ability of spermatozoa to survive during cryopreservation (Holt, 2000). This could be one of the reasons for improved post-thaw viability in the present study.

Sperm abnormality (%)

The mean sperm abnormality percent using different concentrations of Taurine in different groups were presented in Table 3. The mean sperm abnormality percent in 50 mM Taurine group was significantly ($P<0.05$) lower as compared to 25 mM Taurine and 75 mM Taurine groups at pre-freeze stage and 25 mM Taurine, 75 mM Taurine and control group at post-thaw stages of cryopreservation. (Table 3). Whereas, sperm abnormality percent was found to be significantly ($P<0.05$) lower in all the groups(25mM,50mM,75mM) at pre-freeze stage as compared to that post-thaw stage of cryopreservation (Table 3). Kishore et al. (2011) have also reported a significantly lower sperm

Table 1 Individual motility Percent in different groups at various stages of cryopreservation in Gir bulls semen (Mean $\pm$ SE)

Groups (n=24)	Stages of cryo-preservation	
	Pre- freeze Stage (PFS) at 4°C	Post-Thaw Stage (PTS) at 37°C
Taurine 25 mM	62.70 $\pm$ 1.27 ^{Ab}	56.66 $\pm$ 0.88 ^{A_a}
Taurine 50 mM	70.20 $\pm$ 1.22 ^{Bb}	62.70 $\pm$ 0.42 ^{B_a}
Taurine 75 mM	64.37 $\pm$ 1.39 ^{Ab}	56.45 $\pm$ 0.87 ^{A_a}
Control (n=24)	64.37 $\pm$ 1.05 ^{Ab}	56.45 $\pm$ 0.97 ^{A_a}

Means with different superscripts within column(A,B) and Means with different subscripts within Row(a,b) differ significantly ($P<0.05$).

Table 2 Sperm viability Percent in different groups at various stages of cryopreservation in Gir bulls semen (Mean $\pm$ SE)

Groups (n=24)	Stages of cryopreservation	
	Pre- freeze Stage (PFS) at 4°C	Post-Thaw Stage (PTS) at 37°C
Taurine 25 mM	69.87 $\pm$ 0.95 ^{A_b}	61.29 $\pm$ 0.63 ^{A_a}
Taurine 50 mM	69.87 $\pm$ 3.09 ^{B_b}	65.66 $\pm$ 0.58 ^{B_a}
Taurine 75 mM	68.58 $\pm$ 0.95 ^{AB_b}	60.29 $\pm$ 0.70 ^{A_a}
Control	70.54 $\pm$ 1.08 ^{AB_b}	61.75 $\pm$ 0.62 ^{A_a}

Means with different superscripts within column(A,B) and Means with different subscripts within Row(a,b) differ significantly ($P<0.05$).

Table 3 Sperm abnormality Percent in different groups at various stages of cryopreservation in Gir bulls semen (Mean $\pm$ SE)

Groups (n=24)	Stages of cryopreservation	
	Pre- freeze Stage (PFS) at 4°C	Post-Thaw Stage (PTS) at 37°C
Taurine 25 mM	17.87 $\pm$ 0.59 ^{BCa}	24.33 $\pm$ 0.87 ^{Bb}
Taurine 50 mM	15.37 $\pm$ 0.89 ^{Aa}	20.29 $\pm$ 0.73 ^{Ab}
Taurine 75 mM	20.08 $\pm$ 0.62 ^{Ca}	24.45 $\pm$ 0.80 ^{Bb}
Control	16.58 $\pm$ 0.68 ^{ABa}	23.54 $\pm$ 0.72 ^{Bb}

Means with different superscripts within column(A,B) and Means with different subscripts within row(a,b) differ significantly (P<0.05).

abnormality at post-thaw stage but at lower concentration of Taurine in cattle bulls. Perumal et al. (2013) have also found significantly decreased sperm abnormality percent at 50 mM Taurine concentration but for the liquid storage of Mithun bull semen. Whereas, a non-significant effect in sperm viability using Taurine, in cattle bull (Serpil et al. 2009) and in ram (Bucak et al. 2007) has been reported. In the present study, decreased sperm abnormality in Taurine treated semen may be due to its ability to maintain structural stability of the sperm and to stabilize the membrane integrity of acrosome, plasma, mitochondria, and flagella of the sperm (Perumal et al. 2013).

Conclusion

The present study demonstrated that taurine supplemented at dose of 50mM appears to be optimal effective dose to reduce cryodamage in Gir bull semen. It improves the post thaw semen characteristics like individual sperm motility and sperm viability and reduces the sperm abnormality. Further studies are needed to test the fertilizing ability of bull spermatozoa frozen with effective additives used in commercial AI. Future research should focus on identification of molecular pathways for bull spermatozoa death during cryopreservation and identification of molecular markers of fertility in bulls.

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RESEARCH ARTICLE

Genetic analysis of trends in birth weight in Sahiwal cows maintained in Chhattisgarh

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Abstract: The objective of the present study was to estimate the trends in birth weight of Sahiwal cows maintained at Bull Mother Experimental Farm, College of Vety. Science and Animal Husbandry, Anjora, Durg. The information related to the present study was collected from records spread over 20 years from 2001 to 2020 on Sahiwal cattle maintained at Bull Mother Experimental Farm, College of Veterinary Science & A.H, Anjora, Durg. All the statistical analysis was done using WOMBAT Statistical Software. The least square analysis was carried out to estimate the effect of sire and non genetic factors i.e. , parity , period of birth and season of birth on the birth weight of Sahiwal calves by using the Mixed Model DFREML function of WOMBAT Statistical Software. The heritability of birth weight was estimated by Mixed Model DFREML function of WOMBAT Statistical Software. The phenotypic, genetic and environmental trend was estimated for birth weight of calves. Phenotypic trend per year was estimated as the linear regression of the phenotypic value (P) on time (year). The genetic trend was estimated by regressing the estimates of breeding values of individual animals on time (year). The breeding values of individual animals were estimated by fitting BLUP Animal Model using WOMBAT Statistical Analysis software. The effect of sire & all the non genetic factors was significant on birth weight. The heritability of birth weight in this study is estimated as 0.15 i.e. medium. The estimate of phenotypic trend was estimated as -0.105 ± 0.02 days/year. The

estimate of genetic trend was positive (0.008 ± 0.002 days/year) exhibiting positive genetic improvement in the trait. The birth weight is the most neglected parameters though it is a novel trait of increasing economic importance, because birth weight of a calf can indicates life time performance of heifers, adult cows and bulls. Thus dairy genetic improvement programmes needed to be redesign appropriate strategies that would be more beneficial to the currently changing scenarios.

Keywords: Birth weight, BLUP Animal model, Genetic Trend, Phenotypic Trend, Sahiwal

Introduction

Livestock production has always been an integral part of the rural livelihood, around 80 percent of marginal and small farmers along with landless folk possess livestock of some species or the other. The most preferred livestock species is cattle, it forms an integral part of agriculture in India and involves about 70% of its population, most of which are small/medium scale holders (Brar and Nanda 2004). Livestock sector contribute about 4 and 30 % to national and agricultural GDPs of India, respectively. It provides livelihood security and employment to millions of small, marginal and landless farmers. The weight of the newly born calf is of great importance to the producer. A calf weight at birth is strongly associated with mortality risk and developmental problems in growing age and the risk of various diseases in adult stage. Careful management of birth weight by breeders is therefore required to ensure that calf death incidence is minimized and to maintain a healthy growth rate for maximum life time production. Recording birth weights is also an important way to identify animals which have low or moderate birth weight as animals with lower birth weights have lower growth rates. Selvan et al. (2018) reported that because birth weight of calves is easily measured and correlated with other performance traits, it should be considered in the genetic improvement programs. There are certain factors that can affect calf birth weight including dam age, body conditions, nutrition, sex of calf etc. Therefore, it is imperative to separate genetic change from environmental as accurately as possible. The estimates of the trends are essential because the value compares the realized and expected one in the experimental situation and assessment of progress in a particular trait. Hence,

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there is a need to periodically evaluate the genetic parameters and trends and monitor whether the trends are progressing in desirable direction for traits of economic importance or not. Sahiwal is considered to be one of the important milch breeds. Though its original breeding tract lies in Montgomery district of Pakistan, the pure Sahiwal herds are maintained in only a few established farms in India. Bull Mother Experimental Farm, College of Vety. Science and Animal Husbandry, Anjora, Durg is one of the farms in India that maintains pure Sahiwal animals. The birth weight is the most neglected parameters though it is a novel trait of increasing economic importance, because birth weight of a calf can indicates life time performance of heifers, adult cows and bulls. Hence this study was undertaken to study the effect of different non genetic factors on birth weight, genetic parameters of birth weight and to investigate genetic and environmental trends in birth weight over years.

Materials and Methods

Source of data

The information related to the present study was collected from records on Sahiwal cattle maintained at Bull Mother Experimental Farm, College of Veterinary Science & A.H, Anjora, Durg. Calving records of Sahiwal cows, spread over 20 years from 2001 to 2020 were collected for the present study.

Editing and normalization of data

The birth weight records of the Sahiwal calves with known pedigree were taken for the present study. The data was edited and normalized by mean $\pm$ 2 S.D. Total 841 Sahiwal calves having normal birth record were selected for the study.

Classification of data

The data on birth weight of calves were classified and coded on the basis of sire (62 sires), parity (05 levels; 1-5), period of birth (4 levels; 2001-2005, 2006-2010, 2011-2015, 2016-2020), season of birth (4 levels; winter, spring, summer, monsoon).

Statistical analysis

All the statistical analysis was done using WOMBAT Statistical Software.

The least square analysis was carried out to estimate the effect of genotype and different non-genetic factors on the birth weight of Sahiwal calves by using the Mixed Model DFREML function of WOMBAT Statistical Software. The significance of the fixed effects was analyzed by using the Tukey test with a significance level of $P < 0.05$.

The mixed linear model equation for the analysis of the effect of genetic and non – genetic factors on birth weight was:

$$Y_{ijklmn} = u + s_i + P_j + M_k + S_l + E_m + e_{ijklmn}$$

where, Y_{ijklmn} , Observation on n^{th} calf that is progeny of i^{th} sire born in j^{th} parity of dam, k^{th} period, l^{th} season and having m^{th} sex; μ , Overall population mean; s_i , Random effect of i^{th} sire; P_j , Fixed effect of j^{th} parity of dam; M_k , Fixed effect of k^{th} period of birth; S_l , Fixed effect of l^{th} season of birth; E_m , Fixed effect of m^{th} sex; e_{ijklmn} , Random error, which is NID $(0, \sigma^2_e)$

The heritability of birth weight was estimated by Mixed Model DFREML function of WOMBAT Statistical Software.

The phenotypic, genetic and environmental trend was estimated for birth weight of calves. Phenotypic trend per year was estimated as the linear regression of the phenotypic value (P) on time (year).

Best Linear Unbiased Prediction estimates of breeding values of individual animals were estimated by fitting animal model using WOMBAT Statistical Analysis software as:

$$Y = XB + Z\mu + e$$

where, Y, Vector of observations for trait; β , Vector of observations of fixed effects; μ , Vector of observations of random effect (animal); X and Z, Incidences matrices pertaining for fixed and random effect.

After predicting the breeding values of animals, genetic trend was estimated by regressing mean of breeding values, on birth year. The environmental trend was estimated by subtracting the genetic trend from the phenotypic trend.

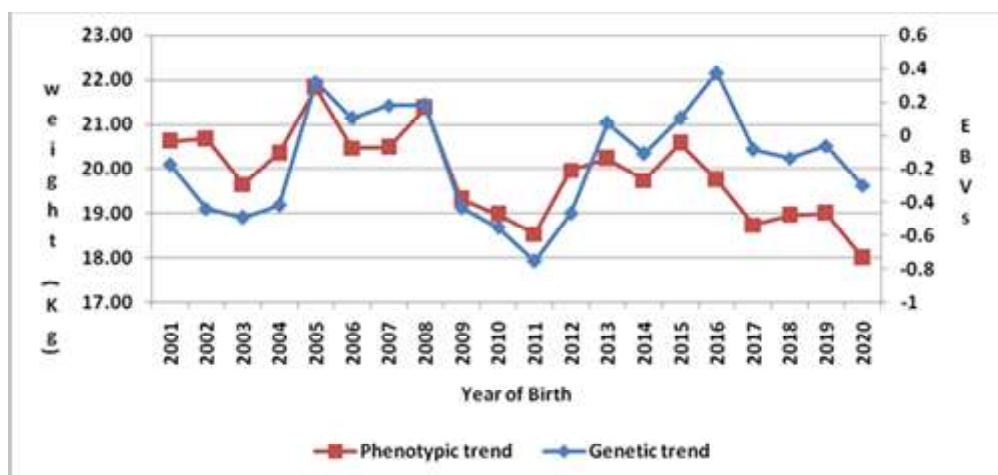
$$E = P - G$$

Results and Discussion

Estimates of genetic and non-genetic factors: The overall least square mean birth weight of Sahiwal calves are presented in Table 1.

Effect of sire was found to have significant effect on birth weight of Sahiwal calves. The effect of parity was also reported significant by Correa et al. (2017) in Mexican Zebu calves, Praharani et al. (2019) in Belgium calves and Saad et al. (2020).

Effect of parity was found to have significant effect on birth weight of Sahiwal calves. Perusal of Table 1 indicated that the birth weight of the calves showed an increasing trend with increasing parity i.e birth weight of calves increases as the parity advances. The reason behind it may be the size of uterus. Heifers have lesser space for embryo development, as age advances with parity the body size increases which in turn also provides more space in uterus for womb development which in turn results in better birth weight. The effect of parity was also reported

Fig. 1 Year wise phenotypic trend and genetic trend for birth weight in Sahiwal calves**Table 1** Least-squares means and SE of birth weight in Sahiwal cattle

Effects	Mean (days)
Overall	20.00±0.49 (841)
Parity of Dam	
First Parity	19.20 ^a ±0.41 (249)
Second Parity	19.96 ^a ±0.42 (188)
Third Parity	20.39 ^b ±0.44 (142)
Fourth Parity	20.40 ^a ±0.45 (111)
Fifth & above Parity	20.74 ^c ±0.53 (151)
Period of Calving	
2001-2005	20.71 ^c ±0.57 (233)
2006-2010	20.27 ^a ±0.57 (226)
2011-2015	20.08 ^a ±0.53 (201)
2016-2020	18.94 ^a ±0.64 (181)
Season of Calving	
Winter	19.95 ^a ±0.50 (372)
Spring	20.26 ^b ±0.52 (162)
Summer	19.53 ^a ±0.52 (180)
Monsoon	20.27 ^b ±0.53 (127)
Sex of calf	
Male	20.74±0.50 (428)
Female	19.26±0.50 (413)

significant by Bakir et al. (2004) in HF calves, Correa et al. (2017) in Mexican Zebu calves where as non-significant effect of parity on birth weight was reported by Naha et al. (2015) in Sahiwal calves Ferdous et al. (2019) in Chittagong calves,

Effect of sex was found to have significant effect on birth weight of Sahiwal calves under study. The significant effect of sex on birth weight was also reported by Correa et al. (2017) in Mexican Zebu calves, Magwaba et al. (2019) in Tuli and calves Umar et al. (2020) in Brown Sindhi calves; non-significant effect was reported by Bakir et al. (2004) in HF calves, Putra et al. (2018) in Ongole calves, Ali et al. (2015) in Red Angus Simmental calves and Praharani et al. (2019) in Belgium calves.

Table 2. Estimates of variance components and genetic parameters for birth weight

Estimate	Birth weight
σ^2_s	0.25
σ^2_e	6.41
h^2	0.15
S.E.	0.08

Effect of season was found to have significant effect on birth weight of Sahiwal calves.. In this study the Sahiwal calves born in the monsoon season had highest birth weight, followed by spring, winter and summer season. The lowest birth weight was observed in calves born in summer season. The effect of calving season on birth weight is highly correlated to environmental factors such as nutrient availability, geological conditions and heat stress. The significant effect of season on birth weight was also reported by Selvan et al. (2018) in Sahiwal and Tharparkar cattle, Putra et al. (2018) in Sumba Ongole cattle, Ferdous et al. (2019) in Chittagong calves, Correa et al. (2017) in Mexican Zebu calves where as non-significant effect was reported by Magwaba et al. (2019) in Tuli cattle, Ali et al. (2015) in Red Angus Simmental calves, Naha et al. (2015) in Sahiwal calves.

Effect of period was found to have significant effect on birth weight of Sahiwal calves. The significant effect of period of calving on birth weight was also reported by Putra et al. (2018) in Sumba Ongole cattle, Ferdous et al. (2019) in Chittagong calves, Correa et al. (2017) in Mexican Zebu calves where as non-significant effect was reported by Ali et al. (2015) in Red Angus Simmental calves and Magwaba et al. (2019) in Tuli cattle.

Estimates of variance components and genetic parameters: Table 2 represents variance components and heritability estimates for birth weight in Sahiwal calves. The heritability estimates is (0.15) i.e. medium. The result implies that birth weight may be influenced more by non additive genetic variability and environmental component and less by additive genetic component of variance.

Thus the results indicates that in birth weight of calves there is a scope for improvement by adopting adequate managemental practices during growth phase and pregnancy phase of dam. Moderate estimates of heritability for birth weight were documented by Bakir et al. (2004) Lbi et al. (2014) Mujibi et al. (2014) Vostry et al. (2015) Ahlberg et al. (2016) Rahbar et al. (2016) Yin and Sven (2018) Selvan et al. (2018) Ferdous et al. (2019) Hanna (2019) Magwaba et al. (2019) and Saad et al. (2020). A comparatively higher estimate of heritability than the present study was reported by Putra et al. (2018) & Khorshidi et al. (2020).

Genetic and Phenotypic trends: The year wise trend for birth weight is presented in Fig 1. The estimate of phenotypic trend was -0.105 ± 0.02 days/year. The estimate of genetic trend was positive (0.008 ± 0.02 days/year) exhibiting positive genetic improvement in the trait. The present study indicates that the desirable genetic improvement was achieved in birth weight at Bull Mother Experimental Farm, Durg over the period of the study. However the environmental trend in negative direction nullified the genetic gain. In other words the negative environmental trends indicate that the genetic potential of the Sahiwal animals for birth weight was increased over years but environmental factors have reduced its effect on phenotypic values. Similar positive trend for birth weight was also reported by Sahin et al. (2012), Chud et al. (2014), Bernardes et al. (2015), Yin & Sven (2018), Saad et al. (2020), Khorshidi et al. (2020), Tomka et al. (2020), Toro et al. (2020) and Nasner et al. (2021).

Conclusions

A calf weight at birth is strongly associated with mortality risk and developmental problems in growing age and the risk of various diseases in adult stage. The estimate of genetic trend was positive exhibiting positive genetic improvement in the trait, however the environmental trend in negative direction nullified the genetic gain. The birth weight is the most neglected parameters though it is a novel trait of increasing economic importance, because birth weight of a calf can indicates life time performance of heifers, adult cows and bulls. Thus, dairy genetic improvement programmes needed to be redesign appropriate strategies that would be more beneficial to the currently changing scenarios. Climate change is also a challenge to the livestock sector in India.

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RESEARCH ARTICLE

Comparative efficacy of three different heat tolerance indices for thermo-adaptability during heat stress in bovines

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Abstract: The present study was conducted on 45 Marathwadi buffaloes and 24 Deoni cows selected from Livestock Farm Complex at COVAS, Udgir of different age groups. Various physiological parameters like rectal temperature, pulse rate and respiration rate of these animals were recorded during the summer season and three heat tolerance indices efficacy viz. Iberia heat tolerance indices, Benezara coefficient of adaptability and Dairy search index were estimated. Highly significant ($P < 0.01$) correlation was observed between the Benezara coefficient of adaptability and Dairy search index in Marathwadi buffalo and Deoni cows.

Keywords: Deoni, Heat Tolerance Indices, Marathwadi, Thermo-adaptability

Introduction

It is well documented that the stress of hot environments lowers productive and reproductive efficiency in farm animals (West, 2003). The physiological tolerance of dairy cows is a strong determinant of the environmental conditions in which they inhabit. Like other homeotherms, cattle regulate their body temperature at a specific range of Environmental temperature with the least involvement of thermoregulatory mechanisms (Aarif et al. 2013). Stress is seen as a symptom resulting from exposure of an animal to a hostile environment. Heat tolerance is the ability of the animals to withstand heat when all other factors are constant. Variance in heat tolerance capability of dairy animals

might be attributed to the differences in the magnitude of change in physiological responses to similar kinds of stressors which depend upon individual adaptive ability (Bianca, 1963).

The variation in climatic variables like temperature, humidity and radiation were recognized as the principal components for reducing the growth and production of all domestic livestock species. The thermal environment is a significant factor that can negatively affect production and reproduction in dairy animals, especially in animals of high genetic merit (Ganaie et al. 2013). Dairy animals regulate internal body temperature by matching the amount of heat produced through metabolism with the heat flow from the animal to the surrounding environment. The three most common heat tolerance indices are the Iberia heat tolerance indices, Benezara coefficient of adaptability and Dairy search index used for measurement of thermo-adaptability of dairy animals. Adaptability is the capacity of any individual to withstand changing environment without significant impairment of its normal operations (Azam et al. 2012). Poor thermal adaptability of dairy animals in a heterologous climate is a limiting factor for optimum production and reproduction performances. Therefore, evaluation of thermal adaptability of dairy animals is an essential prerequisite for selection, culling, formulation of suitable breeding plans, estimation of production and reproduction efficiencies, etc. The Marathwada region of Maharashtra, a perennially drought-plagued area and the most water-deprived regions of the state, possess Deoni cattle and Marathwadi buffalo breeds. These breeds are well adapted to the harsh climate of Marathwada. The present investigation aims to understand their thermo-adaptability in such an environment which could help for better management and will open a new vista for further research. Therefore, an attempt has been made to evaluate the efficacy of these three most common heat-tolerant indices for measurement and evaluation of thermo-adaptability during heat stress in bovines so that better management practices can be planned in the area.

Materials and Methods

The present study has been conducted on 45 Marathwadi buffaloes and 34 Deoni cows of different age groups from the Livestock Farm Complex at COVAS, Udgir. Animals were

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maintained as per routine management practices of Livestock Farm Complex in a uniform environment and identical conditions. The study was carried out in March- April-May-June months in the year 2017. The observations were recorded in the summer season. Deoni cattle and Marathwadi buffaloes were grouped into three categories based upon their age as follows:

Groups	Age	Number of Animals	
		Deoni Cow	Marathwadi buffalo
Group 1	0 -1 year	10	17
Group 2	1 - 3 yr	14	12
Group 3	3 year & above	10	16

Meteorological Observation

Meteorological data for both the experimental seasons were collected from the Observatory managed by the Department of Animal Physiology, College of Veterinary and Animal Sciences, Udgir. All the meteorological variables are presented in (Table 1). The observatory is located within the college campus. Temperature Humidity Index (THI) (morning and afternoon) has been calculated as per NRC (2001) using the following formula:

$$THI = 0.72 (Cdb + Cwb) + 40.6$$

where, Cdb and Cwb = dry and wet bulb temperature in centigrade respectively.

Heat Tolerance Coefficient

The heat tolerance coefficient of bulls has been measured as per methods of Iberia heat tolerance coefficient (Rhoad, 1942), Benezara coefficient of adaptability (Benezara, 1954), and a Dairy search index (Bonsma, 1949). Rectal temperature, pulse rate and respiration rate were recorded at 6.00 to 7.00 A.M and again at 1.30 to 2.30 P.M on the bulls under observation. Heat tolerance coefficients were calculated using the following formulae.

Iberia Heat Tolerance Coefficient (IHTC)

$$IHTC = 100 - 10(BT - 101)$$

where, BT is the body temperature (°F).

The average value of rectal temperature at 6.00 to 7.00 A.M and 1.30 to 2.30 P.M of a day was taken as BT. An ITHC value of '100' indicates the measures of perfect adaptability.

Benezara Coefficient of Adaptability (BCA)

$$BCA = BT/38.33 + NR/23$$

Where, BT is the body temperature (°C) and NR is the respiration rate/min (average of 6.00 to 7.00 A.M and 1.30 to 2.30 P.M). An increase in co-efficient from '2' indicates a reduction in thermal adaptability.

Dairy Search Index (DSI)

$$DSI = 0.5(X_1/X) + 0.2(Y_1/Y) + 0.3(Z_1/Z)$$

X_1 , Y_1 and Z_1 are the observed rectal temperature (°C), respiration rate and pulse rate (per min) respectively. X, Y, and Z are normal temperature, respiration and pulse rate (per min). An increase in DSI value from '1' indicates a decrease in thermal adaptability.

Statistical Analysis

To study the effect of non- genetic factors on heat tolerance coefficients the data were analyzed using mixed model least-squares analysis for fitting constants (Harvey, 1982). The mean and standard error were calculated using standard statistical procedure. Other statistical analysis like correlation etc. was calculated as per standard statistical techniques (Snedecor and Cochran, 1967).

Spearman's rank correlations

The Spearman's rank correlations between predicted live body weights of cattle and buffaloes were derived from various methods used to judge the effectiveness of different methods. The rank correlation was estimated as per Steel and Torrie (1960):

Table 1 The average meteorological data for the months March, April, May and June 2017

Sr.no.	Parameters	March	April	May	June
1	Dry bulb temperature(°C)	29.0	32.2	32.4	25.0
2	Wet bulb temperature(°C)	21.4	20.0	23.6	23.0
3	Maximum temperature	36.4	39.8	38.6	34.4
4	Minimum temperature	20.6	26.0	23.4	21.5
5	Relative humidity	50.0	31	47.0	84.0
6	Vapour pressure	19.8	14.5	22.6	26.5
7	Dew point temperature(°C)	17.4	12.6	19.5	22.1
8	Sunshine period (hrs.)	9.0	10.0	8.0	6.0
9	Evaporation	3.0	5.0	4.5	2.8
10	Wind speed difference	0.2	0.4	0.1	0.1

$$r_s = 1 - \left(\frac{6 \sum d_i^2}{n(n^2 - 1)} \right)$$

Where, r = rank correlation coefficient
 n = Number of sires under evaluation
 d_i = Difference of rank between paired items under two methods

The significance of the rank correlation was tested by t-test with $n-2$ degrees of freedom as given below:

$$t = r \sqrt{\frac{(n-2)}{(1-r^2)}}$$

Results and Discussions

The descriptive statistics revealed that the coefficient of variance was slightly higher for respiration rate in both Deoni cattle and Marathwadi buffaloes. It was observed that the means for respiration rate, heart rate, pulse rate and rectal temperature (Fahrenheit) in Marathwadi Buffaloes were 32.95 ± 0.60 , 65.72 ± 0.87 , 64.46 ± 0.80 and 38.81 ± 0.08 while the case of Deoni cattle it is 34.32 ± 0.97 , 64.05 ± 1.58 , 66.77 ± 1.02 and 102.04 ± 0.22 , respectively (Table 2 and 3).

Table 2 Biometric measurement of cattle and buffalo

AGE (IN MONTH)	Cattle				Buffalo			
	Body Length (cm)	Body Height (cm)	Chest Girth (cm)	Body Weight (Kg)	Body Length (cm)	Body Height (cm)	Chest Girth (cm)	Body Weight (Kg)
0-1 year	86	90	98	78.0	78	82	98	69.0
1-3 yr	111	116	142	209.2	89	96	118	114.4
3year & above	136	129	165	341.4	129	125	181	388.4

Table 3 The descriptive statistics of various parameters in Marathwadi Buffaloes and Deoni cattle

Parameters	Marathwadi Buffalo			Deoni cattle		
	Respiration rate (per min)	Pulse rate (per min)	Rectal temperature (degree celcius)	Respiration rate (per min)	Pulse rate (per min)	Rectal temperature (degree celcius)
Mean	32.95	64.46	38.81	34.32	66.77	102.04
Standard Error	0.60	0.80	0.08	0.97	1.02	0.22
Standard Deviation	4.10	5.44	0.54	4.53	4.77	1.01
Sample Variance	16.84	29.63	0.29	20.51	22.76	1.02
Range	18.00	22.00	2.38	16.00	17.00	3.70
Minimum	22.00	52.00	37.50	28.00	59.00	100.20
Maximum	40.00	74.00	39.88	44.00	76.00	103.90
CV (%)	12.45	8.44	1.39	13.20	7.14	0.99

Iberia Heat Tolerance Coefficient was significantly higher ($p < 0.01$) i.e. 98.41 ± 1.88 and 93.30 ± 2.24 in 3 years and above age group as compared to other age groups in Marathwadi buffaloes and Deoni cattle, respectively. However, Benezara Coefficient of adaptability is higher in the 1 - 3 year age group in Marathwadi buffaloes. In Deoni cattle, it was similar for 0 - 1 year and 1 - 3 year age groups. Dairy Search Index was significantly higher (1.10 ± 0.01) in 1 - 3 year age group in Marathwadi buffaloes, while similar (1.13 ± 0.02) for 0 - 1 year and 1 - 3 year age groups in Deoni cattle (Table 3).

It was observed that the IHTC in Age groups 3 years and above in Marathwadi buffaloes incline towards better adaptability compared to the age groups 0-1 year and 1-3 years, It indicates that the adaptability of the Marathwadi buffalo to the harsh climate of Marathwada increased with an increased in the age. However, in Benezara Coefficient of Adaptability (BCA) and Dairy Search Index (DSI) better adaptability was observed among group 1-3 years compared to the other groups. (Kumari et al. 2018) reported similar trends which are related to our findings that, the Benezara coefficients of adaptability were highest in Murrah (2.26 ± 0.01) followed by Gir and Sahiwal 2.19 ± 0.01 , Jersey cross 2.18 ± 0.01 and Holstein-Friesian cross 2.17 ± 0.01 . Similar findings were also reported by Lalrengpui et al. (2017) that the Analysis of variance (ANOVA) of linear model for dependent variable HTC in Sahiwal and Karan Fries cows was found to be highly significant at 1% level of significance

Fig.1 Comparison amongst different methods of heat tolerance indices in Marathwadi buffaloes (X axis= No. of Animals; Y axis= Heat tolerance indices by different methods)



Fig. 2 Comparison amongst different methods of heat tolerance indices in Deoni cows (X axis= No. of Animals; Y axis= Heat tolerance indices by different methods).

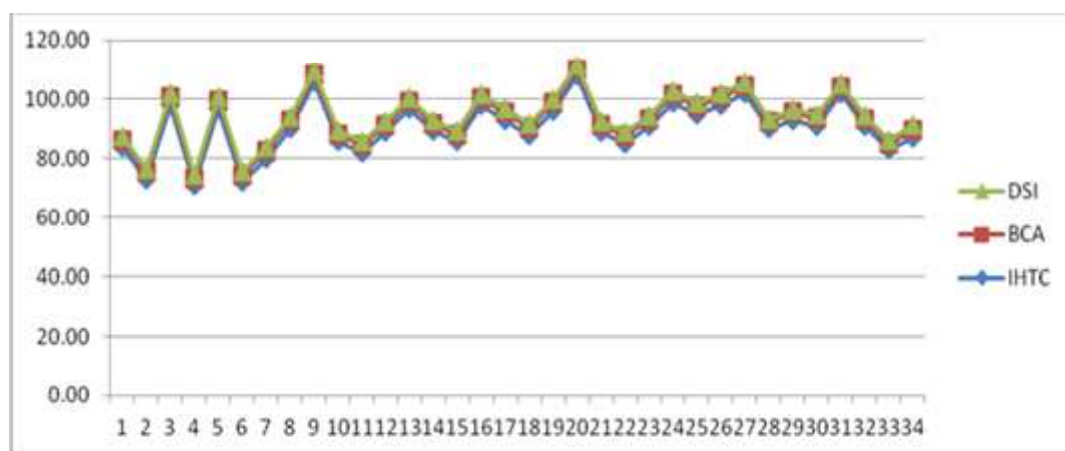


Table 4 Heat tolerance indices of Marathwadi buffalo and Deoni cattle (Mean $\pm$ S.E.)

Species	Age group	Iberia Heat Tolerance Coefficient (IHTC)	Benezara Coefficient of Adaptability (BCA)	Dairy Search Index (DSI)
Marathwadi Buffalo	0 -1 year	86.66 $\pm$ 1.98 ^a	2.43 $\pm$ 0.05	1.06 $\pm$ 0.01 ^a
	1 - 3 year	88.10 $\pm$ 3.25 ^a	2.55 $\pm$ 0.06	1.10 $\pm$ 0.01 ^b
	3 year & above	98.41 $\pm$ 1.88 ^b	2.38 $\pm$ 0.03	1.06 $\pm$ 0.01 ^a
Deoni Cattle	0 -1 year	85.66 $\pm$ 3.81	2.52 $\pm$ 0.07	1.13 $\pm$ 0.02
	1 - 3 year	89.33 $\pm$ 3.06	2.52 $\pm$ 0.08	1.13 $\pm$ 0.02
	3 year & above	93.30 $\pm$ 2.24	2.48 $\pm$ 0.05	1.12 $\pm$ 0.01

Mean having different superscripts (in capital letters) within the same column differs significantly ($P < 0.01$)

Similarly, In Deoni cattle the adaptability to the harsh climate of the Marathwada region was found to be better in the third group (i.e. 3 years of age) as than in other groups. However, in Deoni cattle contradictory results were observed in age groups 3 and above. Age groups 3 and above were found to be less adapted than in the other groups. The variation found might be due to the failure of these animals to acclimatize to stressful weather conditions and resulted in deviation from perfect values of thermoadaptability.

(Mandal and Tyagi 2008) reported that, Holstein Friesian and Sahiwal crossbred bulls was highest thermal adaptability during the winter season followed by summer and rainy. (Das, 2012)

also reported HTC was significantly lowered (1.884 ± 0.045) during December in crossbred calves (Holstein Friesian x Indigenous Local) indicating the highest adaptability of calves in winter. Therefore, HTC may help rank individual animals in respect of adaptability to existing environmental conditions.

The correlation analysis revealed a highly significant ($P < 0.01$) correlation (0.874) between BCA and DSI methods of heat tolerance indices in Marathwadi buffaloes. Similarly in Deoni cattle highly significant ($P < 0.01$) correlation (0.910) between BCA and DSI methods of heat tolerance indices (Table 4 & 5 and figure 1&2). Similar findings were recorded by Vaidya et.al (2018) in sheep and goat that artificial neural network was best fitted with

Table 5 Correlations amongst different methods of heat tolerance indices in Marathwadi buffaloes

		IHTC	BCA	DSI
IHTC	Spearman's rank correlations	1	-.110	-.227
	Sig. (2-tailed)		0.471	0.133
	N	45	45	45
BCA	Spearman's rank correlations	-.110	1	.874**
	Sig. (2-tailed)	.471		0.000
	N	45	45	45
DSI	Spearman's rank correlations	-.227	0.874**	1
	Sig. (2-tailed)	.133	0.000	
	N	45	45	45

in goat and sheep, with the adjusted R^2 of 0.93, explained by its linear relationship with the explanatory variables in goat. However, the prediction accuracy (R^2 value) was observed as 94.21% with 2.35 kg error. While in sheep, the adjusted R^2 was 0.82 and the prediction accuracy (R^2 value) was observed as 85.29% with 3.48 kg error.

Conclusions

The present study revealed that as per IHTC methods of Heat tolerance indices, Deoni cattle and Marathwadi Buffaloes showed better adaptability compared to another age group of animals at 3 years and above age to the harsh climatic conditions of the Marathwada region of Maharashtra. However, as per BCA and DSI, both Deoni cattle and Marathwadi buffaloes showed less stress before 3 years of age. However, the reliability of the Iberia Heat Tolerance Coefficient (IHTC) over BCA and DSI for assessing the adaptability of marathwadi buffaloes and Deoni cattle is better.

Further, it reveals that BCA and DSI indices showed highly significant correlations. IHTC is considered as best amongst the three indices studies as it explained better probability values as compared to the rest. Based on the result it can be concluded that the rectal temperature is the most reliable physiological parameter to assess the adaptability of Marathwadi buffaloes and Deoni cattle since, the rectal temperature is used for calculating adaptability in IHTC.

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RESEARCH ARTICLE

Milk production in productive life of selected dairy breeds in central region of Bihar: An economic analysis

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Abstract: The indigenous dairy breeds occupy a significant position in the national milk production. The share of milk production from indigenous breeds is about 41 per cent of the total milk production. These breeds have additional productive, physical and management advantages which can be captured by analyzing the milk production in whole productive life of an animal. The present study estimates the costs and returns of milk production in different lactations of productive life of selected dairy breeds namely, Graded Sahiwal, Graded Murrah and Crossbred in the Central Region of Bihar. Primary data was collected in respect of 108 animals of the selected dairy breeds in their different lactation groups using a pre-tested structured schedule. In order to choose animals of different lactation groups, Snowball sampling technique was used. The budgetary technique was employed to work out the costs and returns. The key findings were that the per litre cost of milk production increased by 1.12 times, 1.24 times and 1.03 times in Graded Sahiwal, Crossbred cow and Graded Murrah, respectively during 7th lactation and above as compared to 1st to 3rd lactation group. The net return per litre declined by 50 per cent, 65 per cent and 21 per cent in Graded Sahiwal, Crossbred cow and Graded Murrah, respectively for the same lactation groups. The increase in cost of maintenance and the decrease in net returns in the later lactations of productive

life were more in the case of Crossbred cow as compared to indigenous breeds. The net returns per animal per day for the Crossbred cow were negative after 8th lactation, while Graded Murrah and Graded Sahiwal were still having positive returns. It can be concluded that, indigenous breeds comprising Graded Sahiwal and Graded Murrah, were having more consistency in returns throughout the productive life as compared to the crossbred cows.

Keywords: Costs, Indigenous breeds, Lactations, Milk Production, Productive life, Returns

Introduction

The total milk production in India has increased more than 12 folds from 17 million tons in 1951-52 to 210 million tons in 2020-21 (GoI, 2021-22). Consequently, the livestock sector is contributing 4.11 per cent to the GDP of the country and 25.6 per cent to the agricultural GDP at constant price (GoI, 2021-22). About 90 per cent of this milk production is coming from milch bovine population of 303 million comprising cattle (63.78%) and buffaloes (36.22%). Among bovine population, India has a large population of Indigenous descriptive breeds which have survived over a long time and fit well into the agro-ecological environment of their habitat. There are about 50 recognized indigenous breeds of cattle and 19 buffalo in India (ICAR-NBAGR, 2022). The major indigenous cattle breeds of India are Gir, Red Sindhi, Sahiwal, Amrit Mahal, Umbalacheri, Deoni, Hariana, Ongole, Rath, Vechur, etc. The major indigenous breeds of buffalo are mainly Bhadawari, Jafarabadi, Mehsana, Murrah, Surti, etc. These indigenous breeds occupy a significant position in national milk production. The share of milk production from indigenous breeds was about 41 per cent of the total milk production in 2020-21 with 8.82 per cent and 32.13 per cent being the contribution of indigenous cattle and buffalo breeds, respectively (GoI, 2021-22). These breeds have additional productive, physical and management advantages, however, these are not captured in the conventional economics of milk production which are mostly based on single lactation studies.

Many studies have been carried out to find the costs and returns from milk production but for a single lactation (Kumawat, 2016;

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Keerthi S and Paramasivam P. 2019; Kumari et al. 2020; Lalrinsangpuii et al. 2016; Singh et al. 2019, and Mohapatra et al. 2020). In order to capture the whole productive life contribution, the present paper attempted to analyze costs and returns of milk production, breed-wise and lactation-wise in different lactations, and compare the same with crossbred cows.

Materials and Methods

Study area and sampling plan

The sampling design consisted of selecting the sample unit, i.e., animals of different age group from each breed. In each species of dairy animals, the three prominent breeds were identified in consultation with officials of the Department of Animal Husbandry and Dairying (DAHD), Bihar on the basis of their milk productivity. The Indigenous breeds selected were- Graded Sahiwal (cattle), Graded Murrah (buffalo) and the Crossbred cow selected was the Cross of Holstein Friesian (HF). The study was target at Central region of Bihar based on the report of Livestock sector analysis in Bihar (Singh et al. 2018) stating that Sahiwal breed of cattle, Murrah buffaloes and Jersey/ HF crossbred dairy animals are the suitable breeds for the region. The central region of Bihar contributed about 58 per cent of the total milk production of Bihar with 44 per cent of the cattle population (132.7 lakhs) and 41 per cent of buffalo population (87.8 lakhs) in the region. Keeping this in view, the study was conducted in the districts namely Vaishali, Patna, and parts of Muzaffarpur and Samastipur in the central region of Bihar.

Collection of data

A sample size of 108 animals were selected from three lactation groups of each selected breed i.e., 1-3 lactation number, 4-6 lactation number and 7 lactations to cover the productive life of a breed. In making these age groups, it was assumed that economic parameters do not vary much within one lactation group. In this process, 12 animals in each lactation group were selected making a total sample size of 36 animals for each breed. The snowball sampling technique was followed to identify animals of different lactation groups with the farmers. Primary data was collected from the respondents during January- March, 2021 by using pre-tested survey schedule.

Analytical technique

Budgetary technique was used to estimate the costs and returns of milk production in each lactation group. The costs and returns of animals falling in each lactation groups were aggregated and average was calculated per day per animal.

Cost of milk production in each lactation group

In estimation of costs of milk production in each lactation group, standard methodology was followed and the same was combined

for all lactation groups to calculate lifetime costs. The total costs involved in milk production comprise both fixed costs and variable costs. In computation of different costs for the selected dairy breeds in different lactation groups, the following methodology was adopted.

Fixed costs (FC)

Various components of fixed cost include depreciation and interest on fixed assets. Fixed costs are the expenditure incurred by the producer irrespective of the level of production. It does not vary with the output and remain unchanged in the short run. Since, Capital Recovery Cost (CRC) method was used for estimating depreciation, interest on fixed capital was not estimated separately as the CRC approach takes care of it. CRC method gives an annual cost of depreciation over the useful life of the asset which in turn provides the economic rate of return on investment. The formula for estimation of CRC is given by:

$$\text{CRC (Rs/year)} = \frac{Z \{(1+r)^n r\}}{\{(1+r)^n - 1\}}$$

where, **Z** is the initial value of the capital asset, **r** is the interest rate and **n** is the useful life of the assets.

The fixed costs were the joint costs per year incurred on all animals in a herd. Fixed assets like cattle shed, feed manger, chaff cutter, milk cans and buckets etc. were used collectively by the farmer for all cattle irrespective of their age and sex which act as a joint cost for all the animals in that herd. In order to apportion it per animal, the animals in a herd were converted to Standard Animal Units (SAUs). Considering the differences in regional endowments of animal wealth and species, the dairy animals have been converted into SAUs using factors suggested by Sirohi et al. (2015) for the Eastern region.

Variable Costs (VC)

Variable costs are those costs that are incurred on the variable factors of production and can be altered in the short run. It includes feed and fodder cost, labour cost, veterinary and miscellaneous expenses. Data on the variable expenses were collected directly from the farmers for animals in different lactations.

Feed and fodder cost

It includes costs of green fodder, dry fodder and concentrates fed to animals. The cost was estimated as a product of quantity of certain feed/fodder fed to animals and the purchase price/imputed cost of respective feed.

Labour cost

The information regarding labour requirements for various farm operations and labour costs were recorded during the personal interview with the respondents. The cost of labour was calculated considering type of work allotted and wages paid, whereas, family labour costs were determined based on the existing wage rate of permanent farm labour.

Veterinary and miscellaneous expenses

Veterinary expenses comprised of medicines, doctors' fees, vaccination charges as well as natural and artificial insemination charges. Miscellaneous expenditure included electricity, water charges, cost of rope, expenses on repairing fixed assets, insurance charges and other related expenses. As these expenses were joint costs, hence, apportioning was done in the same way as mentioned above in case of the fixed costs.

Calculation of Gross and Net costs

Gross Cost (GC)

It is the total cost incurred by the producer which was estimated by adding all the cost components including fixed and variable costs.

Gross Cost (GC) = Total Fixed Cost (TFC) + Total Variable Cost (TVC)

Net Cost: The net cost was estimated by deducting the imputed income earned through dung, from the gross cost.

Net Cost (NC) = GC – Value of the dung

Value of dung

This was a joint product in milk production. Dung was either used as manure or for fuel purpose after drying. In the available studies, the approach for ascertaining the quantity of dung produced by the milch animal stock has been ad-hoc in nature. The method which was applied to calculate the amount of dung produced was based on the digestibility of various feed and fodder fed to the animals. The digestibility of prevalent feed and fodder of a region were available from literature on animal nutrition. Based on this, the dung output has been computed for individual animals (Sirohi et al. 2015).

Cost of milk production

To estimate the cost per litre of milk in each lactation, the average net maintenance cost per animal per day in that lactation was divided by the average milk of animal per day in the same lactation.

Cost per litre of milk

$$(\text{₹}/\text{L}) = \frac{\text{Net cost per animal per day}}{\text{Average milk yield of animal}}$$

Estimation of returns of milk production in each lactation

The return in this case includes the value of milk only. Gross returns were obtained by multiplying the milk yield of an individual animal with the price received by farmer for milk of that particular breed in lactation group. The total milk output was taken as milk obtained in pail after feeding the calf.

Gross Returns (GR) = (Milk produced/animal /day) × Price of Milk received by farmer

Net Returns

Net returns were calculated by subtracting net costs from gross returns.

$$\text{Net Returns} = \text{Gross Returns} - \text{Net Cost}$$

Net return per litre of milk

It was calculated by dividing net returns per day by the average milk yield (AMY) of the respective breed in given lactation group.

Results and Discussion

The milk is the main output in dairying and its value is the major component of returns of a dairy breed. On the other side, costs depend on breeding, feeding and management practices followed in individual selected breeds namely, Graded Sahiwal, crossbred and Graded Murrah. So, an analysis of costs and returns was done for each breed in different lactation groups.

Costs and returns of milk production from Graded Sahiwal in different lactation groups.

The total gross cost of maintenance of Graded Sahiwal animal was ₹ 155.96 per day during 1st to 3rd lactation group (Table 1). It increased to ₹ 162.15 per day during 4th to 6th lactation and then decreased to ₹ 119.77 per day. It got decreased by almost 26 per cent during ≥7th lactation group as compared to cost during 1st to 3rd lactation. The major reason for increase and subsequent decrease in maintenance cost was mainly due to the change in total feed and fodder costs. The feed and fodder cost counted for 71 to 75 per cent in the gross costs of maintenance of Graded Sahiwal cow during different lactation groups. There was a dip of about 31 per cent in cost of feed and fodder in last lactation group as compared to previous lactation group. Among other costs, changes were similar but reduction was not substantial. On the contrary, the veterinary cost was found the highest during ≥ 7th lactation (₹ 3.51 per day) which was only ₹ 2.07 and ₹ 2.68 per day during 1st to 3rd and 4th to 6th lactation, respectively mainly because of frequent health issues during ≥ 7th lactation. The total variable cost which ranged between 89 to 91 per cent of the gross cost, was ₹ 138.35, ₹ 147.36, and ₹ 106.97 per day during 1st to 3rd, 4th to 6th and ≥7th lactations, respectively. The gross return

was estimated by multiplying the average milk yield by the price of milk received by the farmer. It was found to be stable up to 4th to 6th lactation but reduced considerably during 7th lactation, mainly because of significant decrease in average milk yield though the farmer received higher price for milk during this lactation group due to high fat percentage. The gross return got reduced by 31 per cent during $\geq 7^{\text{th}}$ lactation as compared to 1st to 3rd lactation. The per litre cost of milk production was ₹ 27.54, ₹ 28.24, and ₹ 30.96 per day during 1st to 3rd, 4th to 6th and $\geq 7^{\text{th}}$ lactation group, respectively. The per litre cost of milk production increased by 1.12 times during $\geq 7^{\text{th}}$ lactation as compared during 1st to 3rd due to lower milk productivity in later lactations. The net return obtained per litre of milk was ₹ 6.61, ₹ 5.76, and ₹ 3.33 per day during 1st to 3rd, 4th to 6th and $\geq 7^{\text{th}}$ lactation groups, respectively. Hence, it can be concluded that the net return per litre declined by almost two times (1.98) during $\geq 7^{\text{th}}$ lactation mainly due to low milk yield but it still remains to be positive.

Costs and returns of milk production from crossbred in different lactation groups.

As evident from the Table 2, the total gross cost of maintenance of crossbred animal was ₹ 230.80 per day during 1st to 3rd lactation group and then it decreased to ₹ 219.74 per day during 4th to 6th lactation and ₹ 156.49 per day $\geq 7^{\text{th}}$ lactation group. It got decreased by almost 32 per cent during $\geq 7^{\text{th}}$ lactation group as compared to cost during 1st to 3rd lactation. The feed and fodder cost counted for about 70 per cent in the gross costs of maintenance of

crossbred cow during different lactation groups. It was ₹ 161.46 during 1st to 3rd, ₹ 152.54 per day during 4th to 6th lactation and ₹ 107.44 per day during $\geq 7^{\text{th}}$ lactation, registering a decrease of about 33 per cent compare to 1st to 3rd lactation group. Among other costs, trend was similar but reduction was not substantial. On the contrary, the veterinary cost was found the highest during $\geq 7^{\text{th}}$ lactation (₹ 7.1 per day) which was almost 38 per cent more than 1st to 3rd lactation. It shows that veterinary cost increased in the last lactation group of the crossbred because of frequent health disorders. The fixed cost ranged between 10 to 15 per cent to the gross cost during different lactation groups. The total variable cost which ranged between 84 to 89 per cent of the gross cost, was ₹ 195.29 during 1st to 3rd lactation, ₹ 191.38 during 4th to 6th lactation and ₹ 139.38 during $\geq 7^{\text{th}}$ lactation. It got declined by about 46 per cent from 1st to 3rd lactation to $\geq 7^{\text{th}}$ lactation mainly because of significant decrease in average milk yield. The gross return was ₹ 284.94 per day during 1st to 3rd, ₹ 250.65 during 4th to 6th lactation, and ₹ 153.10 during $\geq 7^{\text{th}}$ lactation. The per litre cost of milk production was ₹ 23.48 during 1st to 3rd lactation, ₹ 25.03 during 4th to 6th lactation and ₹ 29.04 per day during $\geq 7^{\text{th}}$ lactation group, respectively. The per litre cost of milk production increased by 1.24 times during $\geq 7^{\text{th}}$ lactation group as compared during 1st to 3rd lactation mainly due to lower milk productivity in later lactations. The net return obtained per litre of milk was ₹ 8.11, ₹ 6.38, and ₹ 2.79 per day during 1st to 3rd, 4th to 6th and $\geq 7^{\text{th}}$ lactation groups, respectively. Hence, it can be concluded that

Table 1: Costs and returns of milk production from Graded Sahiwal in different lactation groups (₹/animal/day)

Particulars	Lactation Groups		
	1 st to 3 rd lactation	4 th to 6 th lactation	$\geq 7^{\text{th}}$ lactation
Total Fixed Cost (TFC)	17.61(11.29)	14.79(9.12)	12.80(10.69)
Green fodder (F1)	11.65(7.47)	13.37(8.25)	10.16(8.48)
Dry fodder (F2)	38.49(24.68)	42.26(26.06)	34.63(28.91)
Concentrate (F3)	67.53(43.30)	68.77(42.41)	40.78(34.05)
Feed and fodder cost (V1=F1+F2+F3)	117.67(75.45)	124.40(76.72)	85.57(71.45)
Labour cost (V2)	17.38(11.14)	18.95(11.69)	16.75(13.99)
Veterinary Cost(V3)	2.07(1.33)	2.68(1.65)	3.51(2.93)
Miscellaneous cost (V4)	1.23(0.79)	1.33(0.82)	1.14(0.95)
Total Variable Cost (TVC=V1+V2+V3+V4)	138.35(88.71)	147.36(90.88)	106.97(89.31)
Gross Cost (A=TFC+TVC)	155.96(100)	162.15(100)	119.77(100)
Value of Dung (B)	16.03	17.83	12.34
Net Cost (C=A-B)	139.93	144.32	107.43
Price of milk (₹/L) (P)	34.15	34	34.29
Average milk productivity (L/ animal/day) (E)	5.08	5.11	3.47
Gross Return (D= AMY*P)	173.48	173.74	118.99
Net Returns (D-C)	33.55	29.42	11.56
Cost of milk production (₹/L) (C/E)	27.54	28.24	30.96
Net Returns (₹/L) (D/E)	6.61	5.76	3.33

Figures in parentheses indicate the percentage of gross cost

the net return per litre declined by almost 65 per cent from 1st to 3rd lactation to $\geq 7^{\text{th}}$ lactation.

Costs and returns of milk production from Graded Murrah in different lactation groups.

The total gross cost of maintenance of Graded Murrah animal was ₹ 224.03 per day during 1st to 3rd lactation group. It increased to ₹ 228.71 per day during 4th to 6th lactation and then decreased to ₹ 153.61 per day. It got decreased by almost 31 per cent during $\geq 7^{\text{th}}$ lactation group as compared to cost during 1st to 3rd lactation.

Table 2: Costs and returns of milk production from crossbred in different lactation groups (₹/animal/day)

Particulars	Lactation Groups		
	1 st to 3 rd lactation	4 th to 6 th lactation	$\geq 7^{\text{th}}$ lactation
Total Fixed Cost (TFC)	35.51(15.39)	28.36(12.91)	17.11(10.93)
Green fodder (F1)	15.86(6.87)	17.27(7.86)	15.44(9.87)
Dry fodder (F2)	51.29(22.22)	54.04(24.59)	44.25(28.28)
Concentrate (F3)	94.31(40.86)	81.23(36.97)	47.75(30.51)
Feed and fodder cost (V1=F1+F2+F3)	161.46(69.96)	152.54(69.42)	107.44(68.66)
Labour cost (V2)	27.29(11.82)	30.63(13.94)	22.74(14.53)
Veterinary Cost(V3)	4.4(1.91)	6.05(2.75)	7.1(4.54)
Miscellaneous cost (V4)	2.14(0.93)	2.16(0.98)	2.1(1.34)
Total Variable Cost (TVC=V1+V2+V3+V4)	195.29(84.61)	191.38(87.09)	139.38(89.07)
Gross Cost (A=TFC+TVC)	230.80(100)	219.74(100)	156.49(100)
Value of Dung (B)	18.97	20.04	16.81
Net Cost (C=A-B)	211.83	199.70	139.68
Price of milk (₹/L) (P)	31.59	31.41	31.83
Average milk productivity (L/ animal/day) (E)	9.02	7.98	4.81
Gross Return (D= AMY*P)	284.94	250.65	153.10
Net Returns (D-C)	73.11	50.95	13.42
Cost of milk production (₹/L) (C/E)	23.48	25.03	29.04
Net Returns (₹/L) (D/E)	8.11	6.38	2.79

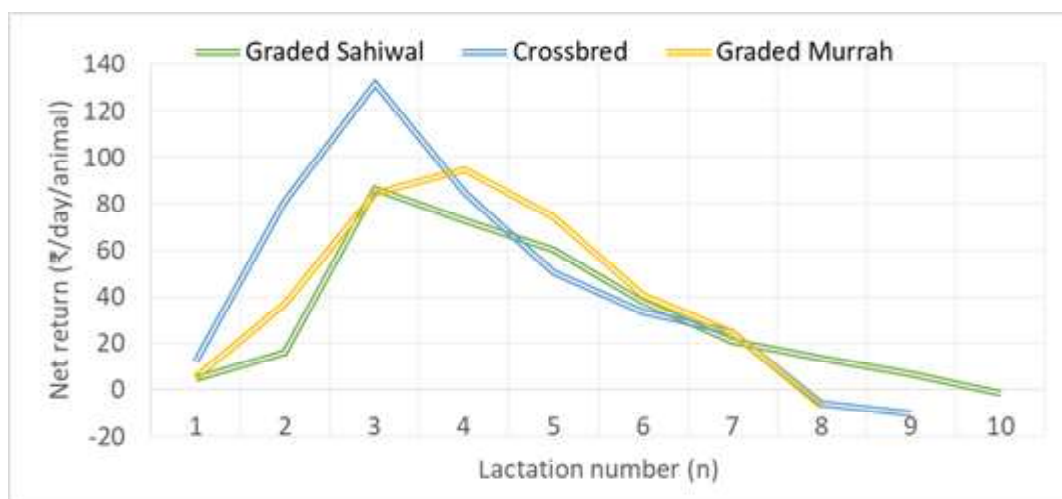
Figures within parentheses indicate the percentage of gross cost

Table 3: Costs and returns of milk production from Graded Murrah in different lactation groups (₹/animal/day)

Particulars	Lactation Groups		
	1 st to 3 rd lactation	4 th to 6 th lactation	$\geq 7^{\text{th}}$ lactation
Total Fixed Cost (TFC)	26.03(11.62)	22.25(9.73)	10.62(6.91)
Green fodder (F1)	17.36(7.75)	17.41(7.61)	14.62(9.52)
Dry fodder (F2)	57.04(25.46)	57.5(25.14)	49.88(32.47)
Concentrate (F3)	98.3(43.88)	100.99(44.16)	51.15(33.30)
Feed and fodder cost (V1=F1+F2+F3)	172.70(77.09)	175.90(76.91)	115.65(75.29)
Labour cost (V2)	20.36(9.09)	24.56(10.74)	20.25(13.18)
Veterinary Cost(V3)	3.53(1.58)	4.77(2.09)	5.91(3.85)
Miscellaneous cost (V4)	1.41(0.63)	1.23(0.54)	1.18(0.77)
Total Variable Cost (TVC=V1+V2+V3+V4)	198.00(88.38)	206.46(90.27)	142.99(93.09)
Gross Cost (A=TFC+TVC)	224.03(100)	228.71(100)	153.61(100)
Value of Dung (B)	23.46	24.17	19.56
Net Cost (C=A-B)	200.57	204.54	134.05
Price of milk (₹/L) (P)	46.30	45.87	46.05
Average milk productivity (L/ animal/day) (E)	5.13	5.36	3.32
Gross Return (D= AMY*P)	237.52	245.86	152.89
Net Returns (D-C)	36.95	41.32	18.84
Cost of milk production (₹/L) (C/E)	39.10	38.16	40.38
Net Returns (₹/L) (D/E)	7.20	7.71	5.67

Figures within parentheses indicate the percentage of gross cost

Fig. 1 Relationship between net return and lactation number of the selected breeds



The major reason behind less expenses was decrease in feed and fodder costs. The feed and fodder cost counted for 75 to 77 per cent in the gross costs of maintenance of Graded Murrah during different lactation groups. It was ₹ 172.70 and ₹ 175.90 per day during 1st to 3rd and 4th to 6th lactation but reduced to ₹ 115.65 per day during $\geq 7^{\text{th}}$ lactation registering a dip of about 33 per cent compared to previous lactation group. The share of concentrate was the highest (almost 40 per cent) because of the high market price of the concentrates. The fixed cost ranged between 6 to 11 per cent to the gross cost during different lactation groups. It was ₹ 26.03, ₹ 22.25, and ₹ 10.62 per day during 1st to 3rd, 4th to 6th, and $\geq 7^{\text{th}}$ lactation groups, respectively. Fixed cost decreased by 59 per cent in last lactation group due to reduce in value of animals. The total variable cost which ranged between 88 to 93 per cent of the gross cost, was ₹ 198.00, ₹ 206.46, and ₹ 142.99 per day during 1st to 3rd, 4th to 6th and $\geq 7^{\text{th}}$ lactations, respectively. The gross return was estimated to be ₹ 237.52 during 1st to 3rd lactation which increased to ₹ 245.86 during 4th to 6th lactation but reduced considerably to ₹ 152.89 during $\geq 7^{\text{th}}$ lactation mainly because of significant decrease in average milk yield. It was estimated that gross return decreased by about 35 per cent during $\geq 7^{\text{th}}$ lactations as compared to 1st to 3rd lactation. The per litre cost of milk production was ₹ 39.10, ₹ 38.16, and ₹ 40.38 per day during 1st to 3rd, 4th to 6th and $\geq 7^{\text{th}}$ lactation group, respectively. The per litre cost of milk production increased by only 1.03 times during $\geq 7^{\text{th}}$ lactation as compared during 1st to 3rd lactation. This increment was less as compared to increment in crossbred and Graded Sahiwal. The net return obtained per litre of milk was ₹ 7.20, ₹ 7.71, and ₹ 5.67 per day during 1st to 3rd, 4th to 6th and $\geq 7^{\text{th}}$ lactation groups, respectively. Hence, it can be concluded that net return per litre declined by almost 21 per cent during $\geq 7^{\text{th}}$ lactation mainly due to lower milk yield. It was observed that, return has not substantially decreased in last lactation groups of Graded Murrah as compared to crossbred (65 per cent) and Graded Sahiwal (50 per cent).

A line chart in Fig. 1 shows the relationship between net returns (₹ /day/animal) to the lactation number of the selected dairy breeds. It depicts that net return per day for the crossbred increased till 3rd lactation and then declined sharply in the subsequent lactation numbers. It increased for Graded Sahiwal till 3rd lactation but was having less return as compared to crossbred which can be inferred from the peak point of the graph. It was further observed that the net return per day initially increased for Graded Murrah till 4th lactation and then started to decline. With passing of 4th lactation, Graded Sahiwal and Graded Murrah were at par in the net return per day as compared to crossbred.

The net returns per day was negative for the Crossbred cow and the curve dipped down to negative quite before Graded Murrah and Graded Sahiwal. It shows that the Crossbred cow was having negative returns after 8th lactation, whereas Graded Murrah and Graded Sahiwal were still having positive returns and thereby, showing the persistency of indigenous breeds (Graded Sahiwal and Graded Murrah) in later lactations. So, it can be concluded net return per day was found to be more for Graded Murrah and Graded Sahiwal as compared to Crossbred cow in the late lactations.

Conclusions

Indigenous breeds were more profitable in later lactations as compared to crossbred. Crossbred though was having the highest net return from milk production per day per animal up to 4th lactation but exhibited a sharp dip in milk production and profit per animal with the increase in lactation number. Graded Sahiwal found to be more consistent in milk production throughout the lactation period as compared to crossbred. Farmers also reported frequent health issues during late lactations in case of crossbred cattle as compared to other two breeds thereby increasing the cost of milk production. The plotting of net returns (₹ /day/animal) against the lactation numbers of the selected dairy breeds

indicated that the net returns per animal from crossbred cow decline sharply after third lactation and turns out to be negative after seventh lactation. In case of Graded Sahiwal, the net return per day per animal becomes even higher than crossbred and Graded Murrah after 4th lactation but also remains positive till 10th lactation. The per day maintenance cost of Graded Sahiwal was the lowest among all the three breeds. Hence, indigenous breeds are the only alternative to farmers having limited resource in the resource scarce areas. These breeds should be conserved as these breeds require lower per day cost of maintenance and thrive better in poor endowment regions. From the study, it can be concluded that indigenous breeds are bound to fetch higher returns to resource poor farmers if production is linked to markets with demand preferences for organic and indigenous cow milk.

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RESEARCH ARTICLE

Strategic framework construction for sustainable livelihood of livestock farmers in drought prone areas: A participatory approach from Odisha

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Abstract: Livestock rearing is a critical component for ensuring sustainable livelihood particularly in drought prone areas. But for those farm households rearing livestock in western dry regions of Odisha, drought poses multiple challenges due to heat stress thus resulting in decreased milk productivity and farmer's income. It is imperative to find out the pertaining issues due to drought among the livestock reared and what suitable strategies are necessitated to minimize the impact of drought holds importance. The state of Odisha was purposively selected for the participatory study due to the incidence of severe drought occurrences predominantly in the western parts of the state. The objective of the study was to construct a framework of strategies in consultation with famers and other stakeholders of livestock sector. Exploratory research design was followed in carrying out this research study involving focus group discussions and transect walk in four randomly selected villages from the drought prone areas. The key problems among livestock found in the study area were anestrus, repeat breeding, lack of pasture grass for grazing and high susceptibility to diseases and decreased feed intake. Pairwise ranking matrix was developed to prioritise and rank the problems. Appropriate strategies were formulated against each problem which can be carried out at farm and community level by the livestock farmers to mitigate the drought impacts and a sustainable livelihood. Some of the important strategies suggested to livestock farm households include

balanced nutrition feeding, restricting wallowing of animals, exploring unconventional feeds, establishing fodder banks and Pani Panchayats, carrying out artificial insemination following AM-PM rule among others.

Keyword: Drought, Framework, Livelihood, Livestock farmer, Strategies

Introduction

Livestock farming plays an important role in poverty reduction and providing food security to millions of people in rural areas. In India, Gupta (2018) reported of about 70 percent rural households rearing livestock; and majority of these livestock owners are landless or small and marginal farmers because of their limited land accessibility for crop cultivation. Considering the climatic vagaries and high concentration of poor people in arid and rain-fed regions, the importance attributed to livestock farming is quite high (Kumar and Singh 2008). Thus livestock farming acts as best insurance agent in the backdrop of occurrence of climate induced natural disasters such as drought.

FAO (2018) study found that among all climate related disasters; droughts, floods and tropical storms seriously affect agriculture sectors. The damage and losses by drought alone amounts to 80 percent; severely impacting agriculture sectors specifically livestock farming followed by crop production. Rojas Downing (2017) study has highlighted critical factors for livestock production affected by droughts. In livestock farming, drought condition is associated with water scarcity, lack of feed and fodder availability, fodder crops inability to grow. Animals suffer from reduced feed intake, poor growth rate, loss of body weight, susceptibility to diseases and infections and issues related to production and reproduction insufficiencies. The production and reproduction issues observed in animals during drought take 2-3 years to be restored if the animals are reared without suitable strategies (Mohanty et al. 2019). Heat stress is also seen among the animals during drought years and the impact is more on high milk producing animals due to increased metabolism levels (Dash et al. 2016). In buffaloes, effects of heat stress is more pronounced due to high thermal load, lack of wallowing places and less number of swallowing glands (Kebede, 2016). Hence for maintaining

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reproductive efficiency, milk production potential and optimum forage utilization; appropriate drought mitigation strategy ought to be carried out by the farmers to sustain their livelihood. Droughts also lead to temporary loss of farmer's livelihood disrupting their income generating activities with a series of hardships.

In India, 75 percent of monsoon (annual rainfall) is received in a short span of four months (June to September). The variability in the quantum, onset and withdrawal of rainfall has long lasting impact on livestock farming (Mohanty et al. 2019). National Agricultural Drought Assessment and Monitoring System (NADAMS) provide real time information on prevalence, persistence and severity level of agricultural drought at state/district/sub-district level. NADAMS (2018) covers 17 predominant agriculture based states which are prone to drought situation including Odisha. The occurrence of droughts in Odisha is a common feature in almost every alternate year while the districts of western Odisha severely suffers from frequent droughts (Panda, 2015). The districts of western Odisha are among the most backward regions in India. Samal et al. (2016) calculated the probability of occurrence of drought in the state of Odisha. The probability was found to be 0.6 implying that out of ten years, the chances of drought would be in six years which indicates the frequent occurrence of droughts in the state. This region faces very high poverty equivalent to 59.10 percent that is 30 percent higher than all India average for rural areas and also are among the most drought prone districts of the country (PACS, 2008). The livelihoods of small and marginal farmers are severely affected because of low asset base in the occurrence of any natural disaster. In Odisha, there are 46.47 lakh operational holdings out of which 91.80 percent are marginal and small holders. On the other hand, about 85 percent of livestock is owned by the landless, marginal and small landholding households (Odisha Economic Survey, 2018). With two-third population of Odisha remaining Below Poverty Line (BPL); in the events of disaster like drought, they are highly vulnerable and act as source of distress for farmers. A holistic development of livestock farmers in drought affected areas can be attained through in-depth location specific participatory study to understand their problems and suggesting suitable strategies. These strategies will help the livestock farmers in the future events of drought occurrences to tackle negative impacts thereby building resilient livelihoods.

Materials and Methods

The participatory study on constructing a framework of strategies for livestock farmers in drought affected areas was carried out in the purposively selected state of Odisha. A comprehensive review of the publications Panda (2015); Rao et al. (2018) and OSDMA (2017) were carried out to identify the drought affected districts of the state. After analysing the reviews, 8 drought prone districts (Sambalpur, Sundargarh, Jharsuguda, Bargarh, Balangir, Kalahandi, Nuapada and Deogarh) were identified predominantly

belonging to western parts of Odisha as depicted in **fig.1**. From the aforementioned 8 drought prone districts, two districts Balangir and Nuapada were selected randomly for carrying out this participatory research. For formulating strategies for livestock farmers in drought prone areas, exploratory research design was followed which included participatory tools such as focus group discussion (FGD), transect walk, experience survey from experts in the field of livestock and climate change research and in-depth study of secondary literature (books, journal articles and reports). The participatory exercises were carried in four villages Khasbahal and Kutenpali (Balangir); Dharambandha and Uparpita (Nuapada).

Strategies are the suggestions provided to the livestock farmers rearing animals in drought areas, thus improving their adaptive capacity in dealing with adverse effects of climate vulnerabilities. This exercise of formulating strategies was a participatory exercise which involved four sequential steps as follows:

- i. Stakeholder's selection
- ii. Meeting with the stakeholders
- iii. Identification and prioritization of problems
- iv. Development of the framework

A thorough discussion elaborating the activities and methods followed in carrying out the selection of stakeholders and meeting them for constructing framework of strategies has been mentioned below-

Stakeholder's selection

Stakeholders are the key persons involved in a specific field/area with variable level of interest and influence. Hence, identifying the key stakeholders and ensuring their fair representation is critical in formulating suitable strategies. The selection of stakeholders was carried out with the help of secondary literature, consulting experts and also the pilot studies conducted in non-sampled areas. The key stakeholders selected for the study were livestock farmers (mix of landless, marginal, small and progressive farmers of different age groups), Veterinary Doctor, Livestock Inspector, Assistant Agriculture Officer, Agriculture Technology Management Agency (ATMA) official and Farmers friend (*Krushak Saathi*).

Meeting with the stakeholders

In the study area villages, the stakeholders were personally contacted so as to fix a suitable date and time to meet. The stakeholders were informed in advance regarding the nature of the project and the agenda for the discussion. The participatory exercises to be carried out were also finalized in consultation with the stakeholders. Meeting with the stakeholders was done

Fig. 1 Depiction of selected drought affected districts (Balangir and Nuapada) of Odisha



Table 1: Places of focus group discussions and participants selected

Study Area	Block	Village	Participants
Balangir	Saintala	Khasbahal	Total 11 Participants 7 livestock farmers, 1 Veterinary Doctor, 1 Livestock Inspector, 1 Assistant Agriculture Officer, 1 <i>Krushak Saathi</i>
	Puintala	Kutenpali	Total 15 Participants 11 livestock farmers, 1 Veterinary Doctor, 1 Livestock Inspector, 1 ATMA official, 1 <i>Krushak Saathi</i>
Nuapada	Nuapada	Dharambandha	Total 12 Participants 8 livestock farmers, 1 Veterinary Doctor, 2 Livestock Inspector, 1 <i>Krushak Saathi</i>
	Khariar	Uparpita	Total 13 Participants 8 livestock farmers, 1 ATMA official, 1 Veterinary Doctor, 2 Livestock Inspector, 1 Assistant Agriculture Officer

on the prefixed date and time. The participatory exercises were transect walk followed by focus group discussion. Personal observations were also a critical activity carried out to gain further clarifications and understanding on various aspects of livestock rearing in the study area and effect of drought on their animals and livelihood. A total of four focus group discussions were carried out; two each in Nuapada and Balangir districts (Table-1). Transect walk were carried out in all the four villages where focus group discussion was conducted.

Preference ranking method

Preference ranking method is a participatory tool to get an idea of what people think are priority problems. Pairwise ranking matrix is used to choose among the pairs of problems, which one of the

problem is bigger compared to the other. A pairwise matrix has two identical set of problems/alternatives one across the x-axis (top) and other across the y-axis (left side). This is a group exercise where discussions are carried out among the pair of problems while exploring the possibility of preferring one problem over the other. The steps are as follows given by Pretty (1995):-

- The FGD participants of each village were asked to delineate the problems affecting livestock rearing due to drought. The key problems identified from the participants in four villages were scarcity of water, lack of pasture grasses, shortage of green and dry fodder, repeat breeding, anestrus problem, high susceptibility to diseases and decreased feed intake.

- The problems highlighted were noted in separate cards for each problem.
- Two cards were placed among the participants for discussion and choose the bigger problem from the pair.
- Note the response in the appropriate box of the pairwise ranking matrix. The pairwise matrix was constructed for four villages of the study area.
- Present a different pair and repeat the comparison until all possible combinations have been considered for selection.
- Prepare a tabular list of the problems depicting total number of preferential choices each problem has received.
- Collating the data from four villages, the final list is prepared showing the total number of preferential choices each problem has received and ranking them accordingly as depicted in Table- 2
- Finally based on the prioritized problems, discussion is carried out among the participants for potential solutions.

Results and Discussion

Framework is defined as a basic conceptual structure with a system of rules or ideas which helps to decide what to do. In order to mitigate drought impact, a framework delineates various steps or procedures which help the livestock farmers to decide what the necessary actions are ought to be taken as ex-ante and ex-post measures. This section discusses how the problems were identified and prioritized and finally the development of framework.

Identification and prioritization of problems

The identified stakeholders in the study area were involved in discussion on the predetermined topics related to breeding, feeding and healthcare management so as to ensure that the discussion does not go off the track. The specific set of problems

related to livestock rearing were identified and duly noted in cards. The identified problems were re-discussed so as to understand how critical their affect was on livestock rearing in the events of drought. Personal observations and experience gained during personal visit to farmers and secondary literature also helped in the gathering thorough understanding of the problems in due consultation with the stakeholders. For livestock reared in the drought affected areas; it was observed that their reproductive and productive parameters were deviating from the normal conditions. The key problems with respect to different aspects of livestock rearing like breeding, feeding, health care management which determine the productive and reproductive performance were delineated. The underlying problems elucidated by the FGD participants were: feeding (scarcity of water, lack of pasture grasses, shortage of green and dry fodder); breeding (repeat breeding, anestrus problem) and management (high susceptibility to diseases and decreased feed intake). The problems observed among the livestock reared in the study area during drought events were in line to the findings of Thornton et al. (2009) and Nardone et al. (2010). Prioritization of the problems is important in formulation of location and disaster specific strategies following pairwise ranking matrices as suggested by Pretty et al. (1995). The two critical issues the participants highlighted in the event of disaster were related to feeding and health care aspects. Ensuring availability of proper feeds and drinking water to animals and disease occurrences during drought was affecting the productivity of the animals and overall livelihood of the farmers.

Development of the framework

For the strategies to be prudent and accepted by the livestock farmers; along with the problems, the necessary suggestions should also come from the stakeholders. The farmers being the ultimate user of the strategies, developing location specific solutions is necessary for mitigating the impact of drought. The problems identified were discussed with experts (Scientists of NDRI, Resource persons at OUAT, Bhubaneswar and KVK, Balangir) to find out perspective solutions. Secondary data sources from research publications, policy papers, institute reports etc. were also referred for searching suitable solutions. The suggestions and perspective solutions obtained at three

Table 2: Preference and priority wise ranking of problems by FGD participants in the study area

Problems	No. of times the problem preferred				Total preferences	Rank
	Khasbahal	Kutenpali	Dharam-bandha	Uparpita		
Scarcity of water	5	3	3	2	13	IV
Shortage of dry fodder	1	2	1	4	8	VI
Repeat breeding	0	2	3	2	7	VII
Lack of pasture grasses	4	5	3	6	18	I
High susceptibility to diseases and decreased feed intake	3	3	5	4	15	II
Anestrus problem	4	4	4	2	14	III
Shortage of green fodder	4	2	2	1	9	V

Table 3: Strategies for breeding management of livestock in drought affected areas

Breeding	Strategies	
	Farmers Level	Community Level
Anestrus Problem	1. Feed vitamins and mineral mixture to lactating animals 2. Allow the anestrus animals for grazing to ensure male exposure 3. Try to maintain the record of estrus and insemination 4. Consult the local veterinarian for check-up and subsequent follow up practices	Organizing regular infertility camps at village level by veterinarian
Repeat Breeding	1. Ensure balanced nutrition feeding to the animals 2. Carry natural service of animal in heat with high pedigree bull 3. Provide mineral mixture @50g/day to repeat breeder livestock 4. Follow correct time of A.I. according to AM-PM rule 5. Providing adequate veterinary service	Awareness among livestock rearers regarding AM-PM rule of A.I.

**** AM – PM Rule:** The time-tested rule suggests when to breed a female bovine (cattle/ buffalo) in estrus for artificial insemination (A.I.).

According to AM-PM rule, a female bovine should receive A.I. 12 hours after being observed in estrus.

1) A female bovine observed in standing heat in the morning should be bred the afternoon of the same day whereas,

2) A female bovine observed in standing heat in the afternoon or evening should be bred the following morning.

Table 4: Strategies for feeding management of livestock in drought affected areas

Feeding	Strategies	
	Farmers Level	Community Level
Lack of Pasture Grasses	1. On agricultural field bunds, planting Sesbania, Subabul or Neem to be encouraged among the farmers	Develop village gauchar (grazing) lands for fodder production.
Scarcity of Water	1. Restrict/minimize wallowing of animals in water bodies 2. Avoid long distance grazing of livestock	Formation of Pani panchayat Desilting of ponds in the village Adopting different water conservation methods at village level to improve the ground water level
Shortage of Green Fodder	1. Explore the use of unconventional feeds such as Acacia pods, jackfruit leaves, babul leaves, banana stem etc. and also crop residues, seed pods 2. Poor quality roughages can be enriched by urea or ammonia treatment before feeding them to animals 3. The crops failed due to lack of rainfall can be utilized for feeding of animals 4. Cultivating short duration fodder varieties of sorghum (Pusa chari, Pant chari etc.) which are drought resistant	Establishing fodder banks in common property resource areas such as waste lands, grazing lands, uncultivated lands etc. Promote cultivation of short duration fodder crops with input subsidy or incentives
Shortage of Dry Fodder	1. Sowing of cereal (Sorghum/bajra) and leguminous crops (Berseem, Lucerne, Horse gram, Cowpea) under dry land system for fodder production 2. After harvest, dry the biomass of crops (Paddy, Black gram, Green gram, Ragi, cow pea etc.) material as fodder 3. Use Groundnut haulms to supplement the feeding of livestock during drought	Promotion of Horse gram, Sunhemp etc. as contingent crop and harvesting it at vegetative stage as fodder

Table 5: Strategies for health care management of livestock in drought affected areas

Health Care	Strategies	
	Farmers Level	Community Level
High Susceptibility to Diseases and Decreased Feed Intake	1. Grazing of animals to be done early morning and late evening 2. Ensure timely feeding i.e. feed green fodder and concentrates during day time and roughages/hay at night time 3. Those farmers with animal sheds should ensure fans and proper ventilation 4. Spread coconut husk/ leaves and straws on the roof of the animal shed 5. Feed sugarcane molasses to enhance the appetite of the animals	Conducting regular health camps at village level for heat stressed animals

stages- village level (FGD), institute level (Experts) and secondary data sources were collated. The problems segregated into breeding, feeding, healthcare management categories while formulation of strategies. Upon discussion with the experts, the strategies so developed were categorised into farm level and community level strategies. Farmer level strategies were those which the livestock farmer can carry out at individual level to smoothen the negative impact of drought. On the other hand, community level strategies (say infertility camps, formation of pani panchayat etc.) were those which requires group participation in carrying out the action. Strategies suggested for repeat breeding and anestrus problem include proper nutrition feeding, grazing for male exposure to cows, feeding mineral mixture, maintaining estrus records and consulting veterinarian at regular intervals. Honparkhe et al. (2022) state that for reproductive livestock management, AM-PM rule of A.I, balanced nutrition feeding, providing adequate water are some of the requisite strategies. Cultivating short duration fodder crops, creating fodder banks, unconventional crop feeding, avoiding long distance grazing and restricted wallowing should be followed to reduce the feeding problems faced by livestock farmers. For western dry areas of Rajasthan, Kant et al. (2017) recommended cultivating drought resistant varieties of fodder crops, growing of perennial grasses, alternate crop cultivation, use of short duration variety as effective strategy for feeding management of livestock. For health management of livestock, feeding sugarcane molasses, proper ventilation and spreading coconut husk on animal shed and early morning grazing of animals are required to ensure productive and reproductive efficiency of livestock reared in drought areas. The health management strategies so developed were in line with the findings of Sankhala et al. (2016) and Kant et al. (2017). The framework of strategies developed for sustainable livestock farming for the drought prone areas is depicted in Table-3, 4, 5.

Conclusion

Traditionally, it is observed that livestock farmers undertake a number of strategies to protect their socio-economic assets to minimize the drought induced shocks. But these traditional methods are usually found to be insufficient thereby failing to protect livestock farmer livelihoods in drought prone areas. However, managing droughts effectively in vulnerable areas requires adoption of suitable strategies in the dimensions of breeding, feeding and healthcare management in livestock rearing in addition to the traditional practices. There is also an urgent need to restructure the dissemination of strategies from individual (farm level) to collective perspective, particularly in drought affected areas since common property resources play a major role in animal husbandry practices. Along with government drought relief measures for livestock farmers which are ex- post in nature; adoption of ex-ante measures before the onset of drought can go a long way in managing drought impact effectively. The prominent problems of livestock rearing faced by farmers in the study area were anestrus, lack of pasture grass and drinking water, high susceptibility to diseases and decreased feed intake. The research was carried out to formulate a mixture of ex-post and ex-ante strategies which can be carried out by livestock farmers in drought prone areas. To mitigate the drought impacts farmers should be encouraged to adopt various drought adaptation strategies developed in the framework with the help of awareness and training initiatives from state livestock department.

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RESEARCH ARTICLE

Participatory assessment of farmer-led adaptation strategies in livestock rearing to climate change in eastern Uttar Pradesh

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Abstract: Climate change is a global phenomenon, but, its effect and impact are local as well as region-specific. Seasonal variations in rainfall and temperature have influenced the agro-climatic conditions as well as populations of pests, weeds, and diseases leading to a loss in livestock productivity. The present study was conducted in three districts of Eastern Uttar Pradesh; namely, Azamgarh, Varanasi & Ghazipur. 180 respondents were interviewed, of which 143 respondents were following at least one adaptation strategy for livestock. 15 adaptation strategies were documented using Climate Change Adaptation Index (CCAI). Quantification of Indigenous Knowledge (QuIK) method given by Di Villers (1996) was adopted to assess the effectiveness of adaptation strategies with the help of 36 key informants (12 from each district). In Azamgarh the most effective adaptation strategy was “following proper sanitation practices” with a mean score $3.81^a (\pm 0.18)$, in Varanasi “Vaccination” was considered the most effective one by farmers with a mean score of $4.13 (\pm 0.15)$, whereas in Ghazipur, “use of jute cloth to cover animals in winter” with mean score $3.44 (\pm 0.12)$ was considered most effective adaptation strategy.

Keywords: Adaptation, Climate change, , Effective, Indigenous, Livestock

Introduction

Agriculture-cum-livestock is the most common farming system followed by the farmers of Uttar Pradesh. Livestock is a source of subsidiary income for many families in India especially the resource-poor who maintain few heads of animals. Cows and buffaloes if in milk will provide regular income to the livestock farmers through the sale of milk. Animals like sheep and goats serve as sources of income during emergencies to meet exigencies like marriages, treatment of sick persons, children's education, repair of houses etc. Livestock is also not untouched by the bearing of climate change. Livestock production systems are sensitive to climate change and at the same are themselves a contributor to the phenomenon, climate change has the potential to offer an increasingly formidable challenge to the livestock sector's development (Sejian et al. 2015). Reduction of milk production in dairy cattle is one of the main economic impacts of climate stress. The negative impact of temperature rise on total milk production for India has been estimated at more than 15 million tonnes in 2050 (Upadhyay et al. 2007). Changes like heat stress, and an increase in average Temperature Humidity Index (THI) above 72, create heat stress conditions in dairy animals and changes in feed intake and other physiological processes. The optimum THI for a dairy animal is about 70. But, most of the regions of Uttar Pradesh experience THI above the optimum during the summer season which directly and indirectly affects the production and reproduction of the dairy animal. Animals exposed to heat stress reduce feed intake and increase water intake, and there are changes in the endocrine status which in turn increase the maintenance requirements leading to reduced performance (Gaughan and Cawsell-Smith, 2015). Reproductive processes are affected by thermal stress. Conception rates of dairy cows may drop 20–27% in summer, and heat-stressed cows often have the poor expression of oestrous (Sejian et al. 2016). As a result, to cope with the long-term effects of shifting weather patterns, farmers may be able to boost their adaptive capacity by adjusting their livestock production systems. Research is needed on assessments for implementing these adaptation measures and

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tailoring them based on location and livestock system (Rojas-downing et al. 2017). Uttar Pradesh is divided into 4 zones or regions i.e. Western, Central, Eastern, and Bundelkhand. Eastern Uttar Pradesh is expected to have a bigger effect on climate change compared to other regions of the state (Tripathi and Mishra, 2017). Eastern Uttar Pradesh may have a larger impact on climate change due to low per capita income. The eastern region, had per capita yearly earnings of Rs 12,741 (lowest among all four regions) while the state's average stood at Rs 17,349 (Times of India Report, 2013). Along with, low educational status, and high population density, the dominance of the small and marginal resource-poor farmers in the eastern region compared to other regions of Uttar Pradesh (World Bank, 2014). Most of the districts affected by flash floods over the last 6-7 years in Uttar Pradesh are from the eastern region (Uttar Pradesh State Disaster Management Plan, 2016-2017). To develop and target appropriate adaptive measures, it is important to identify regions that are relatively more affected by climate change (Radhakrishnan et al. 2018); henceforth the study was carried out in the eastern region of Uttar Pradesh to bring the focus on policymakers and researchers on this particularly vulnerable region of the state.

Methodology

Eastern Uttar Pradesh was the locale of the study. Three districts namely; Azamgarh, Varanasi and Ghazipur were randomly selected from a total of 28 districts of eastern Uttar Pradesh. Two blocks were randomly selected; from each block, two villages were selected; 15 farmers were randomly selected, making the sample size 180 respondents. Out of 180 respondents, 143 respondents have adopted at least one adaptation strategy. The farmers who were having at least one milch animal were selected for the study. Adaptation strategies were operationalised as the measures adopted by the farming community to cope with the adverse impact of climate change on crop farming for sustainable agricultural production. A set of probable fifteen adaptation strategies was prepared using the snowball technique during the pilot study and a total of nine adaptation strategies were documented. 'Climate Change Adaptation Index (CCAI)' was developed at two-level i.e. adaptation strategies wise and respondent wise using the following formula:

$$\text{Climate Change Adaptation Index} = \frac{\text{Obtained Score}}{\text{Maximum Obtainable Score}}$$

The ranking of these adaptation strategies was done according to their higher index value. Adaptation strategies with higher index values indicated that these adaptation strategies had comparatively more coping capacity than the adaptation strategies with the lower index value. The effectiveness of these adaptation strategies was assessed by using the participatory technique; Quantification of Indigenous Knowledge (QuIK) given by De Villiers (1996). The tool of Participatory Rural Appraisal (PRA) matrix ranking was combined with semi-

structured interviews to elicit numerical data from experienced farmers. Going for an experimental trial for these adaptation strategies would have been a time-consuming procedure, QuIK method is beneficial in evaluating the performance of farmers' practices without experimental trials. To conduct this particular method, key informants were selected based on the snowball technique and by interviewing the farmers from each village. Accordingly, three key informants were selected from each village of the sampled districts. Therefore a total of 36 key informants were used to study the effectiveness of the adaptation strategies. Key informants were asked to weigh practices in comparison among the identified strategies according to the following four criteria of effectiveness: (1) Effect on yield performance (2) Associated cost (3) Reducing climate sensitivity (4) Ease in availability and application. In each block of the matrix, key informants were asked to position the necessary number of pieces of stone out of five to each adaptation strategy for every criterion aforementioned. Following the QuIK to identify the most effective adaptation strategies methodology, the collected data were subjected to a one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) as modified by Kramer (1957).

Results and Discussion

Table 1 depicts the index score of each adaptation strategy in livestock-rearing in the district as well as the overall sampled region (eastern Uttar Pradesh). 'Use of cattle shed for cattle and buffaloes' was the most preferred adaptation strategy by the farmers of Azamgarh district, Varanasi district, Ghazipur district, and also the overall region. Farmers have highly perceived the negative impact of heat stress on their livestock; therefore, 93% of respondents were adopting this particular practice (use of cattle shed) to reduce heat stress on the animals. The second most preferred strategy by the farmers of Azamgarh district and the overall region was 'providing extra fresh drinking water during summer', whereas it was preferred as the third and fourth most important adaptation strategy in Varanasi and Azamgarh districts, respectively.

'Providing extra baths to cattle and buffaloes' was the third most important adaptation strategy by the farmers of Azamgarh, Ghazipur, and the overall region. It could be noted that all the top-three most preferred adaptation strategies were related to alleviating heat stress in the animals because farmers have a high perceived reduction in milk production due to extreme heat stress and therefore, practices help in reducing heat in animals and their surrounding was the most preferred ones. The result was in line with the study of Shahbaz et al. (2022) where he found that shelter management is a strategy adopted by livestock farmers mostly to address heat stress, heat waves, humidity, and unpredictable rains.

Correlation between the ranks provided adaptation strategies to climate change in three sampled districts

To check the correlation between different ranks given to adaptation strategies in each district Kendall Tau Rank Correlation was calculated using the SPSS software. It was calculated by setting the following hypothesis

H_0 : There is no correlation of ranks given to adaptation strategies between all three districts

H_1 : There is a correlation of ranks given to adaptation strategies at least in between two districts

From the table, we can infer that the ranks between Azamgarh and Ghazipur are highly correlated ($R_{AG}=0.82$) with a p-value = 0.00 (H_0 is rejected at a 1 percent level of significance). Hence respondents in districts Azamgarh and Ghazipur are alike in their judgments, and their choices for adaptation strategies are in similar directions. In the case of Azamgarh and Varanasi, ranks are correlated ($R_{AV}=0.46$) with p-value = 0.00 at a 5 percent level of significance and in between Varanasi and Ghazipur ranks are correlated ($R_{VG}=0.42$) with p value = 0.03 at 5 percent level of

significance. Here also in both the cases, the order preferences of adaptation strategies are somewhat alike. The Climate Change Adaptation Index and Correlation simply provided insight into the order of preferences for adaptation strategies while just answering the “What” section of the research, i.e. what adaptation strategies farmers were using. However, the effectiveness of the top-five most effective adaption strategies was calculated using the Quantification of Indigenous Knowledge (QuIK) methodology to address the “Why” component of the research, i.e. “Why” farmers choose a certain strategy.

Effectiveness of Farmer-Led Adaptation Strategies Related To Livestock-Rearing

Based on the ranks obtained by the adaptation strategies which were followed by the farmers in livestock-rearing, the top-five most preferred strategies in livestock-rearing were selected separately for all three districts to study the effectiveness of these adaptation strategies. Finally, the effectiveness of the selected adaptation strategies in crop farming and livestock-rearing for all three districts was analyzed with the principle of De Villiers (1996) where the performance of the farmers’ practices

Table 1: Index score and ranking of adaptation strategies in livestock rearing followed by the farmers of Eastern Uttar Pradesh

Adaptation strategies	Azamgarh (n=38)	Varanasi (n=53)	Ghazipur (n=52)	Overall (n=143)
Shower/extra bath to cattle and buffaloes	0.62 (III)	0.47 (VII)	0.77 (III)	0.62 (III)
Proper sanitation for cattle and buffaloes	0.50 (VII)	0.40 (XII)	0.60 (VI)	0.50 (VII)
Vaccination	0.61 (IV)	0.58 (V)	0.54 (VIII)	0.61 (IV)
Reduction in herd size	0.34 (XII)	0.47 (VII)	0.31 (XIII)	0.34 (XII)
Use of shed for cattle and buffaloes	0.93 (I)	0.92 (I)	100.00 (I)	0.93 (I)
Deworming of cattle and buffaloes	0.41 (X)	0.72 (II)	0.32 (XII)	0.41 (X)
Extra concentrate	0.54 (VI)	0.59 (IV)	0.62 (V)	0.54 (VI)
Fumigation of cattle shed with <i>Ajwain</i> and neem	0.32 (XIII)	0.28 (XIV)	0.33 (XI)	0.32 (XIII)
Feeding of extra green fodder	0.38 (XI)	0.47 (VII)	0.44 (X)	0.38 (XI)
Feeding of crop-residue and hay	0.32 (XIII)	0.28 (XIV)	0.27 (IV)	0.32 (XIII)
Use of jute cloth to cover animals	0.58 (V)	0.55 (VI)	0.81 (II)	0.58 (V)
Massage of the body with mustard oil	0.45 (IX)	0.40 (XII)	0.48 (IX)	0.45 (IX)
Feeding of mustard cake to cattle and buffaloes	0.46 (VIII)	0.45 (X)	0.58 (VII)	0.46 (VIII)
Shifting to indigenous breeds	0.28 (XV)	0.43 (XI)	0.21 (XV)	0.28 (XV)
Providing fresh drinking water to animals	0.63 (II)	0.62 (III)	0.69 (IV)	0.63 (II)

(Values in parenthesis indicate column-wise rank)

Table: 2 Kendall Tau Rank (R) Correlation Coefficients of ranks to adaptation strategies in sampled three district

	R_{AV}	R_{AG}	R_{VG}
Kendall Coefficients	0.46*	0.82**	0.42*
p value	0.02	0.000	0.03.

*Correlation is significant at the 0.05 level (2-tailed)
 **Correlation is significant at the 0.01 level (2-tailed)
 R_{AV} : Correlation of ranks to adaptation strategies between Azamgarh and Varanasi
 R_{AG} : Correlation of ranks to adaptation strategies between Azamgarh and Ghazipur
 R_{VG} : Correlation of ranks to adaptation strategies between Varanasi and Ghazipur

as well as indigenous knowledge was assessed without field trial.

Effectiveness of adaptation strategies related to livestock-rearing in Azamgarh district

The five most important adaptation strategies related to livestock-rearing used by the farmers of Azamgarh district were 'extra bath/shower to animals', 'proper sanitation practices for cattle and buffaloes', 'vaccination', 'use of cattle shed for cattle and buffaloes', and 'providing extra fresh drinking water to animals'. Table 3 depicts 'providing proper sanitation practices for cattle and buffaloes' as considered the most effective (mean score = 3.81) adaptation strategy. But, it was observed that 'providing proper sanitation to animals' (mean score = 3.81), 'vaccination' (mean score = 3.65) and, 'extra bath/shower to animals' (mean score = 3.48) did not differ significantly between them. Hence, all three practices combined could be considered an effective adaptation strategy. The productivity loss due to heat stress can be reduced substantially by adopting the heat stress management coupled with good health management (Anjali et al. 2022). 'Extra bath/shower to animals' (mean score = 4.08) was most effective in improving yield performance. Vaccination (mean score = 4.42)

was considered the most cost-effective adaptation strategy and also effective in reducing climate sensitivity (mean score = 4.17). Farmers believed that they were getting better results on milk output when they are going for proper vaccination of their animals as compared to the input and hard work they are giving in providing extra drinking water to cattle and buffaloes, as they had to switch on the electric motor to provide fresh water to animals, raising electricity bill.

Effectiveness of adaptation strategies related to livestock-rearing in Varanasi district

The five most important adaptation strategies used by the farmers of Varanasi districts were, regular 'vaccination', 'use of cattle shed for cattle and buffaloes', 'deworming of animals', 'providing extra concentrate to animals' and, 'providing extra fresh drinking water to animals'. Table 4 shows that out of five adaptation strategies, regular 'Vaccination' (mean score = 4.13) was considered the most effective adaptation strategy. Due to erratic rainfall conditions, the incidence of pests and diseases has increased in the district, and regular vaccination proves to be more beneficial in controlling pests and pathogen attacks. Vaccination was considered cost-effective due to assistance from

Table 3: Effectiveness of adaptation strategies related to livestock-rearing in Azamgarh

Adaptation strategies	Effect on Yield performance	Associated cost	Reducing climate sensitivity	Easy in availability and application	Overall effect
Extra bath/Shower to animals	4.08 ^a ±0.26	2.917 ^{bc} ±0.34	3.42 ^a ±0.36	3.50 ^a ±0.31	3.48 ^a ±0.14
Proper sanitation practices	3.75 ^a ±0.40	3.75 ^{ab} ±0.33	3.50 ^b ±0.34	4.25 ^a ±0.18	3.81 ^a ±0.18
Vaccination	2.58 ^b ±0.47	4.42 ^a ±0.26	4.17 ^a ±0.27	3.42 ^a ±0.36	3.65 ^a ±0.23
Use of Cattle shed for cattle and buffaloes	2.16 ^b ±0.32	1.83 ^d ±0.24	1.33 ^c ±0.19	1.67 ^b ±0.35	1.75 ^b ±0.15
Providing extra fresh drinking water to animals	2.417 ^b ±0.31	2.08 ^{cd} ±0.36	2.50 ^b ±0.31	1.92 ^b ±0.19	2.23 ^b ±0.15

Mean with different superscripts in a column differ significantly at 5 percent level of significance. Multiple comparisons were based on DMRT.

Table 4: Effectiveness of adaptation strategies related to livestock-rearing in Varanasi

Adaptation strategies	Effect on Yield performance	Associated cost	Reducing climate sensitivity	Easy in availability and application	Overall effect
Vaccination	4.25 ^a ±0.22	4.33 ^a ±0.19	3.92 ^a ±0.29	4.00 ^a ±0.35	4.13 ^a ±0.15
Use of shed for cattle and buffaloes	2.75 ^{bc} ±0.43	2.67 ^{bc} ±0.38	1.92 ^b ±0.29	2.42 ^b ±0.36	2.44 ^c ±0.23
Deworming	3.17 ^b ±0.39	3.33 ^b ±0.43	3.42 ^a ±0.45	3.25 ^{ab} ±0.43	3.29 ^b ±0.15
Extra Concentrate	1.92 ^c ±0.38	2.50 ^{bc} ±0.38	2.83 ^{ab} ±0.34	3.08 ^{ab} ±0.36	2.58 ^c ±0.16
Providing extra fresh drinking water	2.67 ^{bc} ±0.26	2.00 ^c ±0.43	3.08 ^a ±0.47	2.25 ^b ±0.41	2.50 ^c ±0.11

Mean with different superscripts in a column differ significantly at 5 percent level of significance. Multiple comparisons were based on DMRT.

Table 5: Effectiveness of adaptation strategies related to livestock-rearing in Ghazipur

Adaptation strategies	Effect on Yield performance	Associated cost	Reducing climate sensitivity	Easy in availability and application	Overall effect
Shower to animals	4.42 ^a ±0.15	2.17 ^a ±0.32	3.75 ^a ±0.37	3.33 ^a ±0.33	3.42 ^a ±0.09
Use of shed for cattle and buffaloes	2.00 ^c ±0.28	3.08 ^a ±0.31	1.50 ^b ±0.26	3.08 ^a ±0.51	2.42 ^b ±0.20
Extra Concentrate	1.58 ^c ±0.19	3.42 ^a ±0.47	3.67 ^a ±0.31	2.83 ^a ±0.39	2.88 ^{ab} ±0.17
Use of jute clothing to cover animals	4.08 ^a ±0.26	3.08 ^a ±0.43	3.17 ^a ±0.34	3.42 ^a ±0.36	3.44 ^a ±0.12
Providing extra fresh drinking water	3.08 ^b ±0.38	3.25 ^a ±0.46	3.00 ^a ±0.41	2.58 ^a ±0.43	2.98 ^a ±0.28

Mean with different superscripts in a column differ significantly at 5 percent level of significance. Multiple comparisons were based on DMRT.

extension agencies and local government incentives to provide services at low cost and a good result in milk production were seen due to the prevention of animals from pest and disease attacks. Some Studies have shown the importance of above mentioned practices as important adaptation strategies to climate change. Regular deworming, preventive vaccination against endemic diseases, dipping and control of intermediate hosts are critical for sustainability of livestock production system in the era of climate change. (Pourouchottamane et al. 2021). Vaccination, deworming, and spraying were considered effective to control diseases caused by changes in climate in Africa (Moenga et al. 2016). Regular vaccination and deworming, and dipping of animals were considered a few adaptation strategies to climate change in Nepal (Koirala and Bhandari, 2018).

Therefore, 'Vaccination' shows a high mean score in all four criteria of effectiveness i.e. improvement in yield performance (mean score = 4.25), cost-effectiveness (mean score = 4.33), 'reducing climatic sensitivity (mean score = 3.92) and ease in availability and application (mean score = 4.00).

Effectiveness of adaptation strategies related to livestock-rearing in Ghazipur district

The five most important adaptation strategies used by the farmers of Ghazipur district were, providing 'extra bath/shower to animals', 'use of cattle shed for cattle and buffaloes', 'providing extra concentrate to animals', use of jute cloth to cover animal's body' and, 'providing extra fresh drinking water to animals.' Table 5 depicts that the 'use of jute cloth to cover animal's body' (mean score= 3.44) was considered the most effective adaptation strategy. As suggested by Kattimani (2020) young calves and animals should be warm by covering with sand bags and gunny (jute) bags. Along with that 'extra bath/shower to animals' (mean score= 3.42) and 'providing extra fresh drinking water to animals' (mean score = 2.98) were also effective strategies as there was no significant difference among all three adaptation strategies.

As indicated by Legrand et al. (2011), water is commonly used to cool cattle during the summer season. Providing 'extra bath/shower to animals' was considered effective in increasing yield performance (mean score = 4.42) and reducing climatic sensitivity (mean score = 3.75), whereas, 'providing extra concentrate to animals' was considered the most cost-effective (mean score = 3.42) and 'use of jute cloth to cover animal's body' was considered most effective in availability and application (mean score = 3.42). Providing clean, fresh adequate amounts of water to lactating animals is used to help to maximize feed intake and milk production while keeping cows comfortable and healthy during heat-stress conditions (Erickson, 2019).

Note: As we could see, vaccination was one such strategy usually preferred in all three districts of the study area for improving the adaptive capacity of their animals towards the impact of climate change. Though Vaccination is not the truly an adaptation strategy *per se* these were coping strategies and show tolerance stress during adverse climatic conditions, because of that it was included in the study as the appreciable number of farmers was seeking vaccination as an adaptation strategy to climate change in all three districts of the study area. The outbreak of diseases like Hemorrhagic Septicemia and Food Mouth Disease along with vector-borne diseases increased over the years due to extreme rainfall conditions and increases in temperature. The diseases were spreading swiftly and widely during monsoons when the humidity and moisture both were high. Earlier there was the trend that the farmers were not regularly going for vaccination practices, but nowadays due to the increase in the outbreak of pest attacks, they were seeking regular vaccination practices.

Conclusions

It was observed that the locally available and cheap remedies, most of which could be prepared in homes formed an integral part of the adaptation strategies by the farmers; many of which lacked a scientific temper. Therefore, providing training that incorporates scientific knowledge along with indigenous

techniques will be of immense help. In addition to the experimental research procedures, a participatory approach was introduced, thereby offering a valuable platform for both farmers and extension service providers to gain insight into different practices led by farmers. The key to the strategy's success is bringing together farmers, consultants, and academics from other fields in an iterative process that generates unique and practical information for coping with climate change. The study illustrated the value of documenting farmers' local livestock-rearing practices, as well as producing a package of local practices and disseminating them to other parts of the country to popularize them. Agro-regional planning for climate-smart agriculture is important because farmers' expectations of adoption levels of various adaptation strategies in response to the impact of climate change vary from region to region, necessitating the creation of strategies based on changing climatic conditions, replacing or incorporating in previously followed practices.

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SHORT COMMUNICATION

Effect of green Azolla (*Azolla pinnata*) supplementation on milk production, constituents and fatty acids profile in mid-lactating Barbari goats

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Abstract: An experiment was conducted to study the effect of fresh azolla supplementation in lactating goats reared under intensive system. Twelve mid lactating Barbari goats were divided into two groups (CON and SUPP) of six each. The CON was control (fed with 500 g concentrate pellets/head/day and *ad libitum* gram straw) while goats in SUPP was supplemented with 250 g fresh azolla (20 gDM/ d) along with g straw and concentrate pellet. Milk production at 0, 30, 60 days of experimental feeding was recorded for two consecutive days. There was 20% improvement in milk production at different stage of lactation on supplementation of 250 g fresh azolla in mid lactating goats. Total solids, moisture, solid not fat (SNFs), pH, ash and protein content of the milk were similar in all the groups. However, milk fat per day was similar in both the group of lactating goats. There was no significant effect of azolla supplementation on different fatty acids, Short chain Fatty acids (SCFA), saturated fatty acids, Mono unsaturated fatty acids (MUFA) and Poly unsaturated fatty acids (PUFA) in lactating goats. Fresh azolla supplementation improved the milk production with no significant effect on milk composition and fatty acid profile in stall fed lactating goats.

Keywords: Azolla, Fatty acids, Lactating goats, Milk

Azolla has been largely used as total feed for fish, including carp and tilapia production, as protein supplement for pigs and poultry (including ducks) and as fermentable nitrogen and mineral

supplement for ruminants (FAO, 2011). Azolla has a symbiotic blue green algae *Anabaena azollae*, which is responsible for the fixation and assimilation of atmospheric nitrogen. This fact makes the Azolla tend to contain relatively high levels of nitrogen and be an attractive protein source for ruminants. Azolla is rich source of good quality protein, phosphorous, calcium and other major minerals, trace minerals (Zn, Se and Cu), Vitamin A, B complex and the bioactive molecules. Kumar et al. (2015) studied the nutrient composition of *Azolla microphylla* and reported crude protein 17.3%, ether extract 2.71% and crude fibre 12.02% on dry matter basis. They reported that azolla contained 0.60% sodium, 0.73% potassium, 0.11% calcium, 16.12 ppm copper and 71.47 ppm zinc. There are at least eight species of Azolla worldwide; *A. caroliniana*, *A. circinata*, *A. japonica*, *A. mexicana*, *A. microphylla*, *A. nilotica*, *A. pinnata* and *A. rubra*. The common species of Azolla in India is *Azolla pinnata*. Supplementation of fresh azolla has been found to improve the lactation performance in animals. Elangovan et al. (2011) reported positive effect of fresh Azolla supplementation on milk yield and FCM yield in lactating cows and buffalo. Kumar et al. (2016) replaced a part of concentrate mixture with sun dried azolla and found that there was increase in the milk production in the lactating barbari goats. Present experiment was conducted to evaluate the effect of fresh azolla supplementation on milk quantity and composition in mid lactating goats.

The experiment was conducted at the Animal experimental shed, Animal Nutrition Management and Product Technology Division of ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura India. Geographically, the institute is located at 27° N latitude, and 78° N E longitude on 176 m above the sea level. Twelve mid lactating Barbari goats (Avg. BW 35.58Kg, in 3-5th lactation at post weaning stage) were divided into two groups (CON and SUPP) of six each. Goats were randomly divided on the basis of body weight, milk yield and parity. CON group was fed with 500 g concentrate pellets/head/day and gram straw *ad libitum* while goats SUPP was supplemented with 250 g fresh azolla (20gDM/ day) along with concentrate pellet and *ad libitum* gram straw. Composition (on % DM basis) of concentrate pellet was maize grain, 20; barley grain, 20; groundnut cake (Expeller), 6; soybean meal, 5, mustard cake (Expeller), 5; guar korma, 5; wheat bran, 15; deoiled rice bran, 14; molasses, 7; mineral mixture,

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2 and Salt, 1%. Does were kept under stall fed system with uniform management conditions by housing them in well ventilated sheds. The duration of experimental feeding was 60 d (January 2020 to March 2020). All the goats were first fed with concentrate pellet. After consumption of pellet gram straw was offered, in SUPP group green azolla was mixed in gram straw and fed to the goats. Water was provided twice daily throughout the experimental feeding.

The representative samples of concentrate pellet, gram straw and green azolla were analysed as per AOAC(2006). Organic matter (OM) was determined by ashing at 550 °C for 5 h. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were discerned by the methods of Van Soest et al. (1991). Crude protein (CP) content ($N \times 6.25$) was measured as per Micro-Kjeldahl method. Ether extract (EE) was estimated using Soxhlet apparatus.

Milk yield of each of the goats was recorded for two consecutive days at 0, 30 and 60 days of experimental feeding. Milk was weighed using electronic balance to estimate amount of milk produced. Representative milk samples were analysed for different milk constituents using automatic milk scanner. For fatty acid analysis in milk method of O'Fallon et al. (2007) was followed for the preparation of fatty acid methyl esters (FAME) of milk samples.

One ml milk sample was taken and placed in 50 mL centrifuge tubes to which 0.7 ml of 10N KOH in water and 5.3ml of methanol (Sigma Aldrich) were added. The tubes were incubated in a 55°C water bath for 90 min with vigorous shaking for 5 sec every 20 min. After cooling to room temperature in tap water, 0.58 ml of 24N H₂SO₄ was added. The tubes were again incubated at 55°C for 90 min in a water bath with shaking for 5 sec every 20 min. The tubes were cooled in tap water. Three mL of hexane (Sigma Aldrich) was added and the tubes were vortexed for 5 min on a multi-tube vortex. The tubes were centrifuged for 10 min at 2000g and the hexane layer was taken out and placed into Eppendorf tubes stored at -20°C till analysis in the GC-MS/MS analysis.

The fatty acid composition of the FAME was determined using GC-MS (GC-MS TQ8030, Shimadzu Corp., Japan) attached with Restek Stabilwax®-MS capillary column (30m × 0.25mm ID × 0.25 µm) and flame ionization detector (FID). 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 held for 60 min. Helium was used as a carrier gas at a flow rate of 1ml/min. Both the injector and detector were set at 260°C. The split ratio was 30:1. GC-Real time Analysis software was used to run the sample for analysis. Fatty acids were identified by comparing their retention

Table 1 Chemical composition of feed (% DM basis)

Attribute (%)	Concentrate pellet	Gram straw	Azolla
Organic matter	93.49	91.75	80.45
Crude protein	18.18	6.11	20.19
Total ash	6.51	8.24	19.55
Ether extract	3.17	0.42	3.22
NDF	23.81	63.24	50.98
ADF	12.70	49.44	31.34
Cellulose	11.11	34.42	14.45

Table 2 Effect of azolla supplementation in lactating goats

Attribute	G1	G2
Initial BW (Kg)	35.68±1.96	35.48±1.70
Final body weight (Kg)	35.36±2.63	35.25±2.09
	Milk yield (g)	
0 d	825.00±92.45	823.33±78.24
30 d	603.33±81.67	748.16±45.71
60 d	524.16±109.91	625.33±39.78
	Milk constituents	
Moisture (%)	84.37±0.54	85.51±0.57
TS (%)	15.62±0.54	14.49±0.57
pH	6.60±0.02	6.55±0.04
Ash (%)	5.24±0.19	5.81±0.43
Fat (%)	5.95±0.34	4.91±0.25
SNF (%)	9.67±0.19	9.57±0.32
Protein (%)	2.39±0.04	2.71±0.11
Fat (g) /day	35.56±4.96	36.50±2.05

Fat (g)/day is calculated as milk yield per day X % fat/100

Table 3: Effect of azolla supplementation on milk fatty acids (%) profile in lactating goats

Milk fatty acids (%)	G1	G2
C6:0	1.19±0.03	1.30±0.08
C8:0	2.33±0.06	2.34±0.13
C10:0	10.21±0.51	10.14±0.80
C12:0	4.89±0.32	4.51±0.32
C14:1	12.56±0.47	11.79±0.67
C16:0	36.53±1.88	34.60±1.87
C16:1	0.60±0.38	0.62±0.21
C18:0	5.55±0.40	6.68±0.53
C18:N9C	23.30±2.30	25.99±2.18
C18:2N6C	2.78±0.39	3.90±1.21
SCFA	13.73±0.57	13.79±0.96
SFA	73.26±2.30	71.36±2.06
MUFA	23.90±2.20	25.99±2.18
PFA	2.78±0.39	2.60±1.12

time with the fatty acid methyl standards (Supelco 37) and were expressed as a percentage of total fatty acids. Blood was collected from the jugular vein of all experimental animals in last week experimental feeding.

The data were analyzed by independent sample t-test as described by Snedecor and Cochran (1989). All the data was analysed by SPSS (1995).

Chemical composition of concentrate pellet, gram straw and Green azolla used for experimental feeding of lactating goats is presented in table 1. The dry matter content of azolla was 7.90% while crude protein content was 20.19% on DM basis. Kumar et al. (2015) also reported crude protein content (%) of *Azolla microphylla* 17.3. The variation may be due to growth condition and nutrient availability in the Azolla pond. Total ash (%) was about 19.55. Azolla is fed to animals mainly as an economic source of protein and minerals. The effect of azolla supplementation on body weight changes of dam and milk production is presented in Table 2. There was no change in the body weight of the lactating goats and nutrient intake was sufficient to maintain the body weight and milk production in both the group of goats. With the advancement of lactation a decreasing trend in milk production was reported in both the group of goats. The milk production was approximately 20% higher in supplemented group of goats as compared to control groups. Improvement in the milk production of supplemented group of goats might be due to high protein and minerals content of Azolla with low lignin content. Azolla supplementation might have improved the nutrient content of feed and better availability of nutrients. Pillai et al. (2002) also reported an overall increase (about 15%) of milk yield in dairy cattle on supplementation of 1.5-2.0 kg of fresh Azolla per day in field trial. They concluded that the increase in the quantity of the milk produced was higher than expected, based on the nutrient content of azolla. Other components of azolla like carotenoids, bio-polymers, unidentified factors might be responsible for the overall increase in the production of milk. Different milk constituents (%) total solids, moisture, solid not

fat (SNFs), pH, ash and protein content of the milk was statistically ($P>0.05$) similar in CON and SUPP group of goats. Lower fat per cent was observed in SUPP group that may be due to more milk production in that group. However milk fat per day was similar in both the group of lactating goats. Supplementation of green azolla had no significant ($P<0.05$) effect on milk fatty acid profile of goats (Table 3). There was no significant effect of azolla supplementation on different fatty acids, Short chain Fatty acids (SCFA), saturated fatty acids, Mono unsaturated fatty acids (MUFA) and Poly unsaturated fatty acids (PUFA) in lactating goats. Milk fatty acid composition is mostly affected by the type of forage, forage to concentrate ratio, lipids supplementation and starch level, together with their interactions (Lopez et al. 2019). The fatty acid composition is in focus nowadays due to the impact of different fatty acids in human nutrition and their relation with various diseases. Supplementation of azolla has no significant effect on the fatty acid profile of goats.

Conclusion

Supplementation of fresh azolla in the ration of stall fed lactating goats did not affect milk production, milk composition and fatty acid profile.

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Production and reproduction performance of Tharparkar cattle in arid region of Rajasthan

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Abstract: A study was conducted on the performance of Tharparkar cattle maintained at Livestock Research Station (LRS), Beechwal, Bikaner. The performance records of first lactation production and reproduction traits of Tharparkar cows were collected from the LRS, Beechwal spread over a period of eight years (2012 to 2020). The overall least-squares means for first lactation 305-days milk yields (FL305DMY), first lactation length (FLL), first dry period (FDP) and age at first calving (AFC) were 2003.52 ± 71.83 kg, 296.76 ± 7.25 days, 119.51 ± 7.44 days and 1417.88 ± 20.59 days, respectively. The non-significant effect of period of birth/calving was found on all first lactation traits under study except age at first calving. Season of birth/calving did not influence these traits in the present study. The random effect of sire was found highly significant ($P \leq 0.01$) on AFC and non-significant on all the production traits. The regression of traits on age at first calving was also found non-significant.

Keywords: Arid-region Production Traits, Reproduction traits, Tharparkar cattle

Animal husbandry is an essential component of Indian agriculture. Livestock plays a vital role in the Indian economy in terms of sustaining income and employment. The levels of production and reproduction traits of the animals are crucial to the success of dairy farming. The phenotypic expression of these traits is influenced by a number of genetic and non-genetic factors. Reliable information on these traits is useful in determining the best selection criterion for improving the overall performance of the animals. Therefore, this study was carried out to assess the influence of important genetic and non-genetic factors viz. sire, period of birth/ calving and season of birth/ calving on production and reproduction traits in Tharparkar cattle.

To estimate least-squares means of first lactation traits, mixed model analysis was carried out using SPSS software. A total of 830 performance records of Tharparkar cows spread over a period of eight years (2012 to 2020) were utilized. Data were classified into three classes according to period of birth P1 (2006-2009), P2 (2010-2013) and P3 (2014-2016) and period of calving viz. P1 (2012-2014), P2 (2015-2017) and P3 (2018-2020). According to season of calving data were classified into three seasons viz. winter (November to February), summer (March to June) and rainy (July to October). Production and reproduction traits were adjusted for effect of non-genetic factors viz. period of birth/calving and season of birth/calving as fixed effects, sire as random effect and AFC as covariate. Various production and reproduction traits were included in the present study viz., first lactation three hundred five days milk yield (FL305 DMY), first lactation length (FLL), first dry period (FDP) and age at first calving (AFC).

To find the effect of various genetic and non-genetic factors on production and reproduction traits, computer package programme, IBM SPSS version 20.0 was used for data analysis.

1. Mathematical model for analysis of age at first calving:

$$Y_{ijkl} = \mu + a_i + S_j + P_k + e_{ijkl}$$

Where,

$$Y_{ijkl} = \text{Observation on the } l^{\text{th}} \text{ progeny of } i^{\text{th}} \text{ sire, related to } j^{\text{th}} \text{ season of birth and } k^{\text{th}} \text{ period of birth}$$

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M = Population mean

a_i = Random effect of i^{th} sire ($i = 1, 2, 3, \dots$)

S_j = Fixed effect of j^{th} season of birth

P_k = Fixed effect of k^{th} period of birth

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

2. Mathematical model for analysis of other production traits:

$$Y_{ijkl} = \mu + a_i + S_j + P_k + b(X_{ijkl} - \bar{X}) + e_{ijkl}$$

Where,

Y_{ijkl} = Observation on the l^{th} progeny of i^{th} sire, in j^{th} season of calving and k^{th} period of calving

μ = Population mean

a_i = Random effect of i^{th} sire

S_j = Fixed effect of j^{th} season

P_k = Fixed effect of k^{th} period of calving

X_{ijkl} = Age at first calving corresponding to Y_{ijkl}

b = Regression of variable on age at first calving

$\bar{X}$ = Average age at first calving

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

Duncan's Multiple Range Test (DMRT): Duncan's multiple range test as modified by Kramer (1957) was used for testing differences

among least squares means (using the inverse coefficient matrix). The differences were considered significant, if

$$(X_i - X_j) \sqrt{\frac{2}{(C^{ii} + C^{jj} + 2C^{ij})}} > \sigma e Z_{pn_2}$$

Where,

X_i and X_j are the least squares means for i^{th} and j^{th} treatment and C^{ii} , C^{jj} and C^{ij} are diagonal and off-diagonal elements in the inverse of coefficient matrix in the least squares normal equations.

The data structure, least squares mean (LSM), standard error (SE) and effect of genetic and non-genetic factors for different first lactation production and reproduction traits under study are shown in Table 1. The overall least-squares means for FL305DMY, FLL, FDP and AFC were 2003.52 ± 71.83 kg, 296.76 ± 7.25 days, 119.51 ± 7.44 days and 1417.88 ± 20.59 days, respectively.

The least-squares analysis was used to estimate least-squares mean and to determine the influence of various genetic (sire) and non genetic factors such as period of birth/calving, season of birth/calving and age at first calving. The differences between means were tested through Duncan's New Multiple Range Test (DMRT) as modified by Kramer (1957).

The random effect of sire was observed non-significant on all the production traits. These findings are in agreement with the results of Verma et al. (2017) in Sahiwal cattle. The random effect of sire was observed to be highly significant on AFC under study. These findings are in agreement with the results of Mishra et al. (2018) in Tharparkar cattle.

Table 1: Descriptive statistics and data structure for production and reproduction traits in Tharparkar cattle

Traits/ Factors	FL305DMY(Kg)	FLL (days)	FDP (days)	AFC (days)
Overall Mean(μ)	2003.52 ± 71.83 (83)	296.76 ± 7.25 (83)	119.51 ± 7.44 (83)	1417.88 ± 20.59 (83)
Sire	NS	NS	NS	**
Season	NS	NS	NS	NS
S1(Winter)	2000.67 ± 93.74 (37)	292.76 ± 9.46 (37)	129.04 ± 9.70 (37)	1441.54 ± 34.95 (27)
S2 (Summer)	2105.99 ± 91.65 (35)	298.12 ± 9.25 (35)	121.96 ± 9.49 (35)	1452.07 ± 29.43 (35)
S3 (Rainy)	1903.88 ± 157.78 (11)	299.39 ± 15.93 (11)	107.53 ± 16.34 (11)	1360.04 ± 37.90 (21)
Period	NS	NS	NS	S
P1	2230.56 ± 204.12 (25)	313.42 ± 20.61 (25)	124.93 ± 21.14 (25)	1589.81 ± 66.96^b (19)
P2	1997.36 ± 164.36 (28)	313.63 ± 16.59 (28)	125.22 ± 17.02 (28)	1319.63 ± 43.38^a (27)
P3	1782.63 ± 242.94 (30)	263.24 ± 24.53 (30)	108.39 ± 25.16 (30)	1344.21 ± 48.91^a (37)
AFC	NS	NS	NS	NS
Regressioncoefficient	0.170 ± 0.217	-0.00018 ± 0.0244	0.0153 ± 0.0214	-

No. of observations are given in parenthesis. Figure with different superscripts differ significantly; ** - Highly significant ($P \leq 0.01$); * - Significant ($P \leq 0.05$); NS - Non-significant

The effect of period of birth on age at first calving of Tharparkar heifer was highly significant ($P < 0.01$) with highest age at first calving in the first period and lowest in second period than other periods. Similar result were also reported by Chand (2011), Pirzada (2012) and Choudhary (2018) in Tharparkar cattle, Singh and Dubey (2005) in Sahiwal cattle and their crosses and Nehara et al. (2019) in Rathi cattle.

The effect of period of calving was observed non-significant on FL305DMY, FLL and FDP. Similar findings on FL305DMY were also reported by Choudhary et al. (2019) in Tharparkar and Verma et al. (2017) in Sahiwal cattle. The non significant effect on FDP was also reported by Pirzada (2012) and Choudhary et al. (2019) in Tharparkar cattle.

Results of present study revealed that season of birth did not influence the age at first calving in the Tharparkar cattle. Similar reports were also reported by Panneerselvam et al. (1990), Gahlot (1999), Chand (2011), Choudhary (2018) and Mishra et al. (2018) in Tharparkar cattle. Japheth et al. (2015) in Karan Fries, Balasubramaniam et al. (2013), Raja and Gandhi (2015) in Sahiwal also found same result. Contrary to present study, Kachwaha (1993), Rahumathulla et al. (1994) and Pirzada (2012) reported significant effect of season of birth in Tharparkar cattle.

FL305DMY was not influenced by season of calving in the present study. Similar results were also reported by Panneerselvam et al. 1990, Pirzada (2012), Hussain et al. (2015) and Choudhary et al. (2019) in Tharparkar, Dongre et al. (2013) and Verma et al. (2017) in Sahiwal, Nehara et al. (2019) in Rathi and Savalia et al. (2020) in Gir cattle.

FLL was not significantly influenced by season of calving. Similar results were found by Hussain et al. (2015), Choudhary et al. (2019) in Tharparkar cattle. Contrary to present study Pirzada (2012) in Tharparkar and Nehara et al. (2019) in Rathi cattle reported significant effect of period of calving.

FDP was also not influenced by season of calving. Similar results were also observed by Pirzada (2012), Hussain et al. (2015) and Choudhary et al. (2019) in Tharparkar cattle.

The least-squares analysis of variance of data revealed that regression of production traits on age at first calving were non-significant for all the production traits. The regression coefficients were positive for FL305DMY and FDP, negative for FLL.

The regression of first lactation 305 days milk yield on age at first calving and first lactation length on AFC were non-significant. The similar results were reported by Singh (2012), Sohal (2016) and Nehara et al. (2019) in Rathi cattle.

The regression of first dry period on age at first calving was also found non-significant. The similar results were reported by

Kachwaha (1993), Rahumathulla et al. (1994), Gahlot (1999) and Choudhary et al. (2019) in Tharparkar cattle.

Conclusions

The study concluded that the effect of non-genetic factors was found non-significant on most of the first lactation production traits; it shows that this cattle breed is adaptive to wide range of climatic conditions in arid region of Rajasthan. The effect of sire was found highly significant on age at first calving; it indicates that this trait can be improved by selecting superior sire directly and sire selection can bring further genetic improvement in the Tharparkar herd for this trait.

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SHORT COMMUNICATION

Impact of biometric characteristics of udder and teats on milk quality of indigenous dairy cattle

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Abstract: The present investigation was carried out to investigate the effect of biometric characteristics of udder and teats with milk Somatic Cell Count which are important for farmers, manufactures and consumers. For this study we collected 50 ml milk from 150 Indigenous lactating cattle breeds viz., Kankrej, Sahiwal and Rathu cattle (50 from each breed) reared under similar management conditions at university cattle farms located in Bikaner city of Rajasthan, India. Udder and teat morphometric parameters, i.e. Normal and pendulous shape of udder, depth of udder, flat, inverted and pointed shape of teat-end, length of teats were measured before milking. We observed that the significant ($P < 0.05$) effect of udder shape, udder depth, teat end shape and teat length on SCC in Kankrej, Rathu and Sahiwal breeds of cattle. It concludes that the udder and teat morphometry characteristics are significantly associated with somatic cell count and prevalence of subclinical mastitis in indigenous breeds of cattle.

Keywords: Milk quality, Somatic Cell Count, Udder and Teat morphometric

In dairy cows' physical characteristics of udder, and having long and thick teats are imperative traits related with occurrences of sub-clinical mastitis (Chrystal et al. 2001). During intra-mammary infection, somatic cell count (SCC) increases as part of the inflammatory response. In detection of subclinical mastitis, measurement of the SCC is the most frequently used indirect measure (Beaudeau et al. 2002). Therefore, selection of dairy cattle

based on the anatomical attributes of udder and teat morphology may contribute in reducing the chance of mastitis. Earlier, various studies have reported the association between managerial, environmental, parity, stage of lactation and quarter factors, including the physical characteristics of the teat, and SCC in dairy breeds (Sharma et al. 2016; Singh et al. 2022). Teat length was not significantly related with SCC (Seykora and McDaniel 1985), whereas SCC mostly increased with increasing teat diameter (Chrystal et al. 1999). However, in Gir cows' negative association was reported between SCC and teat diameter (Porcionato et al. 2010). To our best knowledge, there are little published studies aiming to determine the association between physical characteristics of udder and teat with SCC of indigenous breeds of cattle.

Therefore, the udder conformation traits with strong arguments can be used to improve udder health. Different breeds have different body characteristics which provide them resistance to udder infection. Aforesaid discussion led us to hypothesize that somatic cell count is affected by different factors including host and environmental. Udder and teat morphometry are major host related factors. All the developed country is using milk somatic cell count as a marker to determine the udder health. The aim study, therefore, was to investigate the associations of udder and teat biometric with SCC in three different indigenous dairy cattle breeds

The Present study was performed at university cattle farms, RAJUVAS, Bikaner, India. The altitude of Bikaner city is 230 meter above mean sea level, latitude and longitude position being 28° 01' 00"N and 73° 18' 43"E", respectively. City is situated in hot semi-arid region with very little rainfall and extreme temperatures.

For this study, a total of 150 lactating cows of Kankrej, Rathu and Sahiwal breed were selected from institute farm. From each breed, a total of 50 healthy lactating cows were selected based on the 4 functional teats and no visible signs of clinical mastitis.

A 50 ml of milk samples were collected from all the four quarters of lactating cows as pool sample of all individual cows in the

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sterilized milk sampling bottle after discarding the first 4-5 streaks of foremilk

The prepared smears for SCC were performed within one hour after collection and methods of calculating to the SCC in milk was done as per reported by Schalm et al. (1971) with slight modification. The dried stained smears were examined under the oil immersion lens of the microscope (Company: Olympus). Thirty different fields per smear were observed, and the average number of somatic cells per field was calculated. The average number of cells per field was then multiplied by the microscopic factor of the microscope, i.e. 393174 to obtain the number of somatic cells per ml of the milk. Microscopic factor was calculated as described by Shukla (1980).

SCC/ml of milk = 393174 × average number of cells per field

Data of SCC were converted in to log scale ($\text{Log}_{10}\text{SCC}$) because SCC not displaying a normal distribution.

Udder shape was classified in to normal and pendulous which was observed visually. Teat-end shape was divided in three categories viz. flat, inverted, and pointed which was observed visually. Udder depth was measured from bases of udder to tip of largest teat. Teat length was measured from the base of the teat to the end of teat. Udder depth and teat length was measured with the help of measuring tape. In present study, grouping of data was done as describe below

Udder Shape – it was divided into Normal udder and pendulous.

Udder depth - it was divided into small (<25 cm), medium (25-35 cm) and large udder depth (>35 cm).

Teat end shape - it was divided into pointed, flat and inverted teat end.

Teat length - it was divided into small (<7 cm), medium (7-10 cm) and large teat length (>10 cm).

The data were analyzed by analysis of variance and General Linear Model Procedure of SPSS software statistical package (version 24. 0) and the means were compared by Duncan's multiple range test. The significance level was set at 95%.

The data on mean values of $\text{Log}_{10}\text{SCC}$ in regular and pendulous shape of udders are presented in table 1. The mean values of $\text{Log}_{10}\text{SCC}$ differed significantly ($P<0.05$) between the regular and pendulous udder shapes in all three breeds of cattle, being significantly lower in regular shape of udders than the pendulous udders in Kankrej, Rath and Sahiwal cattle breeds, respectively. Somatic Cell Counts are influenced by cow productivity, health, parity, lactation stage, and udder and teat morphometry of an animal. In this study, milk SCC was positively associated with udder morphometry (viz. udder shape, udder depth). Sharma et al. (2016) who stated that pendulous udder and larger udder depth have a strong positive association with high SCC. A pendulous shaped udder and larger udder depth is associated with a predisposition to sub clinical mastitis because decreasing distance between teat and floor, increased chance of injury with floor. Several investigations observed low depth and normal udder having lower SCC (Nmcová et al. 2007; Ptak et al. 2011; Singh et al. 2014).

The data pertaining to the mean values of $\text{Log}_{10}\text{SCC}$ in udder depth viz., small, medium and large udders are presented in table 1. The mean values of $\text{Log}_{10}\text{SCC}$ in Kankrej cattle were significantly ($P<0.05$) lower in small and medium udder depth udders than large depth udders. Similarly, in Rath cows the mean $\text{Log}_{10}\text{SCC}$ was significantly ($P<0.05$) lower in small and medium depth udders as compared to large depth udders. However, in Sahiwal cows the average $\text{Log}_{10}\text{SCC}$ was significantly ($P<0.05$) lower in small depth udders than those having large depth. Present study results are in general agreement with the earlier studies who had reported that the incidence of mastitis increases with increasing depth of udder (Singh et al. 2014; Bharti et al. 2015 and Sharma et al. 2016).

Table 1 Mean $\pm$ SE of $\text{Log}_{10}\text{SCC}$ in different udder and teat morphometry characteristics of three indigenous breeds of cattle (50 cows of each breed)

Udder and teat morphometry		Kankrej	Rathi	Sahiwal
Udder shape	Regular	5.13 $\pm$ 0.066 ^a	5.17 $\pm$ 0.060 ^a	5.21 $\pm$ 0.070 ^a
	Pendulous	5.77 $\pm$ 0.116 ^b	5.74 $\pm$ 0.175 ^b	5.62 $\pm$ 0.101 ^b
Udder depth	Small	5.08 $\pm$ 0.102 ^a	5.14 $\pm$ 0.075 ^a	5.14 $\pm$ 0.086 ^a
	Medium	5.15 $\pm$ 0.090 ^a	5.17 $\pm$ 0.096 ^a	5.31 $\pm$ 0.112 ^{ab}
	Large	5.73 $\pm$ 0.108 ^b	5.71 $\pm$ 0.159 ^b	5.62 $\pm$ 0.101 ^b
Teat end shape	Pointed	5.04 $\pm$ 0.083 ^a	5.07 $\pm$ 0.089 ^a	5.18 $\pm$ 0.069 ^a
	Flat	5.27 $\pm$ 0.134 ^{ab}	5.32 $\pm$ 0.102 ^{ab}	5.22 $\pm$ 0.171 ^a
	Inverted	5.59 $\pm$ 0.098 ^b	5.47 $\pm$ 0.144 ^b	5.64 $\pm$ 0.151 ^b
Teat length	Small	5.21 $\pm$ 0.132 ^a	5.07 $\pm$ 0.089 ^a	5.15 $\pm$ 0.087 ^a
	Medium	5.05 $\pm$ 0.081 ^{ab}	5.32 $\pm$ 0.102 ^{ab}	5.19 $\pm$ 0.115 ^a
	Large	5.56 $\pm$ 0.105 ^b	5.47 $\pm$ 0.144 ^b	5.57 $\pm$ 0.055 ^b

Mean with different superscript in columns differ significantly from each other ($P<0.05$)

The data on average values of $\text{Log}_{10}\text{SCC}$ in different teat end shapes viz. pointed, flat and inverted are presented in table 1. The average SCC in Kankrej and Rathī was significantly ($P<0.05$) higher in the inverted end teats than pointed shape teat ends. While it was statistically similar in pointed and flat end teats and in flat and inverted shape of teat ends in both Kankrej and Rathī cows. However, in the Sahiwal cows the average $\text{Log}_{10}\text{SCC}$ was significantly ($P<0.05$) lower in pointed and flat end teats as compared to inverted end teats. In present study, we assumed that animal having flat and inverted teat-end shape may be under the risky of intra-mammary infection. The present study results are in general agreement with findings of Sharma et al. (2016) who had reported a significant effect of teat end shape on SCC and found higher count of Somatic cell in cows with inverted teat end as compared to flat and pointed teat end in Tharparkar breeds cows. Similarly, Bardakcioglu et al. (2011) and Bharti et al. (2015) had observed that the chance of mastitis varies considerably between different teat morphology. Further, Kaur et al. (2018) indicated that teat shape and teat end shape associated with sub clinical mastitis. In respect to teat shape, conical teats were least associated with sub clinical mastitis then bottle shaped teats. According to Chrystal et al. (2001), susceptibility of intra-mammary infection increases in pointed and inverted teat-ends, this is possibly because of greater amount of residual milk in these teats, which act as media for growth of bacteria.

The data pertaining to mean $\text{Log}_{10}\text{SCC}$ in small, medium and large teat lengths are presented in table 1. In Kankrej and Rathī cows the average $\text{Log}_{10}\text{SCC}$ was significantly ($P<0.05$) higher in teat having large length as compared to small length teats. It was statistically similar in small and medium length teats and in medium and large length teats. While, the $\text{Log}_{10}\text{SCC}$ in Sahiwal cows was significantly ($P<0.05$) lower in small and medium length teats than large teats. In the present study, we have observed positive association between teat length and SCC. Sharma et al. 2016 was found significant association of teat length with SCC in Tharparkar cow. Similarly, Berry et al. (2004) suggested that dairy cows with longer teats are genetically predisposed to a higher incidence of mastitis. These findings are in support with the present study results. Usually, it is assumed that longer teats are more prone to physical injuries as they are placed closer to floor and teat lesions (Butto et al. 2010).

Conclusion

The present study concludes that the udder and teat udder and teat morphometry characteristics were significantly associated with somatic cell count and prevalence of subclinical mastitis in indigenous breeds of cattle. Therefore, their inclusion in selection and breeding program as indicator trait may help to reduce the incidence of mastitis in indigenous cattle. Present study is useful for farmers during selection of dairy animals.

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