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RESEARCH ARTICLE

Development of process protocol to enhance the phospholipid content of ghee residue

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Abstract: Many by-products of the dairy industry contain nutritive and commercial value, which with appropriate technological interventions can be exploited for improving the profitability of the industry. Ghee residue (GR) is one such by-product reported to contain considerable amount of polar lipids dominated by phospholipids (PLs). An attempt was made to develop a stepwise protocol to enhance the PL content in ghee residue, to facilitate its efficient and economical extraction from the residue matrix. As a first step, remnant ghee (about 13%) in the residue was expelled by pressing the ghee residue matrix at 5 kg/cm^2 for 5 min. This was followed by treating the ghee residue with two solvents, namely n-hexane and water. Treatment with n-hexane at solvent to solid ratio of 1:4, 1:3 and 1:2, for a contact time of 30 min, resulted in a PL content 26.22, 24.68 and 21.29% (on lipid basis), respectively, in the ghee residue samples. Steeping of the ghee residue in hot water (boiled to 100^{o}C) at a solid to water ratio of 1:4 for 60 min resulted in retention of 27.03% lipids in the residue, corresponding to a removal of 16.93% fat with the solvent stream. This resulted in an appreciable enrichment of phospholipids in the ghee residue matrix to the tune of 30.56% on lipid basis; corresponding to the initial PL content of 8.26%. Increase of surface area of the solid matrix through size comminution was also explored to enhance the efficacy of solvent treatment. When the ghee residue was ground to a particle size of 0.25 mm and 0.30 mm and then subjected to hot water treatment, its PL content was found to increase to 9.56% and 9.32% (ghee residue basis), respectively. Thus, the physical and chemical interventions significantly improved the phospholipid content of ghee residue.

Key words: Ghee residue, boiling water treatment, polar lipids, phospholipids, neutral lipids

Introduction

Milk lipids are globular macrostructures composed of triglycerides with different melting points and covered by three layers of milk fat globular membrane (MFGM) (Martini et al. 2016). Out of the total lipids present in milk, phospholipids (PLs) represent up to 1% of the composition (Lopez et al. 2017) and variations in their percentage are attributed to season, lactation stage and type of feed (Liu et al. 2017). Many dairy by-products are reported to have considerable amounts of polar lipids (Ravindra et al. 2022) such as beta serum, whey protein phospholipids concentrate, butter, milk, cream and ghee residue.

Polar lipids from biological matrices can be extracted using conventional techniques but the process is reported to have challenges such as excess time, expensive solvents and energy consumption (Traversier et al. 2018). These processing hurdles have been eased by adopting assisted extraction techniques to separate polar lipids from non-polar fraction across different dairy products. Some of the assisted techniques reported for dairy products include ultrasonication, filtration, supercritical fluid extraction, switchable solvent extraction and solid phase extraction. Literature reports on extraction of dairy based polar lipids are majorly focussed on buttermilk as the substrate, due to the presence of significant amounts of phospholipids (polar) in its composition. In this context, ghee residue can also be considered as a dairy by-product with significant polar lipids in its matrix.

Ghee residue is a dark brown residue separated during melting of butter or cream while manufacturing ghee. Compositionally, it is rich in lipids, proteins with fractions of lactose and minerals and negligible amount of moisture (Wani et al. 2022). While

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manufacturing ghee, butter or cream is subjected to different temperature protocols, leading to varied proportion of polar lipids (Krishnegowda et al. 2022). Butter heated at 110 to 120°C leads to generation of volatile aromatic compounds which is attributed to heat catalyzed reaction (Aneja et al. 2002). Combination of temperature and time of heating are known to influence the presence of volatile components in ghee. This is due to the fact that processing at low temperature retains more volatile compounds whereas, high temperatures and extended heating time results in loss (Sserunjogi et al. 1998). It has been reported that duration and temperature of ghee clarification alters the phospholipid content owing to the migration of phospholipids from GR to ghee at higher clarification temperatures (Santha and Narayanan, 1979).

Lipids present in ghee residue have a complex structure with both neutral and polar lipid fractions. Unlike plant based matrices, ghee residue is different in its physical features with a solidified structure in its core. Perturbing ghee residue using heat or mechanical action would facilitate loosening of the matrix and movement of its constituents. The application of solvent and hot (boiling) water treatment could be a simple approach to separate polar lipids from neutral lipids in such matrices. Since the amphiphilic activity of this polar lipid fraction could potentially aid in its utilization as an emulsifier, this could ultimately lead to the use of ghee residue as a substrate to yield a green emulsifier. Hence, the present study was undertaken to optimize different treatment interventions in ghee residue to extract its polar lipids fraction using different solvents.

Materials and Methods

Preparation of ghee and collection of ghee residue

Cow milk was procured from the Livestock Production Centre (LRC) of ICAR - National Dairy Research Institute (NDRI), Southern Research Station (SRS), Bangalore. Cream separation from milk was carried using centrifugal cream separator. Fat content of cream was determined using the standard Gerber test (IS: SP-18 1981)) and found to vary between 52 to 60% across different batches. Ghee was prepared by direct cream and creamery butter methods as outlined in Aneja et al. (2002). Ghee was carefully decanted from the kettle through muslin cloth to separate the ghee residue. Adhered ghee in residue was expressed by gentle hand press. Resultant ghee residue was stored under refrigeration (4±2°C) until it was used for further experiments. As a comparative study, ghee residue was also prepared by direct cream method (Santha and Narayanan, 1979) to evaluate the influence of preparation method on yield of lipids and PLs.

Mechanical pressing of ghee residue

Ghee residue obtained from previous step contained higher and varied proportion of lipids due to its particulate structure and multiple batch processing. The stored (4°C) ghee residue was heated (tempered) to 40±2°C in a hot air oven (Falcon Scientific Co., Bangalore, India) . To remove excess surface lipids and to account for the uneven draining of ghee from residue during straining, it was subjected to compression using a mechanical press. Ghee residue was firmly confined inside a muslin cloth and pressed between the plates of a hydraulic press (Multipurpose machine, Milk Tech Engineers, Bangalore, India). This helped to eliminate loosely adhered lipids in the particulate material. Compression pressure was evaluated at 3 levels (3, 4, and 5 kg/cm^2) for duration of 5 min. The pressed ghee residue was removed from muslin cloth and stored at 4±2°C. The hydraulic press used to apply the pressure on the residue is depicted in Figure 1.

Pre-treatment with n-hexane

Ghee residue obtained from creamery butter method contained larger proportions of lipids which include neutral and polar lipids. In order to eliminate the neutral lipids and concentrate the proportion of polar lipids in the matrix, the ghee residue was pre-treated with two solvents.

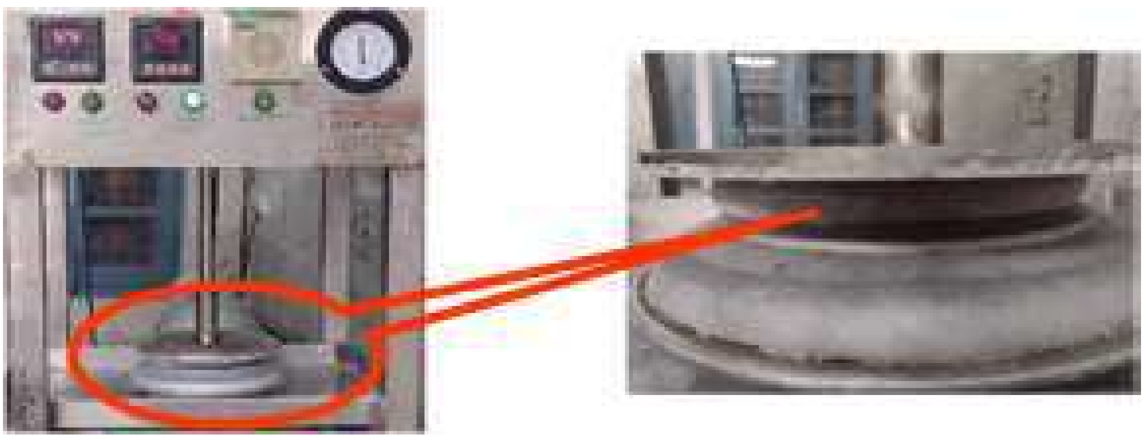


Fig. 1 Hydraulic press used for extraction of lipids from ghee residue

Ghee residue stored after pressing was tempered at 40±2°C for 4 h to melt lipids entrapped in the matrix. Tempered ghee residue was mixed with n-hexane at 1:2, 1:3 and 1:4 solid to solvent ratio (w/v) in a wide mouth glass beaker (15 cm dia.). The beaker was covered with aluminium foil and agitated frequently (every 5 min.) for better solid-solvent contact for varied time periods (20, 30 and 40 min.). After a defined time of contact, the solvent was drained out through Whatman filter paper 1 (185 mm Ø). The retentate residue was then analysed for its lipids and PLs content using phosphorus estimation methods (Sharma et al. 2007). Time of exposure and solid to solvent ratio were considered as the independent parameters. The experiment was aimed to optimize combination of time and solid to solvent ratio to eliminate maximum non-polar lipids.

Hot water boiling

An alternate pre-treatment approach for separation of neutral lipids from ghee residue evaluated in the study was by using boiling water treatment. The ghee residue sample obtained from the creamery butter ghee was pressed using the hydraulic press as mentioned in the above section and tempered to 40°C before mixing with boiling water in solid to solvent ratio of 1:2, 1:3 and 1:4 in a glass beaker. The beaker was immersed in boiling water bath for varied time periods of 20, 30 and 40 min. After the scheduled time, the beaker (with the contents) was placed in a deep freezer (-4 to -12°C) for 5 h to facilitate solidification of lipids separated from ghee residue during the boiling water treatment. The solidified fat layer was carefully skimmed off the surface using a sharp-edged knife. The residual mixture (ghee residue and water) was poured into a stainless-steel tray and dried at 43±1°C for 48 h. Resultant dry fraction was coarsely powdered using pestle and mortar followed by the estimation of its lipids and PLs. Solid to solvent ratio and time of contact were considered as independent factors in this analysis. The data for both the pre-treatments (using n-hexane and boiling water) were compared for removal of neutral lipids from the ghee residue.

Comminution of ghee residue particles

Ghee residue obtained from the solvent treatment method (boiling water) was subjected to size comminution to pass through two sieve sizes (0.25 mm and 0.30 mm). Yield of lipids and PLs from the comminuted samples were analyzed.

Lipid estimation by gravimetric method

Lipids from the ghee residue samples after treatment was recovered using method detailed by Cheng et al. (2019) with few modifications. Ghee residue was taken in Mojonnier flask and added with 2 mL ammonia solution (30%) and 1 g of NaCl, followed by light mechanical shaking. After few minutes, 10 mL of ethyl alcohol (95%) was added and mixed thoroughly to facilitate better access to solvent. Diethyl ether and petroleum ether 15 mL each was added into the flask and plugged with rubber stopper to agitate the contents. The flask was allowed to rest for 60 min. and the solvent was carefully transferred to a pre-weighed glass beaker. In the second stage of solvent extraction ethyl alcohol (5 mL), diethyl ether (10 mL) and petroleum ether (10 mL) were added to flask and allowed to rest for 60 min. Solvent was pooled to previously collected sample followed by one more extraction with same proportion of solvents. Solvent obtained in all three steps were pooled and solvent was evaporated by hot water bath maintained at 75°C. After major quantity solvent was evaporated, beaker was placed in hot air oven maintained at 90±2°C till constant weight was obtained.

Estimation of Phospholipids content

Phospholipids content of ghee residue after two different treatments was estimated based on phosphorus content in fat extracted using organic solvents in previous step. The method used by Murthy and Narayanan (1966) with slight modifications was adopted for estimation of PLs. The lipids obtained were digested with nitric acid and sulphuric acid (5 mL each) using a heating mantle till the colour of the mix turned light yellow or colourless. Digestion flask was cooled and added with 10 mL of distilled water and heated till the evaporation of water. Further, 10 ml water was added and heated till its evaporation. After cooling, 5 mL of this aliquot was mixed with 0.44% ammonium molybdate and 0.4 mL of reducing agent (1-amonia-2-napthol-4 sulphonic acid, sodium sulphite, sodium bisulphite) in a test tube and immersed in boiling hot water bath for 7 min. For blank, 0.5% sulphuric acid was used whereas, 1 µg/mL potassium dihydrogen phosphate was used as standard. Optical density was measured at 720 nm using UV/VIS spectrophotometer (LABINDIA Analytical UV 3200XE, India). PLs were estimated as the ratio of optical density of sample and standard by multiplying with 25.9 as conversion factor for phosphorus to PLs.

Table 1: Effect of ghee preparation method in the yield, lipids and phospholipid content of the ghee residue

Method	GR yield (%)	Lipids (%)	Phospholipids (% GR basis)	Phospholipids (% on lipids basis)
Creamery butter method	5.16±1.53[b]	54.55±6.03[a]	4.98±1.26[a]	9.08±1.88[a]
Direct cream method	13.56±2.52[a]	35.90±3.83[b]	0.95±0.08[b]	2.68±0.47[b]

Same alphabet in column indicates no-significant difference ($p<0.05$) through Tukey's test.

Fig. 2 Lipid content of pressed ghee residue at varied pressure levels

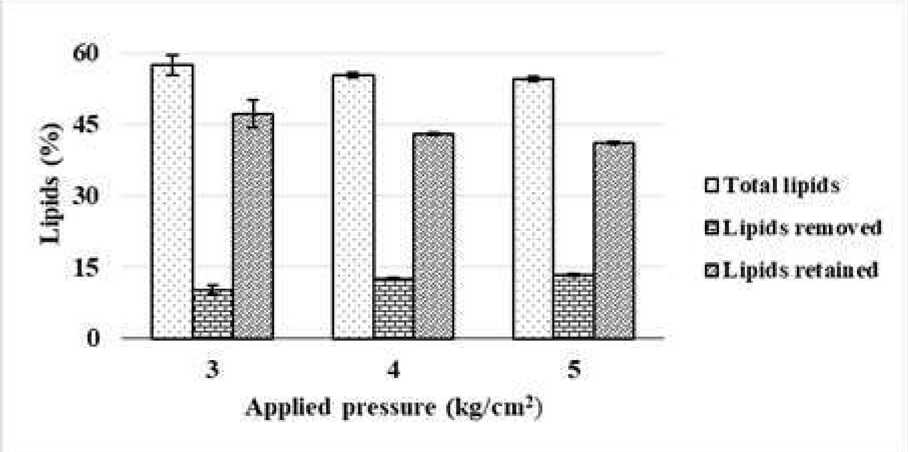
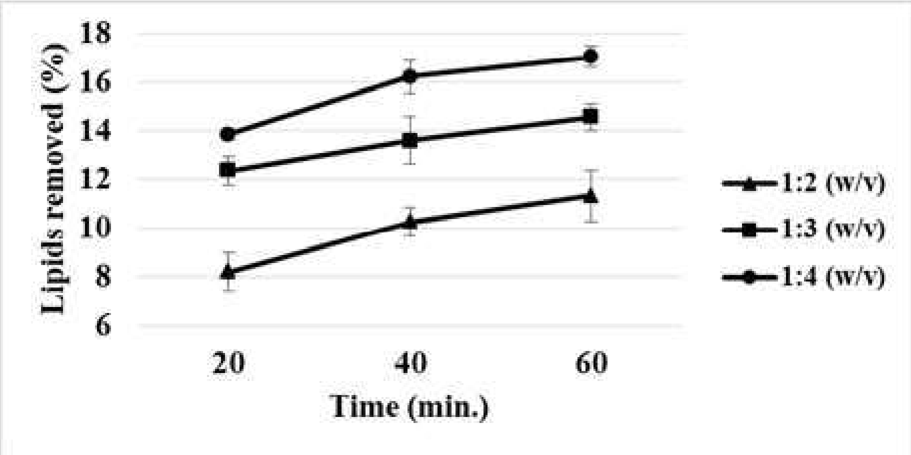


Fig. 3 (a) Lipids (%) removed in ghee residue by n-hexane treatment at different time and solids to solvent ratio



Results and Discussion

Comparison of ghee residue yield by creamery butter and direct cream method

The results for the PL content of ghee residue samples prepared by different methods are tabulated in Table 1. It can be seen that though there was 2.62 folds increase in the yield of ghee residue in the direct cream method, a 51.94% decrease in total lipids can be seen compared to creamery butter method. The PLs content was lower (0.95±0.08%) for the GR obtained from direct cream method than the creamery butter method (Table 1). Thus, it can be observed that the method of preparation also affected the PL content. The similar findings for higher PL content in the GR obtained from creamery butter method than direct cream were also reported by Sangma et al. (2023). The results are also supported by the findings of Santha (1977) and Janghu et al. (2014). It is postulated that the high serum solids present in ghee residue contributed to lesser lipids and PLs yield when expressed on total ghee residue weight basis. While working with different fractions of fat in cream, Pal and Rajorhia (1975) observed increase in ghee yield corresponded with a reduction in amount of ghee residue. This was attributed to the yield of ghee residue proportional to solids-not-fat (SNF) fraction in the raw material. Based on the above findings, it was deduced that the ghee residue obtained from the creamery butter method yielded higher PLs content in the residue.

Optimization of pressure to remove excess lipids from ghee residue

The pressure applied on ghee residue when it was strained through the muslin cloth to remove residual ghee was very low. Hence, ghee residue was subjected to varied pressure levels using a hydraulic press. With increase in applied pressure, more lipids were observed to be removed from the residue matrix resulting in a reduction of lipids present in ghee residue. However, pressing beyond 5 kg/cm^2 pressure did not show any considerable change in lipids content of the residue Based on this investigation, pressing at 5 kg/cm^2 for 5 min. was adopted for the expulsion of ghee from ghee residue. The total lipid content in the pressed ghee residue was recorded to be 41%±0.28% after hydraulic pressing (Figure 2). Similar methods are often used in the extraction of fat/oil from the oilseeds by pressing the flaked/grounded oilseeds in a hydraulic or screw press. The application of pressure is known to create a driving force that squeezes and facilitates in oozing out of loosely held oil in the matrix (Savoire et al. 2013).

Figure 3 (b) Lipids (%) retained in ghee residue by n-hexane treatment at different time and solids to solvent ratio

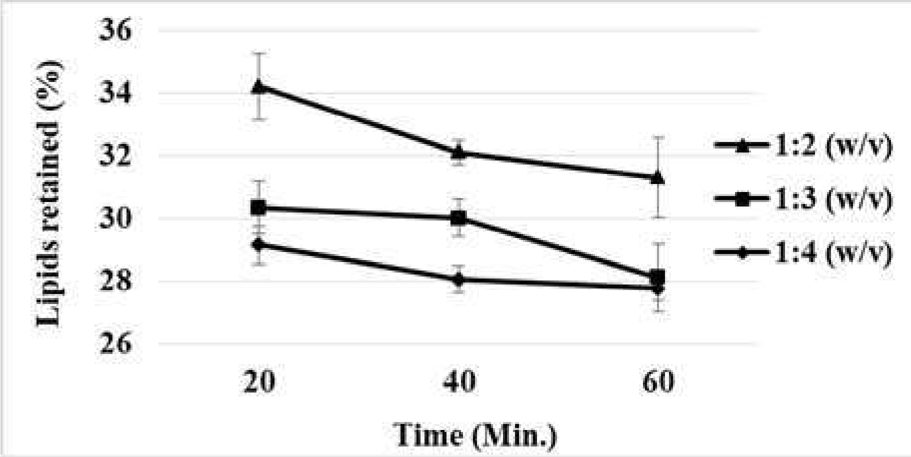
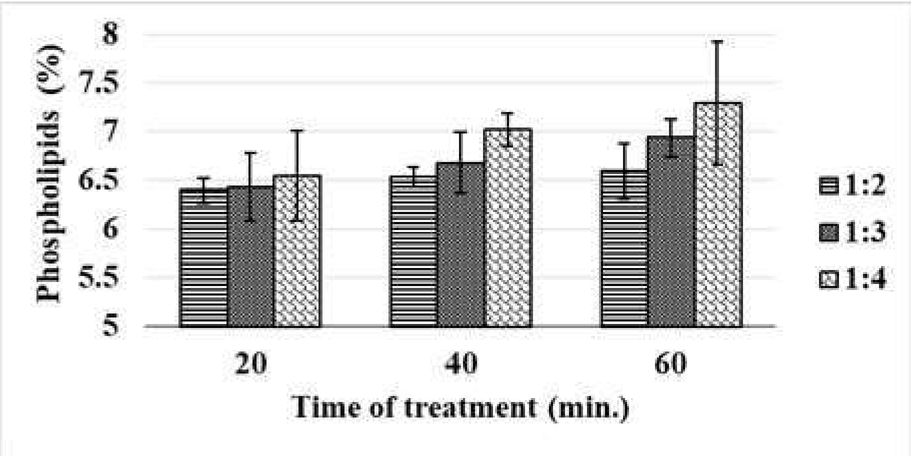


Fig. 4 (a) Phospholipids expressed on dry weight basis after n-hexane treatment



Treatment with n-hexane to remove neutral lipids

Two factors, namely contact period (20, 40 and 60 min.) and solids to solvent ratio (1:2, 1:3 and 1:3) were considered and the results are presented in Figure 3 and 4. Both factors were observed to positively influence the separation and removal of lipids from the residue through n-hexane. Solids to solvent ratio of 1:2% (w/v) led to removal of 8.21% of lipids after a contact period of 20 min. against 11.32% for 60 min. However, when solids to solvent ratio were increased to 1:4 (w/v) and the contact period was 60 min., 17.04% lipids were removed from the residue and the treated ghee residue retained 27.78% of lipids (Figure 3 a & b). The above observations affirmed the influence of contact time and solids to solvent ratio on the mass transfer phenomena i.e. lipids movement from the residue.

Weller and Hwang (2005) also emphasized on the improvement in lipid yield with increase in solvent to solid volume from 3 to 5 mL/g while extracting lipids from sorghum using n-hexane as solvent. In the same experiment, increase in time of exposure from 1 to 6 h also resulted in enhanced lipid extraction. This was attributed to higher contact time and concentration gradient for diffusion of lipids into solvent.

Phospholipids in ghee residue after n-hexane treatment

The ghee residue after treatment with n-hexane was evaluated for PLs content, which was expressed on ghee residue and lipid wt. basis (Figure 4 a & b). From the figure, it is evident that PLs content in the residue also improved with increase in solids to solvent ratio. Short exposure time (20 min.) resulted in 6.39% of PLs in ghee residue and it was 21.20% on lipid basis. As time of treatment increased to 60 min, PLs present in sample also increased to 7.29% which amounts to 26.22% on lipids basis. The reason attributed to increase in PLs content is due to the movement of neutral lipids from the ghee residue matrix into the solvent (n-hexane). Vale et al. (2019) studied the separation and extraction of lipids using a three-phase lipid extraction protocol which included hexane, methyl acetate, acetonitrile and water in 4:4:3:4 ratios. The study demonstrated the efficacy of the solvents in classifying the neutral lipids in upper phase and polar lipids in middle phase of the solvent.

Statistical analysis of the data indicated significant difference among the PLs content in solvent due to solid to solvent ratio and time factors ($p<0.05$). However, interactive effect of the two factors was found to be statistically insignificant ($p<0.05$). The study concluded that there was significant improvement in the

Fig. 4 (b) Phospholipids expressed on lipids weight after n-hexane treatment

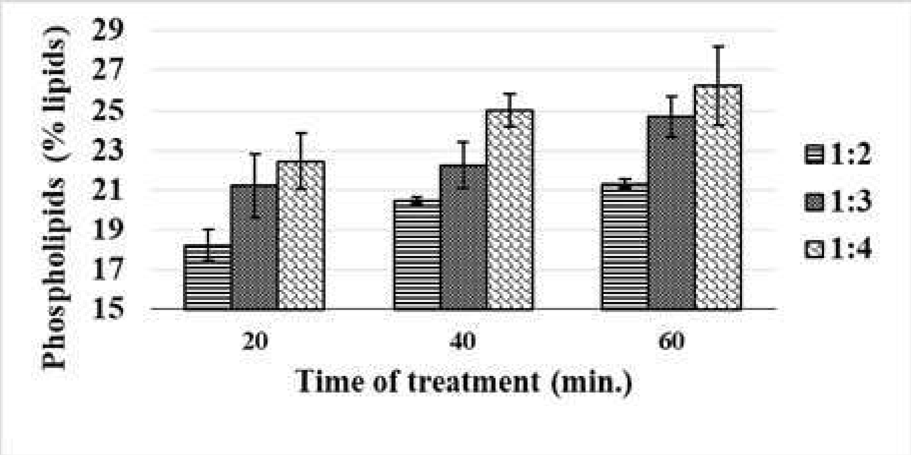
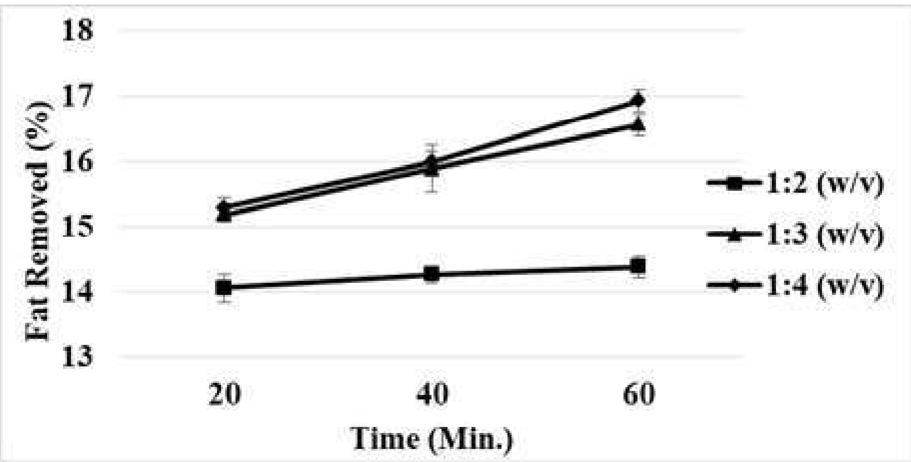


Fig. 5 (a) Lipids (%) removed in ghee residue by boiling water treatment at different time and solids to solvent ratio



PLs content of ghee residue with increase in time and solid to solvent ratio when treated with n-hexane.

Boiling water treatment of ghee residue

Boiling water treatment of the pressed ghee residue was also considered for the contact time of 20, 40 and 60 min. and solids to solvent ratio 1:2, 1:3 and 1:4. The total lipids removed and retained in ghee residue after boiling water treatment are depicted in Figure 5 a & b. Nearly 17% of lipids were removed from the residue at solids to solvent ratio of 1:2 for 60 min (Figure 5 a). Solids to solvent ratio of 1:3 and 1:4 followed similar trend lines for the removal of total lipids from the ghee residue under this treatment. The effect of solids to solvent ratio on retention of lipids in ghee residue was also found to be significant ($p<0.05$).

Chemat (2015) indicated that water could be employed as a good solvent to remove polar lipids from lipids complex at high temperature (100 and 374°C) and pressure (22.1 MPa). At elevated temperature and pressures, water undergoes self-ionization leading to decreased viscosity, surface tension and increase in diffusivity (Shitu et al. 2015). Even at normal atmospheric conditions, water demonstrates the ability to act as polar solvent; this is ascribed to its dielectric constant of 80. Based on these points, water can be considered as a solvent to diffuse polar components. It was hypothesized that this would facilitate free movement of non-polar fraction as a floating layer which could then be separated due to density gradient.

It is assumed that during boiling water treatment polar lipids of the ghee residue matrix migrate to and is held by water whereas; the non-polar lipids would be concentrated in the top layer, which could be skimmed off. Thus, evaporation of water and drying of the ghee residue after boiling water treatment was expected to result in the retention of polar lipids in ghee residue matrix. During pre-treatment, it was observed that wider diameter containers facilitated better lipids flotation than narrow diameter (Figure 5 a). Lipids were carefully removed along with little fraction of ghee residue after freezing for easy skimming (Figure 5 b).

Phospholipids in ghee residue after hot water boiling treatment

The PLs content of the dried fraction of the ghee residue was estimated post-treatment with boiling water and expressed on ghee residue and lipids basis (Figure 6 a & b). PLs retained in ghee residue reported an increasing trend with increase in time and solids to solvent ratio. Maximum PLs content (8.26% ghee residue basis) was reported with contact time of 60 min. at solids

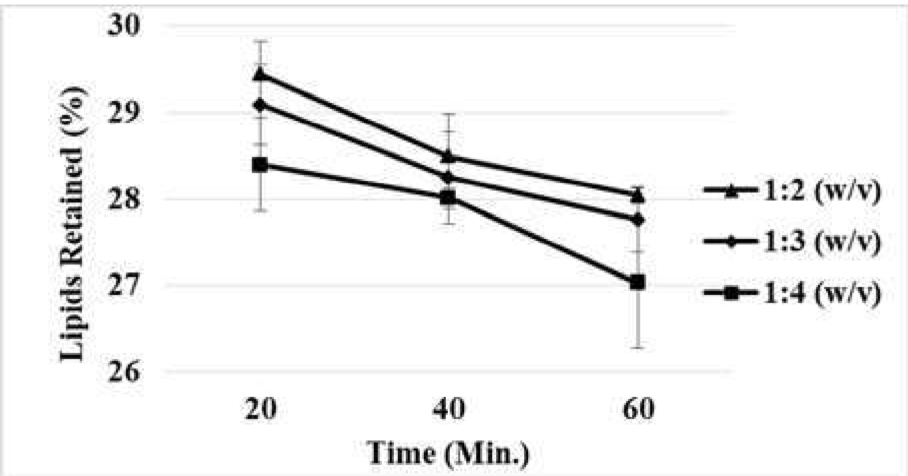


Fig. 5 (b) Lipids (%) retained in ghee residue by boiling water treatment at different time and solids to solvent ratio

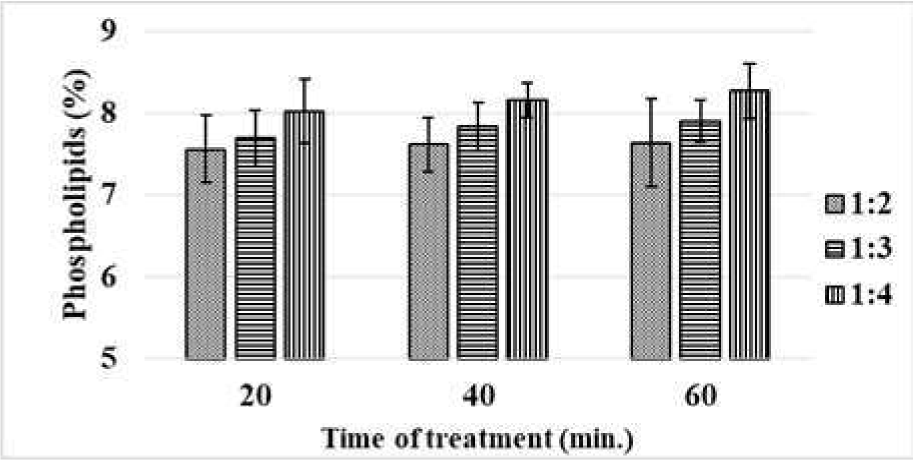


Fig. 6 (a) Phospholipids expressed on dry weight after boiling water treatment

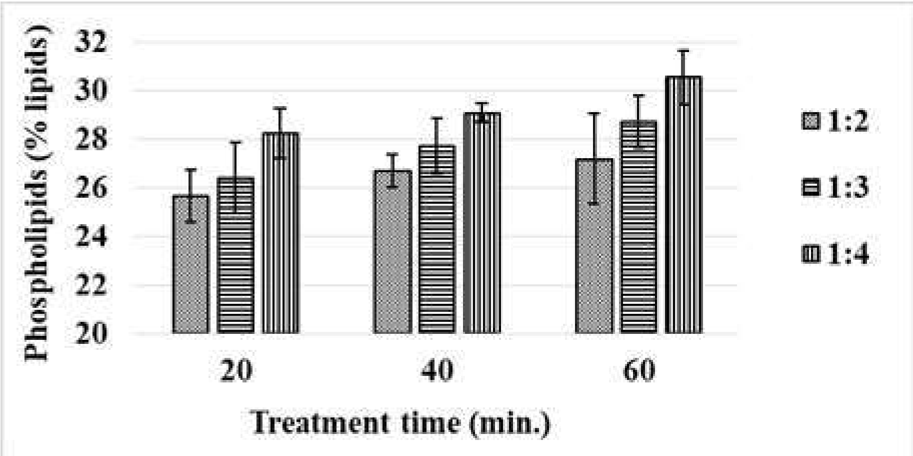


Fig. 6 (b) Phospholipids expressed on lipids weight after boiling water treatment

to solvent ratio of 1:4. This corresponded to a PLs content of 30.56% when reported on lipid weight basis.

No significant influence ($p>0.05$) was noted for contact time for retention of PLs ($p<0.05$). However, solids to solvent ratio had significant influence on retention of PLs in the treated ghee residue. As the F value for this factor was greater that F-critical value, two factor comparison was analysed by Tukey's test at 20 min. contact time. Results from post-hoc test indicated no significant difference among treatments. Hence, solid to solvent ratio at the lower level of 1:2 can be considered optimal over extended solvent ratio.

Table 2: Effect of various pre-treatments on the lipids and phospholipid content of the ghee residue

Stage of ghee residue	Lipid (%)	Phospholipids (GR wt. basis)	Phospholipids (lipids wt. basis)
After filtering	54.55±6.03[a]	4.98±1.26[b]	9.08±1.88[b]
After pressing	41.48±0.41[b]	6.29±1.32[b]	15.14±3.02[b]
After hot water treatment	29.46±0.45[c]	7.4±0.73[ab]	25.09±2.10[a]
After comminute to 0.25 mm	29.55±1.52[c]	9.56±0.82[a]	32.47±4.03[a]
After comminute to 0.30 mm	28.54±1.45[c]	9.32±1.16[a]	32.74±4.74[a]

Comminution of the pre-treated ghee residue

The pressed ghee residue after boiling water treatment and drying was subjected to size reduction by passing through a mechanical mill. The size reduced particles were then classified by passing through 0.30 and 0.25 mm sieve followed by estimation of its lipids and PLs. The progressive improvement in lipids and PLs profile of the pre-treated ghee residue, as it was subjected to different steps of the pre-treatment protocol, is depicted in Table 2.

A perusal of the data indicated a definite improvement in the PLs content with each step of treatment with a slightly higher yield of PLs when the particle size was reduced to 0.25 mm (9.56%) over 0.3 mm (9.32%). The PLs content in the ghee residue was enriched from 4.98% at freshly prepared stage to 9.56% after comminution to 0.25 mm particle size. The results indicated successive improvement in PLs content that could be ascribed to the elimination of non-polar lipids. Between boiling water treatment and comminution to 0.25 mm particle size, sizable increase in PLs was noticed. This could be due to increased surface area which facilitated gradient for movement of lipids from particle surface to solvent. From overall treatments, there was an increase of 91.96% in PLs content expressed on ghee residue basis. This increment could be mainly due to elimination of non-polar lipids fraction and physical processing.

Conclusion

Ghee residue is an untapped by-product of the dairy industry, with most avenues for its downstream utilization being restricted to as an ingredient in either the confectionary or animal feed industry. The presence of phospholipids in the residue and the potential industrial use of this valuable component as an emulsifier opens alternate avenues for the utilization of ghee residue. The present study formulated a stepwise protocol, targeting a stepwise removal of neutral lipids from the residue matrix leading to progressive improvement of the PL content in the ghee residue. The developed protocol included steps such as mechanical pressing of the fresh ghee reside matrix at 5 kg/cm^2 for 5 min., extraction with n-hexane at solids to solvent ratio of 1:4 for 30 min, treatment with boiling water at a solid to water ratio of 1:4 for contact time of 60 min. and size comminution to 0.25 mm. The progressive enhancement of PLs in the ghee residue with each treatment step ultimately resulted in a PL content of 9.56% in the residue. Thus, the developed protocol can be suggested as a preliminary treatment of ghee residue, for the effective and economical extraction of PLs from the residue for further use.

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RESEARCH ARTICLE

Physico-chemical characteristics of butter prepared from the milk of selected indigenous and crossbred cows

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Abstract: This study aimed to investigate the effects of the season in relation to breed and the butter preparation method on chemical composition, β-carotene content, conjugated linoleic acid (CLA) content, color attributes, texture profile, and keeping quality. Fat content showed minor variation in butter types, conventional butter had significantly ($p<0.05$) lower free fatty acid (FFA) levels than desi butter. Tharparkar butter, especially in summer batches, had the highest β-carotene content. CLA levels varied with breeds and seasons; increased during summer due to outdoor grazing on green fodder. The colour analysis highlighted breed-specific variations, with Tharparkar butter displaying superior visual characteristics. Texture analysis demonstrated that conventional butter was harder, less adhesive, and showed higher cohesiveness compared to desi butter. The study emphasizes the need for careful breed selection and seasonal considerations to ensure the nutritional quality, appearance, texture, and quality of butter products. Monitoring free fatty acid levels is crucial for assessing butter shelf life and quality.

Keywords: Desi butter, spreadability, firmness, β-carotene, conjugated linoleic acid (CLA).

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Introduction

The milk production in India for the year 2021-22 was reported to be 221.01 MT, with a CAGR (compound annual growth rate) of approximately 6.2% (NDDB Report, 2022). Butter is the second most widely consumed fat-rich dairy product in India, accounting for 6.5% of total milk conversion, following ghee (Battula et al. 2020). Butter is a water-in-oil type emulsion, wherein water is dispersed within the oily medium (Patel et al. 2015). In comparison to vegetable oil-based margarine, butter is more expensive, leading to adulteration practices by profit-oriented food business operators. It has been reported that A1 milk protein from exotic cow breeds may have adverse health effects on humans, such as juvenile diabetes, coronary heart disease, irritable bowel syndrome, schizophrenia, and autism (Parashar and Saini 2015; Chia et al. 2017). Indigenous cow milk contains the A2 variant of milk protein, suggesting that focusing on A2 milk and milk products may be beneficial for better health outcomes. Cow milk butter appears more yellowish than buffalo milk due to its higher β-carotene content, which is converted into vitamin A by an intestinal mucosal enzyme (β-carotene-15,152 -monooxygenase) present in buffalo (Patel et al. 2015). β-carotene has been reported to play a role in preventing night blindness, xerophthalmia, strengthening the immune system against infections, promoting gastrointestinal function, and supporting proper growth, development, and functioning of the reproductive systems (Grune, 2010). Various flavoring compounds, including diacetyl, δ-octalactone, δ-decalactone, skatole, and butanoic acid, contribute significantly to the flavor of butter, with each compound having a threshold concentration in relation to butter flavor. Butter primarily consists of fat, serving as a repository for bioactive lipids such as short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA), and phospholipids. Butterfat is a major carrier of phospholipids (such as lecithin and glycoprotein) and fat-soluble vitamins like vitamins A, D, E, and K, which play essential roles in regulating various biological activities, including bone mineralization, tissue differentiation, cell growth, and coenzyme activity (Park et al. 2021). While butter consumption offers health benefits, it is important to consider the potential risks associated with saturated fatty acids, trans fatty acids (TFA), and cholesterol found in butter (Givens, 2017). In India, butter is commonly used as a cooking medium at the household level, where household butter is typically prepared using the desi method. Exploring the composition and therapeutic value of butter from indigenous cow milk is a relatively new area of research, could provide valuable insights. However, there is limited data available on the composition of butter from selected indigenous cow breeds and crossbreeds, including their β-carotene, CLA content and rheological properties.

Materials and Methods

Chemicals and reagents

Fatty acid methyl ester (FAME), β-carotene, and linoleic acid-conjugated reference standards were procured from Sigma-Aldrich Co, St. Louis, USA. All solvents (chloroform, methanol, hexane, diethyl ether, petroleum ether, ethanol absolute, n-butanol, Tetrahydrofuran, acetonitrile and milli-Q water) used were HPLC grade. Oxalic acid, sodium carbonate, sodium chloride, potassium hydroxide pellets, sodium hydroxide pellets, sodium bisulfate, sulphuric acid, potassium dichromate, and phenolphthalein reagent were AR grade and procured from Qualigens Fine Chemicals, Mumbai, India. Starter culture (NCDC 167) was obtained from the National Collection of Dairy Culture, ICAR-NDRI, Karnal.

Sample collection

Fresh milk samples were collected from individual mid-lactating cattle of various breeds, including Tharparkar, Sahiwal (an indigenous breed), Karan Fries (a crossbred breed), as well as pooled cow milk from all three breeds. These samples were obtained from the Livestock Research Centre (LRC) of the National Dairy Research Institute (NDRI) in Karnal. The collected milk samples were stored at 4! until further analysis. Before analysis, the samples were warmed to 40! and analyzed within a 2-hour time frame. To evaluate the effect of seasonal variation, the sampling period was divided into two seasons: Winter (November-January) and Summer (February-April). The dairy animals were maintained under identical feeding and management conditions throughout the study. They were provided with concentrate feed during all seasons, while berseem, oats, and alfalfa were additionally supplied during the winter season.

Preparation of Butter

Desi and conventional butter samples were prepared using starter culture (NCDC 167), following the unsalted butter preparation technique described by De (2012).

Chemical parameters

The chemical parameters, including free fatty acids, moisture, fat, and curd content, of both conventional butter samples and desi butter samples were determined using standard methods outlined in IS 548-1 (1964) for free fatty acid analysis and IS 3507 (1966) for moisture, fat, and curd content determination.

β-carotene measurement

The estimation of β-carotene was performed using the HPLC method described by Dhankhar (2017). The analysis was conducted using an Agilent 1260 infinity system (Agilent Technologies, USA) equipped with a Diode Array Detector (DAD) and a reversed-phase C18 column (ZORBAX 300 SB-C18, 4.6 x 250 mm, 5 µm). The wavelength used for detection was set at 450 nm. A mobile phase consisting of a mixture of methanol/THF/water was utilized, and the column flow rate was set at 0.8 ml/min. To ensure the purity of the solvent mixture, proper filtration using a 0.22 µm pore size filter and sonication at 40 Hz for 10 minutes were performed prior to the HPLC test.

CLA measurement

Methyl esters were prepared following the guidelines outlined in ISO/IDF (2002). CLA methyl esters was injected at volumes of 1, 2, 3, 4, and 5 µl using an auto sampler (AOC-20i), into a Shimadzu GCMS TQ 8030 gas chromatography system coupled with a flame ionization detector. Chromatographic separation of fatty acid methyl esters (FAMEs) was achieved using an SPTM-2560 fused silica capillary column (100 m x 0.25 mm I.D. x 0.20 µm film thickness, Supleco). Nitrogen was used as the carrier gas with a flow rate of 0.8 ml/min. The oven temperature program initiated at 140°C with a hold time of 5 min, followed by a ramp to 240°C at a rate of 3°C/min (hold time of 15 min). The injector and ion source temperatures were both maintained at 250°C throughout the analysis. A detailed regression line was plotted based on the peak areas obtained at different concentrations of the FAME mixture. The fragmentation spectra of the standard linoleic acid conjugated methyl ester were compared with those obtained from the sample run.

Colour measurement

The colour value of butter was determined using the method outlined by Sert and Mercan (2020) in terms of L*, a*, and b* values. A Hunter Colourflex colourimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) equipped with a xenon flash lamp as the light source was employed for this analysis. Each sample was tempered at 10°C and tested in triplicate in this system, L* indicates the position of the sample on the dark-light axis, a* represents the position on the green-red axis, and b* signifies the position on the blue-yellow axis.

Texture profile analysis (TPA)

The textural attributes of the butter samples were evaluated using a Texture Analyser TAXT2i (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 25 Kg load cell. A typical two-bite compression test was performed on the product using a P-75 platen compression probe attached to the texture analyser. Three replicates were conducted for each sample. Before testing, the butter samples were stored at a temperature of 10ºC. The parameters measured included hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. The Texture Expert for Windows software version 1.20 (Stable Micro Systems) was used for data analysis.

Statistical Analysis

Statistical analysis was performed using SPSS 20 software (IBM Corporation, USA). The two-way ANOVA (Analysis of variance) and Tukey's HSD post-hoc test ($P < 0.05$) were used for the evaluation of differences in concentration of fatty acids, β-carotene and conjugated linoleic acid of butter between breeds and seasons. The results were expressed as mean with standard deviation.

Results and Discussion

Chemical composition of butter samples

The chemical composition of butter prepared through desi and conventional methods was investigated, and the results are presented in Table 1. The fat content of the butter samples showed no significant variations ($p \geq 0.05$) in the fat percentage among Karan-Fries, Sahiwal, Tharparkar, and conventional butter. Notably, conventional butter exhibited the highest fat percentage, attributed to lower fat loss in buttermilk during the conventional preparation process, as noted by previous studies (Panchal and Bhandari 2020). The free fatty acid (FFA) content of conventional butter was significantly lower ($p \leq 0.05$) compared to desi butter derived from indigenous cow milk. Among the desi cow milk butter samples, non-significant differences ($p \leq 0.05$) were observed in the FFA content of Karan-Fries, Sahiwal, and Tharparkar cow butter. Moreover, no significant differences ($p \geq 0.05$) were noted in the FFA content among butter samples from Karan-Fries, Sahiwal, and Tharparkar cow milk. The curd content of butter, derived from Karan-Fries, Sahiwal, Tharparkar milk, and conventional sources, exhibited non-significant differences ($p \geq 0.05$). These results indicate the uniformity in curd content across the tested butter samples.

β–carotene content of butter

The β-carotene content of freshly prepared butter using desi and conventional methods is presented in Fig. 1(a). Statistical analysis revealed significant differences ($p \leq 0.05$) in the β-carotene content among the butter samples. Butter derived from Tharparkar milk exhibited the highest β-carotene content, followed by conventional butter and Sahiwal butter, with the lowest content found in Karan Fries-derived butter. This observation aligns with the findings of Kumar et al. (2022). Notably, the β-carotene content in Karan-Fries butter differed significantly ($p<0.05$) from all other types. Furthermore, a significant increase in β-carotene content was noted in butter prepared during the summer season, attributed to outdoor grazing on green fodder (Antone et al. 2015). The average β-carotene content in butter derived from conventional, Karan Fries, Sahiwal, and Tharparkar milk was 5.90 µg/g fat, 4.63 µg/g fat, 5.8 µg/g fat, and 6.11 µg/g fat, respectively. β–carotene content of butter during summer season (5.3 – 6.77 µg/g fat) was significantly ($p \leq 0.05$) higher than butter in winter season (3.95-5.44 µg/g fat). This seasonal variation in β-carotene content is consistent with previous studies (Marino et al. 2012), which reported a 25% to 68% decrease in β-carotene content in milk samples collected during the winter season compared to those collected in the summer season. Additionally, the shift from a summer diet to a winter diet led to a rapid decrease in β-carotene content, as demonstrated by Noziere et al. (2006).

The decrease in β-carotene content in milk fat with the changing seasons is well-documented in the literature (Noziere et al. 2006, Marino et al. 2012). These findings underscore the importance of seasonality in influencing the nutritional quality of butter, particularly concerning β-carotene content.

CLA content of butter

The conjugated linoleic acid (CLA) content in butter samples derived from various breeds is presented in Fig. 1(b). Statistical analysis revealed significant variations in CLA content among the different breeds. Butter prepared from Karan Fries milk exhibited the highest CLA content (15.7 mg/g fat), followed by Tharparkar (12.91 mg/g fat), and Sahiwal (12.12 mg/g fat) breeds.

Table 1 Chemical composition of butter prepared from indigenous and crossbred cow milk

Butter	Moisture %	Fat %	FFA %	Curd %
CBPM	15.69 ± 0.172^{ab}	82.25 ± 0.69^{a}	0.14 ± 0.002^{b}	1.11 ± 0.06^{a}
DBKF	15.57 ± 0.073^{b}	82.09 ± 0.38^{a}	0.27 ± 0.05^{a}	1.07 ± 0.08^{a}
DBSW	15.79 ± 0.111^{a}	82.07 ± 0.34^{a}	0.27 ± 0.03^{a}	1.15 ± 0.07^{a}
DBTP	15.76 ± 0.098^{b}	82.01 ± 0.27^{a}	0.29 ± 0.01^{a}	1.13 ± 0.05^{a}

Results are expressed as means ± S.D (n=3). a-b means within columns with different superscript are significantly different ($p \leq 0.05$) from each other.

CBPM- conventional butter from pooled milk ; DBKF- desi butter from Karan Fries ; DBSW- desi butter from Sahiwal; DBTP- desi butter from Tharparkar

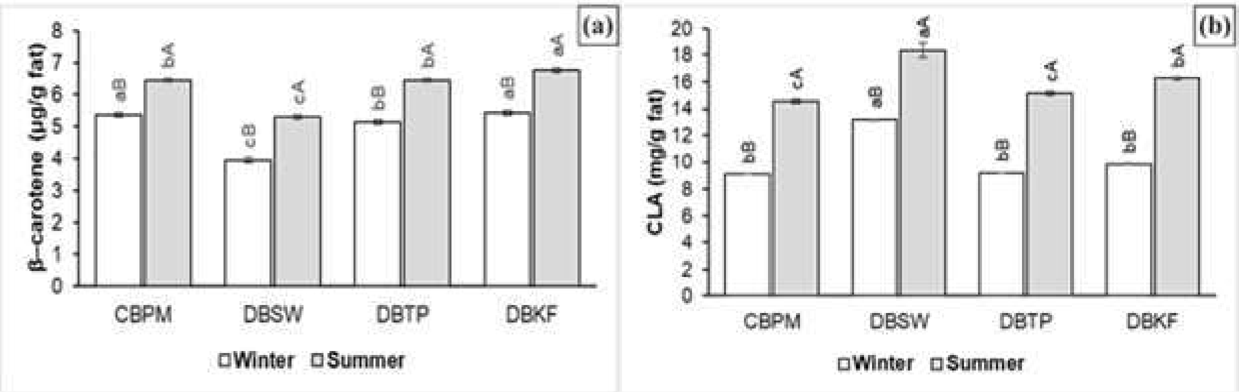

Fig. 1 Effect of breed and season on β-Carotene and CLA content in butter
(a) β-carotene content of butter (b) CLA content of butter
Results are expressed as means ± S.D (n=3). A-B Means within a season and a-b means within treatment (CBPM, DBKF, DBSW, and DBTP) with different superscripts were significantly different (p≤0.05) from each other.

Table 2 Effect of season on colour values of butter prepared from indigenous and cross bred cow milk

	L*		a*		b*	
Breeds	Winter	Summer	Winter	Summer	Winter	Summer
CBPM	78.35± 0.25[bA]	75.47 ± 0.15[cB]	5.61± 0.06[aA]	3.79 ± 0.06[aB]	26.67 ± 0.19[cB]	31.42± 0.13[bA]
DBKF	81.06 ± 0.32[aA]	77.99 ± 0.33[aB]	3.89 ± 0.06[dA]	2.01 ±0.06[cB]	23.69±0.29[dB]	30.84 ± 0.20[cA]
DBSW	79.14 ± 0.29[bA]	76.31 ± 0.16[bB]	4.08 ± 0.06[cA]	3.68 ± 0.05[aB]	29.71 ± 0.18[bB]	31.68 ± 0.20[bA]
DBTP	76.88 ± 0.94[cA]	75.91 ± 0.33b[cA]	4.45 ± 0.09[bA]	3.47 ± 0.08[bB]	30.24±0.1[aB]	37.18 ± 0.13[aA]

Results are expressed as means ± S.D (n=3). A-B Means within rows and a-b means within columns with different superscript were significantly different (p≤ 0.05) from each other.

CBPM- conventional butter from pooled milk; DBKF- desi butter from Karan Fries ; DBSW- desi butter from Sahiwal; DBTP- desi butter from Tharparkar

Conventional butter significantly differed from both Karan Fries and Tharparkar milk butter. The average CLA content in butter derived from conventional, Karan Fries, Sahiwal, and Tharparkar milk was 11.78, 15.7, 12.12, and 12.91 mg/g fat, respectively. These values fall within the range reported in the literature (2 to 37 mg/g fat) (Stanton et al. 2020).

Significant seasonal differences ($p \leq 0.05$) were observed in the CLA content of butter, with higher levels during the summer (14.54-18.34 mg/g fat) compared to the winter season (9.1-13.14 mg/g fat). This finding is consistent with previous studies that reported higher CLA levels in summer months compared to winter (Frelich et al. 2009). Ahmad et al. (2019) noted elevated CLA content (22.1 mg/g fat) in milk from pasture-fed cows compared to cows with one-third (8.9 mg/g fat) or two-thirds (14.3 mg/g fat) of their diet as pasture. The increase in CLA during pasture feeding is attributed to the effects on biohydrogenation and the provision of z#-linolenic acid as a lipid substrate, leading to the formation of cis-9, trans-11 CLA in the mammary gland (Indu and Jayaprakasha 2021, Stanton 2020). These observations underscore the impact of both breed variation and seasonal factors on CLA content in butter, emphasizing the need for comprehensive understanding and careful management in dairy practices.

Colour value of butter

The colour characteristics of butter prepared from selected indigenous and crossbred cow milk, employing both conventional and desi methods, are detailed in Table 2. The colour attributes, namely lightness (L*), redness (a*), and yellowness (b*), were analyzed. During the winter season, Karan-Fries butter displayed the highest lightness value (81.06), whereas Tharparkar butter recorded the lowest value (76.88). There was a significant ($p<0.05$) difference in lightness values between butter from Karan-Fries, Sahiwal and Tharparkar milk. Notably, there was statistically significant higher lightness values ($p<0.05$) observed during the winter season compared to the summer season.

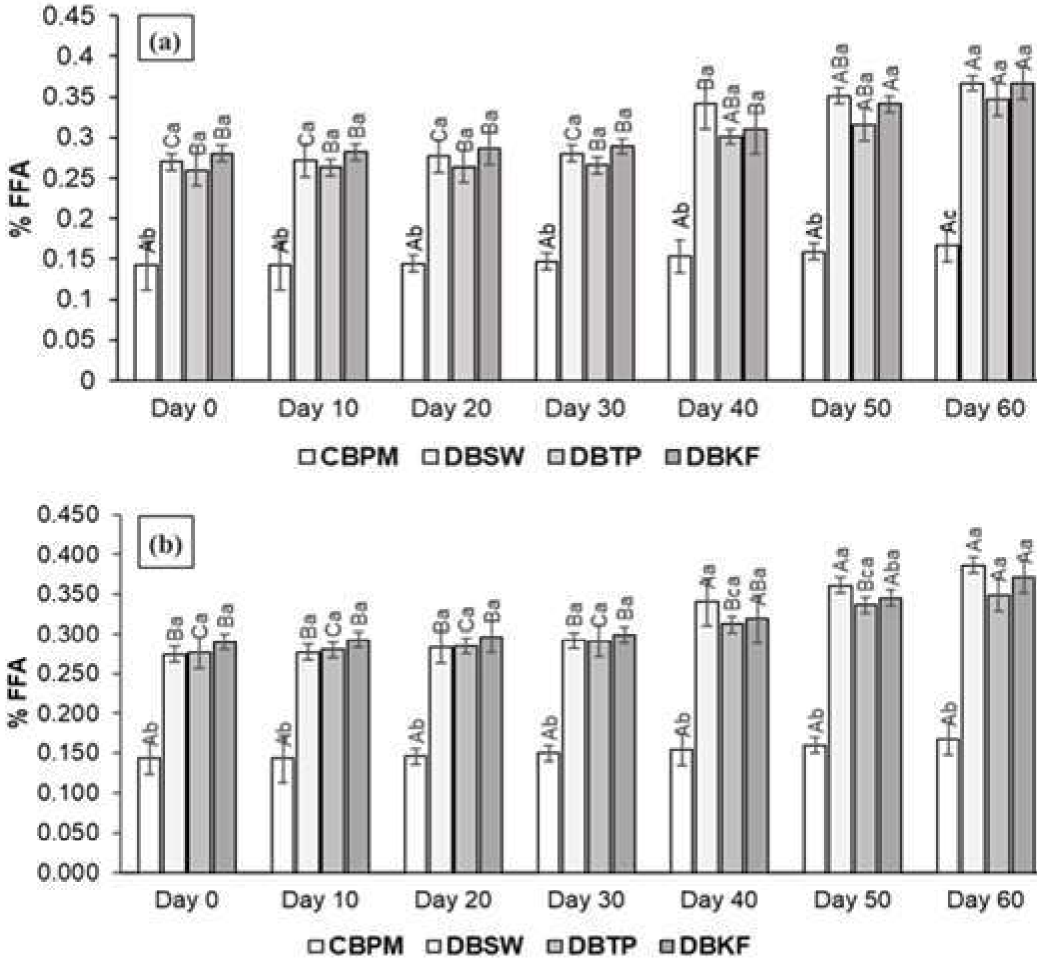

Fig. 2 Effect of breed on self-life of butter prepared from indigenous and crossbred cows
(a) Butter prepared in winter season (b) Butter prepared in summer season
Results are expressed as means ± S.D (n=3). $^{A\text{-}B}$ *Means within storage (days) and* $^{a\text{-}b}$ *means within treatment (CBPM, DBKF, DBSW, and DBTP) with different superscripts were significantly different (p≤0.05) from each other.*

Butter conventionally prepared from pooled cow milk consistently exhibited significantly higher ($p<0.05$) redness values in both seasons. Among the various breeds analyzed, Tharparkar butter showcased the highest redness, whereas Karan-Fries butter displayed the lowest. Moreover, a significant and consistent difference in redness values ($p<0.05$) was observed among butter from Karan-Fries, Sahiwal, and Tharparkar milk throughout various seasons. However, a significant difference in yellowness values was noted between Sahiwal, Tharparkar and Karan-Fries butter.

Yellowness is a crucial attribute in butter due to its naturally yellowish hue, mainly due to β-carotene content (O'Callaghan et al. 2016). The yellowness values of butter increased as summer advanced, as indicated by the lower b* values in winter (23.69 to 30.24) compared to summer (30.84 to 37.18). These colour analyses provided valuable insights into the visual characteristics of butter, demonstrating the influence of breed, season, and preparation methods on its appearance. Consequently, butter from Tharparkar milk displayed the highest yellowness, whereas the lowest yellowness was observed in Karan-Fries butter. In comparison, Ponnal (2021) reported L* value of 85.76, a* value of -4.00, and b* value of 32.07 for salted butter. Significant differences ($p\leq 0.05$) were observed in the lightness (L*) and redness (a*) values between butter prepared in winter and summer seasons, with higher values noted in winter and lower values in summer. Conversely, in terms of yellowness values (b*), the trend was reversed, with higher values observed in summer and lower values in winter.

Texture profile analysis (TPA)

Table 3 Effect of breed on texture profile of butter prepared from indigenous and crossbred cows

	CBPM	DBKF	DBSW	DBTP
Hardness	11.69 ± 1.20^{a}	8.64 ± 0.91^{b}	3.94 ± 0.30^{d}	6.03 ± 0.47^{c}
Fracturability	4.58 ± 0.89	-	-	-
Adhesiveness	-322.73 ± 36.60^{c}	-287.66 ± 27.57^{b}	-178 ± 8.29^{a}	-198.12 ± 12.46^{a}
Springiness	0.20 ± 0.07^{a}	0.19 ± 0.015^{b}	0.16 ± 0.010^{d}	0.18 ± 0.007^{c}
Cohesiveness	0.020 ± 0.002^{c}	0.025 ± 0.005^{bc}	0.035 ± 0.005^{a}	0.029 ± 0.002^{ab}
Gumminess	0.23 ± 0.018^{a}	0.21 ± 0.026^{a}	0.14 ± 0.01^{c}	0.17 ± 0.003^{b}
Chewiness	0.046 ± 0.014^{a}	0.039± 0.004^{ab}	0.022 ± 0.0008^{c}	0.031 ± 0.001^{bc}
Resilience	0.006 ± 0.0009^{a}	0.005 ± 0.0013^{c}	0.005 ± 0.0006^{d}	0.005 ± 0.001^{b}

Results are expressed as means ± S.D (n=3). a-b means within rows with different superscript are significantly different ($p \leq 0.05$) from each other.
CBPM- conventional butter from pooled milk; DBKF- desi butter from Karan Fries ; DBSW- desi butter from Sahiwal; DBTP- desi butter from Tharparkar

The texture profile of butter prepared from selected indigenous and crossbred cow milk by conventional and desi method is illustrated in Table 3. Statistical analysis revealed significant differences ($p < 0.05$) in hardness, adhesiveness, cohesiveness, gumminess, and chewiness within breeds. Notably, butter prepared through the conventional method in winter exhibited a fracture point, whereas no fracturability was observed in butter prepared using the desi method. Conventional butter was found to be significantly harder ($p < 0.05$) than butter prepared through the desi method, with Sahiwal butter displaying the lowest hardness.

During the double-byte test, conventional butter exhibited higher adhesiveness compared to butter prepared using the desi method. The hardness values for butter prepared in winter were significantly higher than those prepared in summer, a phenomenon attributed to differences in fatty acid composition as reported by Blaško et al. (2010). Additionally, Sert and Mercan (2020) highlighted that butter made through continuous methods was harder than conventionally churned butter. Summer butter displayed significantly higher adhesiveness values compared to winter butter, possibly due to the presence of more unsaturated fatty acids, promoting softer texture and enhanced adhesive properties, a finding corroborated by Pădureţ (2021). Cohesiveness values remained relatively constant for Sahiwal butter across both seasons, whereas cohesiveness increased in summer for conventional, Karan Fries, and Tharparkar butters. The cohesiveness value for winter and summer butter were 0.019 to 0.035 and 0.021 to 0.035, respectively. Gumminess exhibited a slight increase in Karan Fries and Tharparkar butter, while a slight decrease was observed in conventional and Sahiwal butter; however, these differences were non-significant ($p \geq 0.05$).

Chewiness, calculated as the multiplication of gumminess and springiness, representing the energy needed to chew solid food until it is ready for swallowing. It remained same for both the winter and summer season in case of Sahiwal and Tharparkar butter but variation found in butter prepared from Karan fries and butter by conventional method, respectively. Chewiness values for butter in winter and summer ranged from 0.022 to 0.048 and 0.022 to 0.044, respectively. Resilience, a measure of how well a product regains its original shape and size, did not exhibit a significant difference ($p \geq 0.05$) between butter prepared in winter and summer seasons.

Texture analyses provided valuable insights into the physical properties of butter, the impact of breed, season, and preparation methods on its texture characteristics.

Assessment of butter keeping quality

The keeping quality of butter is characterized by changes in % FFA. Butter lipolysis, the process of breaking down triglycerides into fatty acids, may increase % free fatty acids with storage time, as illustrated in Fig. 2 (a, b). Karan Fries butter exhibited higher initial free fatty acid levels than conventional butter. By the 60th day of storage, conventional butter showed no significant difference in %FFA, while desi butter displayed appreciable differences between trial days. On the 60th day, the FFA released from both conventional and desi butter was below the regulatory limits outlined in the Food Safety and Standard Regulations (2017).

Fig. 2 (a, b), illustrates how storage duration affects free fatty acids in winter and summer butter. At the beginning of storage, the levels of free fatty acids were 0.142%, 0.270%, 0.260%, and 0.280% for conventional, Karan Fries, Sahiwal, and Tharparkar butter prepared during the winter season, respectively. By the end of storage, these levels had changed to 0.166%, 0.367%, 0.347%, and 0.367%. For summer butter, the initial free fatty acid levels were 0.143%, 0.274%, 0.277%, and 0.290%, increasing to 0.168%, 0.386%, 0.349%, and 0.371% by the end of storage for conventional, Karan Fries, Sahiwal, and Tharparkar butter, respectively.

After 30 days at 4°C, the FFA content of desi butter increased rapidly, unlike conventional butter, which remained relatively stable. This rapid increase in FFA in desi butter might be attributed to lipolytic enzymes secreted by the starter culture, leading to the breakdown of triglycerides into di- or mono-glycerides, releasing free fatty acids. In contrast, conventional butter, with its inherent lipase activity, showed a more controlled release of free fatty acids. From Fig. 2 (a, b), it is evident that FFA% significantly impacts butter shelf life. Karan Fries butter exhibited the highest increase in FFA content, followed by Tharparkar, Sahiwal, and conventional butter. These findings underscore the importance of monitoring free fatty acid levels for assessing butter quality and shelf life.

Conclusion

In conclusion, the findings of the present study highlight significant potential for the promotion of milk and milk products derived from indigenous breeds within the dairy sector. Butter prepared from indigenous breeds demonstrates favourable characteristics, including lower hardness, excellent spreadability, appealing colour values, and notable content of β-carotene and CLA. These attributes not only enhance the organoleptic qualities of the butter but also contribute to its nutritional value. The results underscore the importance of preserving and promoting indigenous breeds in the dairy industry, emphasizing the diverse benefits they offer in the production of high-quality dairy products. This research provides valuable insights for stakeholders, policymakers, and dairy industry professionals, supporting informed decision-making to foster the sustainable development of the dairy sector. Future studies could further explore specific genetic factors and breeding techniques to optimize these desirable traits in dairy products, ensuring the continued growth and success of indigenous breeds in the dairy market.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest in the presented research work.

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RESEARCH ARTICLE

Effect of *Njavara* rice bran on physico-chemical, sensory, and textural properties of sweetened yoghurt

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Abstract: *Njavara* rice and rice bran possess numerous therapeutic applications, including their utilization in alleviating diabetes, cervical spondylitis, rheumatoid arthritis, neuromuscular diseases, psoriasis, skin lesions, and stomach ulcers. In recent decades, numerous studies have shown increasing attention towards functional foods which is positively associated with better nutritional status and quality of life, and the decreased incidence of noncommunicable diseases. This can be attributed to the growing awareness among consumers regarding the correlation between nutrition and health, leading to an increased emphasis on personal well-being. Therefore, to improve the health properties of sweetened yogurt containing 10% cane sugar, *Njavara* rice bran was added in amounts ranging from 0% to 10%. The impact of these additions on the quality of the product, specifically in terms of physicochemical properties, sensory qualities, and texture, was evaluated. A standard method was followed for the preparation of rice bran-fortified sweetened yoghurt. The addition of rice bran was found to have a significant impact on the physico-chemical characteristics of sweetened yoghurt. An increase in rice bran was associated with a decrease in yoghurt's titratable acidity. Conversely, the yoghurt's syneresis, water activity, and antioxidant activity were found to increase. Furthermore, the inclusion of rice bran in the yoghurt formulation resulted in a reduction in L^* and an increase in a^* values. The addition of increasing quantities of rice bran (7% and 10%) was found to have a negative impact on the sensory and textural properties of the yoghurt. Based on the results it can be concluded that fortification of sweetened yoghurt with 5% rice bran could produce a functional product with healthy properties besides its valuable nutritive value.

Keywords: Njavara, Rice bran, Yoghurt, Functional foods, Fermented foods

Introduction

The science of nutrition is shifting its focus from defining daily dietary guidelines to gaining knowledge of nutrient functions and metabolism, promoting health, and mitigating illness risk through consumption. Functional food science is a subfield of nutrition science that seeks to enhance dietary requirements by incorporating new knowledge regarding the relationships between food ingredients and physiological processes. Functional foods are industrially processed or naturally occurring foods that, when regularly consumed as part of a varied diet at adequate levels, may offer health benefits beyond essential nutrition (Granato et al. 2020). The modern trend in developing functional foods is to enhance the health benefits of commonly consumed foods by adding functional components such as phytochemicals, prebiotics, probiotics, dietary fiber, vitamins, minerals, etc. Looking at the importance of functional foods, FSSAI came out with Food Safety and Standards (Food or Health Supplements, Nutraceuticals, Foods for Special Dietary Uses, Foods for Special Medical Purposes, Functional Foods, and Novel Food) Regulations, 2016. The global functional foods market size was estimated at USD 304.2 billion in 2022 and is expected to expand at a compound annual growth rate (CAGR) of 8.5% from 2022 to 2030, projected to reach USD 586.1 billion in 2030 (Grand View Research (n.d.)).

Humans have consumed fermented foods for several years. Although they were most likely intended for preservation, it would have been evident that these foods had other desirable qualities. Fermented foods have distinct aromas, body and textures, and functions compared to the raw components from which they are prepared (Tamang et al. 2020). Yoghurt is a popular dairy product made by the bacterial fermentation of milk

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which involves thermophilic strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Fermented dairy products are recognized for their exceptional nutritional and health-related characteristics, such as their ability to exhibit antimutagenic properties and prevent lower serum cholesterol levels and gastrointestinal infections. These are advantageous for consumption by individuals who are lactose intolerant and those who have atherosclerosis (Rasane et al. 2017). Yoghurt is a rich source of calcium in its bio-available form. It is also a good source of potassium, phosphorous, vitamin A, and vitamin B, especially vitamin B2, and vitamin B12. It also provides essential fatty acids and valuable proteins (Hadjimbei et al. 2022).

The *Oryza sativa* ssp. *indica* variety Njavara (GI Kerala, India 2007) is an early maturing (60–70 days) red rice variety with medicinal properties (Solanki et al. 2019). Njavara rice is slightly sweet and it is considered a magical rice which boosts immunity. Its bran is rich in fiber and is beneficial for all people who are suffering from digestion problems. Also, it is rich in bioactive compunds, especially phenolic and phytochemical compounds, which are excellent antioxidants (Nayeem et al. 2021). Njavara rice and rice bran are used to treat diabetes, cervical spondylitis, rheumatoid arthritis, neuromuscular disorders, psoriasis, skin lesions, and stomach ulcers (Kowsalya et al. 2022). It is also recommended in the preparation of weaning food for underweight babies. Broth made by adding Njavara rice is recommended to pregnant women as it increases the weight of the foetus. Cooked with milk and herbs, it can treat internal wounds (Rathna-Priya et al. 2019). Red and black rice grains have the maximum concentration of phenolic and anthocyanin compounds compared to other rice grains (Haskito et al. 2020). Rice bran contains a diverse array of phytochemicals, such as phytic acid, ferulic acid, tocotrienols, γ-oryzanol, flavonoids, and phytosterols which makes them excellent antioxidants (Law et al. 2017). With the provision of using rice bran as a nutraceutical as per Food Safety and Standards (Health Supplements-Nutraceuticals) Regulations (2022), there is a scope for new dairy product (functional food) development. In view of the above, the present investigation was carried out to develop *Njavara* rice bran-fortified sweetened yoghurt and to study the effect of red rice bran on the physicochemical, sensory, and textural attributes of yoghurt.

Materials and methods

Materials

The buffalo milk used in the study was sourced from the Experimental Dairy of ICAR-National Dairy Research Institute (NDRI), Karnal. Starter culture NCDC-263 containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was obtained from the National Collection of Dairy Cultures (NCDC) at ICAR-National Dairy Research Institute (NDRI), Karnal (India). The starter culture was maintained in autoclaved reconstituted skimmed milk (12g/100mL) by sub-culturing once a fortnight to attain high activity. The *Njavara* rice bran used in the study was purchased directly from a farmer in Kerala (details can be provided on request). Skim milk powder (Brand: Dairy Best) manufactured by M/s Pashupati Dairies Pvt. Ltd., Dehradun was procured from the local market. Polystyrene (PS) cups with lids (aluminum) of 100 mL capacity were procured from M/s Rajlaxmi Trading and Manufacturing Co., Karnal, and used for the packaging of the product.

Preparation of Rice Bran fortified Yoghurt

For preparing *Njavara* rice bran-fortified sweetened yoghurt, the fresh raw buffalo milk was standardized to 3% fat and 8.5% solids-not-fat (SNF) using fresh raw buffalo milk and skimmed milk powder (SMP), respectively and 10% sugar was added. Then the milk was subjected to two-stage homogenization (2000/500 psi) after heating to 60°C. Further, rice bran was added to the homogenized milk at different levels (0%, 1%, 3%,5%, 7%, 10%) and subjected to high shear mixing. Then it was heat treated at 90°C for 15 min and then cooled to 45°C. The starter culture was added at 1% and filled into pre-sterilized 100 mL cups. Incubation was carried out at 45°C for about 5-6 hours till a final titratable acidity of 0.6% expressed as lactic acid was reached. Once the desired acidity was obtained, without disturbing the set product, cups were transferred to refrigerator storage. All the trials were carried out in triplicate.

Physico-chemical analysis

Titratable acidity

The AOAC (2005) method was used to determine the titratable acidity. A yoghurt sample of around 10 g was weighed in a conical flask and diluted with double-distilled water to twice its volume. After adding two mL of phenolphthalein indicator, the mixture was titrated with 0.1 N NaOH until a persistent pink colour developed. The following formula was used to determine acidity, which was expressed as % lactic acid by weight.

$$Acidity\ (\%\ LA) = \frac{9NV_1}{V_2}$$

Where N = The normality of the NaOH used, V_1 = The volume (mL) of the sample used for the test, and V_2 = The volume (mL) of 0.1 N NaOH required for the titration.

Water activity

The water activity of rice bran fortified sweetened yoghurt was measured using the water activity meter "Aqua Lab" (Model series 3 TE) supplied by Decagon Devices, WA, USA. Prior to measurement, the samples were tempered to 25°C.

pH

The pH of the samples was measured using a digital pH meter (EUTECH Instruments, Singapore) at 20°C using a combined glass electrode fitted in association with a temperature probe. Before use, the pH meter was calibrated using standard buffers of pH 4.0 and 9.0 at 20°C. The pH values were measured in duplicate at three different times to monitor the fermentation.

Antioxidant activity (DPPH)

With a few minor adjustments, the method described by (Brand-Williams et al. 1995) was used to test the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity. 1 g sample was diluted in 25 ml of 60% methanol. Further, 2.9 ml of 60 mM DPPH in methanol was added to 100 μL of the diluted sample and kept in the darkness for 30 min. The absorbance of the reaction mixture was then measured at 517 nm. As a blank, methanol was utilized. Triplicate measurements were taken. DPPH radical scavenging capacity was calculated by using the following formula:

$$\text{Radical Scavenging Activity (\%)} = \frac{(\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}) \times 100}{\text{Absorbance}_{\text{blank}}}$$

Syneesis

After taking it out of the refrigerator, a cup of yoghurt was stirred 20 times in a clockwise and counterclockwise direction. Using a 5 mL pipette, about 30 g of the sample was placed into a 50 mL polypropylene conical centrifuge tube and stabilized for 2 hours at 5 °C. To observe whey separation, the agitated samples were centrifuged at 3000 rpm for 15 minutes at 10 °C. Syneresis was expressed as percent syneresis (Hussain et al. 2016).

$$\text{Percent syneresis} = \frac{\text{Weight of separated whey}}{\text{Initial weight of sample}} \times 100$$

Colour analysis

The surface colour of yoghurt was measured using a "Colourflex" colorimeter and software (version 4.10) supplied by Hunterlab (Hunter Associates Laboratory, Inc., Reston, VA, USA), and the results were expressed using the CIELAB system. 75 mL of the pasteurized and cooled yoghurt mixture was poured into a sterilized glass beaker with a diameter of 10 cm and a height of 6 cm, and incubation was performed. After approximately 16 hours of storage at 6-8°C, the cups were removed from the refrigerator and tempered at 25°C for 2 hours. Then, using a spatula, the contents of the beakers were loosened from the sides and transferred to the sample container attached to the Colourflex device. For each sample, measurements were taken in triplicate (Raju and Pal, 2011).

Texture measurement

Using the texture analyzer, TA-XT plus (M/s Stable Micro Systems, UK), equipped with a 50 kg load cell and back extrusion method the textural properties were determined. For determining the textural attributes, inoculated milk was filled (~140 mL) up to 5 cm in a sterilized glass tumbler (10 cm height and 6 cm diameter), and incubation was carried out in the tumblers itself. Before analysis, the beakers were tempered at 25°C for 2 h. The back extrusion probe (A/BE) with a 5 cm diameter disc and extension bar was used. The extrusion disc was positioned centrally over the sample container. At a crosshead speed of 1.0 mm/s, it penetrated 10 mm (20% compression) into the yoghurt. Through compression and back extrusion, the probe moved the material, which caused the fluid to rise through the annular space. From the resulting force-time curves, firmness, i.e., the 'peak' or maximum force (g), consistency, i.e., the area under positive region (g.sec), cohesiveness (g), i.e., maximum negative force, and work of cohesion (g.sec), the area of the negative region of the curve were calculated using the instrument attached software (Exponent Connect™). All measurements were done in triplicate for each sample.

Sensory analysis

The sensory evaluation of the yoghurt samples was done using a 9-point Hedonic scale. The specimens were instantly removed from the refrigerator before being presented to the evaluators. A sensory review panel of five judges was chosen from the Dairy Technology Division of ICAR-NDRI, Karnal, who were well-versed in sensory evaluation procedures and product qualities. The sensory qualities of yoghurt were evaluated in the study, including colour and appearance, flavour, body and texture, and overall acceptance. The sensory evaluation tool used a nine-category hedonic grading system: dislike excessively (1), dislike very much (2), dislike moderately (3), dislike slightly (4), neither like nor dislike (5), like slightly (6), like moderately (7), like very much (8), and dislike severely (9) (Hussain et al. 2016).

Statistical analysis

Data obtained from various experiments were recorded as mean ± standard deviation (SD) and subjected to statistical analysis. Triplicate samples with triplicate measurements from each group (n = 3) were taken to estimate the physico-chemical, sensory, and textural properties of each of the different treatments for consistency of the results. Data were analyzed using one-way analysis of variance (ANOVA) at a 5% level of significance. The mean values were compared using Duncan's paired comparison test using SPSS software (version 26, M/s IBM corporation).

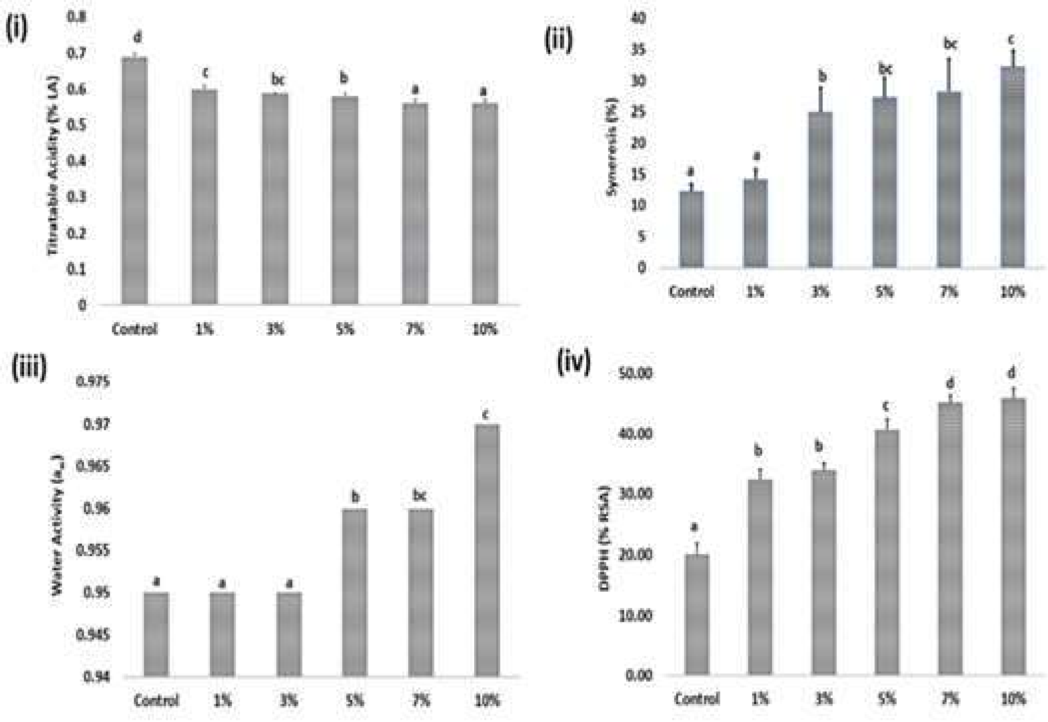

Fig. 1 Effect of *Njavara* rice bran on the physico-chemical properties of sweetened yoghurt: (*i*) acidity; (*ii*)syneresis; (*iii*) water activity; and (*iv*) antioxidant activity (DPPH, % Radical scavenging activity). Each observation is a mean ± SD of three replicates (*n*=3). [abcd] means values of treatments within a graph with different letters significantly differ ($P \le 0.05$)

Results and Discussion

Effect of *Njavara* rice bran on the physico-chemical properties of sweetened yoghurt

The effect of the incorporation of *Njavara* rice bran on the physicochemical characteristics of sweetened yoghurt is given in Figure 1 and Figure 2. It can be observed that rice bran incorporation significantly (*P*d”0.05) affected most of the physico-chemical properties. Incorporating rice bran into the yoghurt resulted in a significant reduction (*P*d”0.05) in acidity. The acidity of the control yoghurt was found to be significantly higher (*P*d”0.05) compared to the samples containing rice bran. A significant reduction (*P*d”0.05) in the titratable acidity of the yoghurt samples was seen with an increase in the quantity of rice bran. The yoghurt sample that did not contain any bran had the greatest acidity level, measuring at 0.69% lactic acid. This was followed by a 0.6% acidity level in the yoghurt sample with 1% bran addition and a 0.58% acidity level in the yoghurt sample with 5% bran addition. The lowest acidity level of 0.56% was seen in the yoghurt samples with 7% and 10% bran concentrations. The incorporation of fibre into yoghurt resulted in a decrease in the acidity levels of the product, potentially due to the dilution impact. In their study, Issar et al. (2016) observed a consistent reduction in titratable acidity as the proportion of apple pomace fibre in yoghurt increased. In contrast, Hasani et al. (2017) observed an elevation in the titratable acidity of the yoghurt after the incorporation of wheat bran. Raju and Pal (2014) also reported an increase in the titratable acidity of *misti dahi* following the addition of inulin and attributed this to the soluble fiber characteristic of inulin that might have easily digested by the bacteria and resulted in the formation of organic acids.

Rice bran addition significantly increased the syneresis (*P*d”0.05) of the sweetened yoghurt compared to the control (Figure 1). The percentage of syneresis seen in the control yoghurt was 12.33%. The addition of rice bran at a concentration of 1.0% resulted in a modest increase in syneresis, although this difference was not statistically significant ($P > 0.05$) when compared to the control yoghurt. It was hypothesized that the inclusion of dietary fibres found in rice bran, such as β-glucan, pectin, hemicellulose, and arabinogalactan, could potentially enhance the water retention properties of yoghurt, thereby reducing the occurrence of syneresis in yoghurt. However, in the current investigation, conversely, elevated quantities of rice bran exhibited a significant increase ($P \le 0.05$) in the syneresis of the yoghurt samples. The observed increase in yoghurt syneresis after the incorporation of rice bran may be attributed to the potential impact of lactic acid bacteria fermentation on the integrity of the crystalline arrangement and the monosaccharide content of the dietary fibre present in rice bran (Wu et al. 2023).

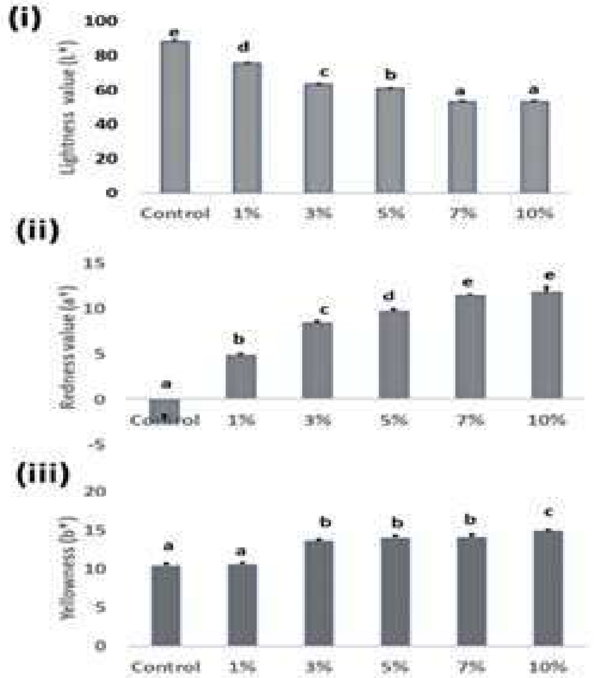

Fig. 2 Effect of *Njavara* rice bran on the colour (CIELAB) of sweetened yoghurt: (*i*) lightness (L^*); (*ii*) redness (a^*); and (*iii*) yellowness (b^*). Each observation is a mean ± SD of three replicates (n=3). [abcde] means values of treatments within a graph with different letters significantly differ ($P \leq 0.05$)

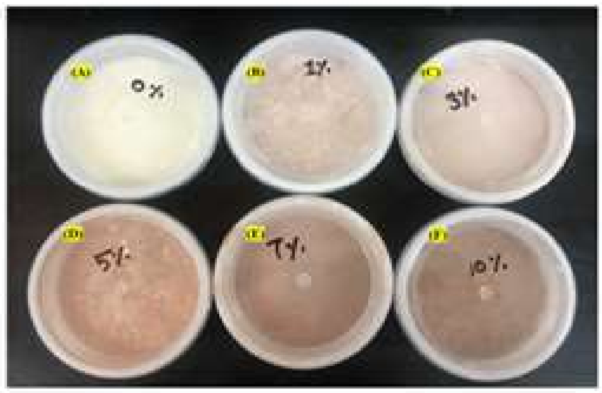

Fig. 3 Picture of yoghurt added with different levels of *Njavara* rice bran: *(A)* Control yoghurt (0% bran); *(B)* yoghurt with 1% bran; *(C)* yoghurt with 3% bran; *(D)* yoghurt with 5% bran; *(E)* yoghurt with 7% bran; and *(E)* yoghurt with 10% bran

Raju and Pal (2014) observed a comparable rise in the whey separation of *misti dahi* after the incorporation of dietary fibres. The slight enhancement in syneresis seen in *misti dahi* containing inulin may be attributed to the disruption of the gel structure, resulting in the separation of whey. In a similar vein, Garcia-Perez et al. (2005) observed that the inclusion of orange fibre at concentrations of 0.6% and 0.8% resulted in a disruptive impact on the gel matrix of yogurt, ultimately leading to an augmentation in syneresis. Demirci et al. (2017) showed that an increase in the content of rice bran resulted in a reduction in the syneresis of yoghurt.

Water activity significantly increased (*P*d"0.05) with rice bran addition to the sweetened yoghurt. Yoghurt with 10% rice bran recorded the highest water activity among all the batches. Measuring water activity provides a more accurate assessment of water's capacity to serve as a reactant and a solvent (Labuza et al. 2020). The increased syneresis seen in bran-fortified yoghurt samples (Figure 1), characterized by the expulsion of water from the gel matrix, can be attributed to the higher water activity resulting from the presence and interaction of bran fibres. Wu et al. (2023) also observed an increase in the syneresis of yoghurt when 2% rice bran was added after fermentation. They attributed this to the change in the monosaccharide composition and damage of the ordered crystal structure of rice bran during the fermentation by lactic acid bacteria.

The present study assessed the impact of fortifying sweetened yoghurt with *Njavara* rice bran on its antioxidant activity, as depicted in Figure 1. The data demonstrate a positive correlation between the concentration of rice bran and the DPPH antioxidant activity, specifically in percentage radical scavenging activity. As the level of rice bran increased, the antioxidant activity increased significantly (*P*d"0.05). Including rice bran at a concentration of 1% resulted in a 1.6-fold increase in DPPH activity. Similarly, adding 5% and 10% rice bran increased DPPH activity by 2.03 and 2.28-fold, respectively. Rice bran possesses a substantial quantity of inherent antioxidants that have the potential to function as scavengers of free radicals (Rao et al. 2010). According to a study conducted by Mohanlal et al. (2012), it was found that the red rice variety has a superior radical scavenging function compared to conventional (white) rice kinds. This study shows that incorporating natural compounds, such as red rice bran, can potentially enhance antioxidant activity. Consequently, these food sources can serve as a valuable means for consumers to defend against diseases resulting from the harmful effects of free radicals and oxidative stress. According to Haskito et al. (2020), including red rice bran and black rice bran in goat milk yoghurt increased its antioxidant activity.

The colour of dairy products significantly determines customer acceptability and can exhibit variations in pigment concentration in yoghurt during storage. Adding rice bran led to significant changes in the instrumental colour of sweetened yoghurt. The findings depicted in Figure 2 illustrate a statistically significant reduction ($P \leq 0.05$) in the lightness (L^*) measurements of yoghurt upon the incorporation of rice bran in comparison to the control group. The yoghurt samples containing rice bran had a reddish colour (Figure 3). The study observed a significant increase in the a^* values of rice bran-fortified yoghurt ($P \leq 0.05$), although the impact on the b^* value was not as pronounced. The a^* and

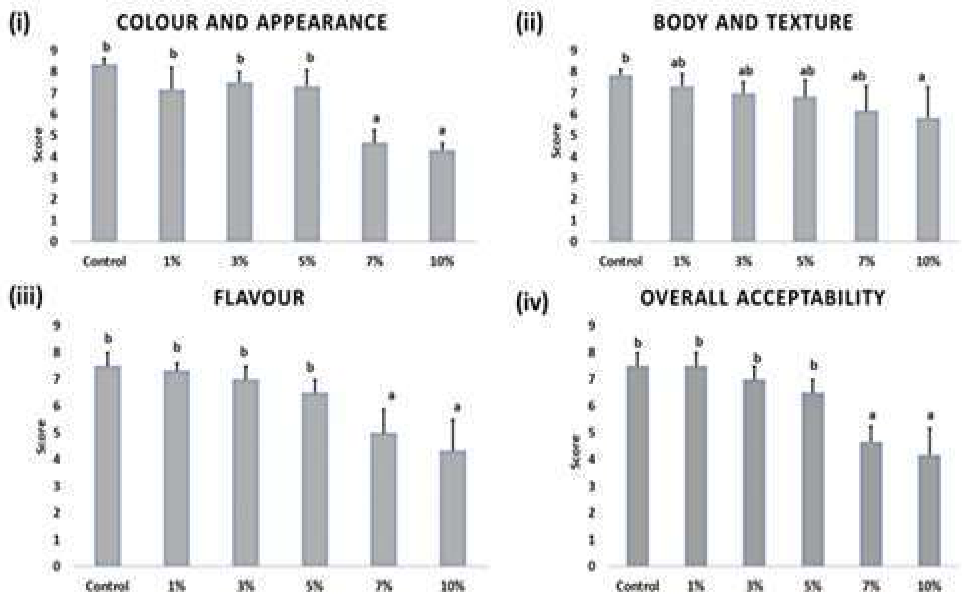

Fig. 4 Effect of *Njavara* rice bran on the sensory properties of sweetened yoghurt: (*i*) colour and appearance; (*ii*) body and texture; (*iii*) flavour; and (*iv*) overall acceptability. Each observation is a mean ± SD of five replicates (n=5). [ab] means values of treatments within a graph with different letters significantly differ (P≤0.05)

b^* values of yoghurt supplemented with rice bran varied from 4.93 to 11.87 and 10.62 to 14.94, respectively. In comparison, the control a^* and b^* values of control yoghurt were determined to be -2.63 and 10.45, respectively. Similar to the current findings Nontasan et al. (2012) documented a decrease in the lightness measurements of yoghurt following the incorporation of black rice extract. Similarly, Aktas and Akin (2020) observed a decrease in the lightness value of *tarhana* when corn bran was used. The authors also documented a decrease in the a^* and b^* values when rice bran and corn bran were used in *tarhana*, contradicting our findings. Similarly, the study conducted by Amjad et al. (2021) documented a notable elevation in the L^* and b^* parameters of yoghurt after the incorporation of barley bran. The observed decrease in the L^* value and increase in the a^* value of the yoghurt containing rice bran can be attributed to the presence of phenolic compounds inherent in the rice bran, contributing to its distinctive colouration. Additionally, the Maillard browning reaction during heat application, particularly in a substantial quantity of added sugar, may have further influenced these colour changes.

Effect of *Njavara* rice bran on the sensory properties of sweetened yoghurt

The sensory qualities of sweetened yoghurt were evaluated concerning the influence of red rice bran, as depicted in Figure 4. There were notable variations seen in the sensory attributes of sweetened yoghurt, with a significant difference (P≤0.05), except for body and texture, which did not display a significant difference (P>0.05). The experimental findings suggest that the control yoghurt demonstrated significantly superior ratings (P≤0.05) regarding colour and appearance compared to the yoghurt samples with rice bran. The samples with larger concentrations of rice bran (7% and 10%) received notably lower scores from the panelists. This was attributed to observing a discernible layer of settled rice bran in all cups. The observed combination of higher syneresis in rice bran-supplemented samples (Figure 1) may be responsible for the comparatively lower colour and appearance score of yoghurt containing rice bran. According to Hasani et al. (2017), using barley bran in yoghurt formulation considerably reduced the appearance score. Similarly, the study conducted by Samilyk et al. (2021) revealed that including rice bran in fermented milk had a notable impact on sensory characteristics. Raju and Pal (2014) observed that using oat fiber in *misti dahi* resulted in a notable decrease in colour and appearance evaluations.

Among the rice bran-fortified samples, the yoghurt fortified with 10% rice bran attained the lowest body and texture scores, with a significant difference (P<0.05) from that of the control. Although the other levels of rice bran fortification reduced the body and texture scores, however, they were comparable to the control (P>0.05) (Figure 4). The findings on the reduced body and texture scores of sweetened yoghurt supplemented with bran align with the outcomes seen in a study conducted by Samilyk et al. (2021) on yoghurt fortified with rice bran. According to their findings, incorporating rice bran into the formulation led to the separation of whey, leading to an inconsistent body and texture of the product and ultimately decreasing its body and texture scores and overall acceptability. The observed decrease in the body and texture scores of rice bran-supplemented yoghurt may be attributable to the interactions between the fiber and milk solids, resulting in a weakened gel formation. The findings regarding the instrumental firmness and consistency of rice bran-enriched yoghurt, as presented in the section on textural qualities, validate this assertion.

Overall, the addition of bran to sweetened yoghurt resulted in a decrease in flavour scores. However, a statistically significant difference (*P*d"0.05) was only found for the samples fortified with 7% and 10% levels of bran. Previous research has documented similar results. A study conducted by Demirci et al. (2017) showed that the inclusion of rice bran in fermented milk resulted in decreased flavour values compared to plain samples. Furthermore, as the amount of rice bran increased, the acceptability of the samples decreased. According to Raju and Pal (2014), the inclusion of fiber in *misti dahi* substantially impacted the flavour ratings. According to Asadzadeh et al. (2020), there was a substantial decline in flavour scores as the concentration of oat bran extract increased.

Adding rice bran resulted in a substantial decrease in the overall acceptability of sweetened yoghurt compared to the control group ($P \leq 0.05$). Among the batches tested, samples containing 7% and 10% rice bran exhibited the lowest acceptability scores, with statistical significance (*P*d"0.05). Although the acceptance scores of yoghurt with 1%, 3%, and 5% rice bran added were lower than the control group, the observed difference was not statistically significant ($P > 0.05$). The diminished overall acceptability ratings of rice bran-enriched samples may also be ascribed to subpar visual appeal and heightened syneresis, as depicted in Figure 1. The current investigation showed that yoghurt containing rice bran exhibited lower scores than the control group. However, the average scores of yoghurt samples containing 1%, 3%, and 5% rice bran were found to be above 6.5. This suggests that it is possible to produce yoghurt of satisfactory quality by incorporating a maximum of 5% red rice bran. Previous research has yielded comparable results. According to Fernandez-Garcia and Mcgregor (1997), the augmentation of fibre quantities resulted in enhanced consistency and texture of yoghurt, but at the expense of a decline in its overall sensory quality. In a study conducted by Zomorodi et al. (2015), it was observed that adding wheat bran and apple fibre to probiotic yoghurt decreased sensory scores. A study conducted by Hashim et al. (2009) showed that the inclusion of 4.5% date fibre in yoghurt resulted in a significant fall in the sensory scores of the product.

Effect of *Njavara* rice bran on the textural properties of sweetened yoghurt

The impact of including red rice bran on the texture of sweetened yoghurt is illustrated in Figure 4. The addition of bran to yoghurt had a significant impact on its firmness ($P \leq 0.05$). Among all the samples fortified with bran, those containing 7% and 10% levels of bran exhibited the lowest firmness values. The observed reduction in hardness values of sweetened yoghurt may be attributable to the disruptive influence of bran fibres on the gel structure. In a study conducted by Wu et al. (2023), it was observed that the inclusion of rice bran reduced the firmness of yoghurt. Furthermore, the researchers found a positive correlation between the bran concentration and the extent of this impact. The yoghurt

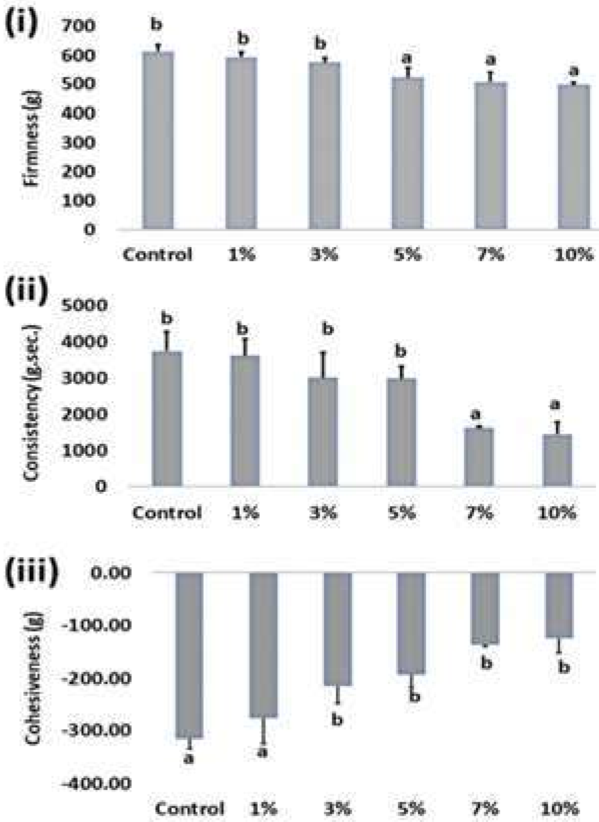

Fig. 5 Effect of *Njavara* rice bran on the textural properties of sweetened yoghurt: (*i*) firmness (g); (*ii*) consistency (g.sec); and (*iii*) cohesiveness (g). Each observation is a mean ± SD of three replicates (n=3). [ab] means values of treatments within a graph with different letters significantly differ ($P \leq 0.05$)

stiffness exhibited a significant decrease of 46.6% compared to the control group when the percentage of rice bran scaled to 3.0%. In their study, Raju and Pal (2014) observed a reduced hardness value of *misti dahi* when supplemented with dietary fibres.

The concept of consistency pertains to the firmness, thickness, or viscosity of a liquid or semi-solid substance. A significant drop in the consistency of the yoghurt was observed as the concentration of rice bran increased ($P \leq 0.05$). The observed loss in consistency when larger levels of rice bran (7% and 10%) were added can be attributed to the induced separation of whey, resulting in an inconsistent firmness and consistency of the product. The decrease in hardness and consistency of yoghurt added with rice bran can be attributed to the interactions between the fibre and milk solids, leading to a compromised gel formation. In a study conducted by Wu et al. (2023), it was shown that the incorporation of rice bran led to a comparable reduction in the overall consistency of the yoghurt. A 46.67% decrease in consistency was seen when the concentration of rice bran increased from 0% in the control group to 3%. In contrast, Fernandez-Garcia and Mcgregor (1997) documented that the increase in fibre quantities led to improvements in the consistency and texture of yoghurt.

The cohesiveness of food exhibits a direct correlation with its tensile and compression strength. The measure of cohesiveness indicates the extent of deformation a material undergoes before rupture and is directly correlated with the internal strength of the material's structure. Cohesion in a product is observed when it demonstrates the ability to maintain adherence to itself when subjected to compressive or tensile stress. The cohesiveness of rice bran-fortified yoghurt was significantly lower (*P*d"0.05) than the control. This observation suggests a potential association with the reduced strength of protein aggregates. Similar results have been reported by Wu et al. (2023). They observed a 42.82% reduction in the cohesiveness as the level of rice bran increased from 0 to 3%.

Conclusion

The addition of rice bran has a significant impact on the physico-chemical characteristics of sweetened yoghurt. An increase in rice bran was associated with a decrease in yoghurt's titratable acidity. Conversely, the yoghurt's syneresis, water activity, and antioxidant activity were found to increase. Furthermore, the inclusion of rice bran in the yoghurt formulation resulted in a reduction in lightness and an increase in redness values. The sensory and textural characteristics of the yoghurt were observed to be influenced when higher quantities of rice bran (7% and 10%) were added. Based on an analysis of the physico-chemical, sensory, and textural qualities, it can be inferred that the optimal level of *Njavara* rice bran integration into sweetened yoghurt is 5%.

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RESEARCH ARTICLE

Coagulase positive Staphylococci contamination and the risk associated with the production of toxin in foods - An Exploratory Study

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Abstract: Food-borne diseases pose a threat to human health and the economy of individuals, families, and nations. *Staphylococcus aureus* is one of the most common causes of foodborne intoxication in most countries of the world. The present study was done to analyse the extent of *Staphylococci* contamination and the risk associated with the production of toxins in foods. A total of 450 Food samples were collected from retail markets, sweet shops, and households in Hyderabad. Among 143 (31.7%) cultures of *Staphylococci,* 106 (74.1%) showed coagulase enzyme production and 37 (25.9%) isolates were coagulase-negative. Only nine cultures (6.3%) showed a positive result for enterotoxin production. It is known that $>10^6$ CFU/g of *S. aureus* is likely to produce an enterotoxin, however, in the present study 17% of food samples have crossed the limit but very a small number of them were able to produce enterotoxin. For the risk assessment of *S. aureus* contamination in foods, coagulase test and toxin production of isolates have to be evaluated. The data will help set standards for the microbiological quality of foods.

Keywords: *Staphylococcus,* Enterotoxin, Coagulase, Safety

Introduction

Staphylococcus species are recognized as significant pathogens responsible for outbreaks of foodborne illnesses (Tohoyessou et al. 2020). In India, the rate of infection is still higher because of the warm and humid climate. *Staphylococcus aureus* food poisoning is an intoxication caused by the ingestion of food containing staphylococcal enterotoxins (SEs), and is one of the most common foodborne diseases in the world. *Staphylococcus aureus* produces a variety of extracellular products including the staphylococcal enterotoxins which have been implicated in human and animal diseases. The heat stability of *S. aureus* is one of the important properties of SEs in food safety (Le Loir et al. 2003). Contamination of foods by *S. aureus* may occur directly from infected food-producing animals or may result from poor hygiene during the production process or retail and storage of foods or from humans who will carry this microorganism (Doyle and Beuchat, 2007).

Coagulase-positive Staphylococci (CoPS) are opportunistic pathogens that can exist as commensals in humans, animals, and food-producing animals but have the potential to cause severe or even life-threatening diseases. They are facultative anaerobic gram-positive bacteria with a non-spore-forming spherical shape. At least nine species of CoPS have been identified, including Staphylococcus aureus, *S. hyicus, S. intermedius, S. pseudintermedius, S. lutrae, S. schleiferi subsp. coagulans, S. delphini, S. argenteus,* and *S. schweitzeri.* (Velazquez-Guadarrama et al. 2017; Gonzalez-Martín et al. 2020).

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Certain pathogenic strains of this bacterium produce heat-stable staphylococcal enterotoxins (SEs). Currently, 23 SEs have been identified, including five major classical types (SEs: SEA to SEE) and non-classical SE-like toxins (SEl: SEG to SEU). (Chajęcka-Wierzchowska et al. 2020; Saif et al. 2019). Approximately 95% of food poisoning outbreaks are caused by classical enterotoxins (Ahmed et al. 2021). Consuming food containing staphylococcal enterotoxins (SEs) can lead to the onset of severe symptoms, such as vomiting, high fever, nausea, and diarrhea, typically occurring rapidly in less than 8 hours (Jett et al. 2001).

Food Safety Standards Authority of India (FSSAI) regulations for microbiological requirements for different milk products indicate that coagulase-positive *S. aureus* should be less than 100 per gram of the milk product. Although enterotoxins are produced mainly by coagulase-positive staphylococci, some coagulase-negative staphylococci are involved in a variety of human and animal infections (Cunha et al. 2006). Taking into account that the toxigenic potential of coagulase-negative staphylococci is often neglected, the present study was done to analyze the extent of *Staphylococci* contamination and the risk associated with the production of toxins in foods.

Materials and Methods

Study area

The study was carried out in Hyderabad which is the capital of Telangana, India. As of now, it is the sixth-most populous city and the sixth-most populous urban agglomeration in India. The twin cities of Hyderabad and Secunderabad come under the ambit of a single municipal unit, the Greater Hyderabad Municipal Corporation. For administrative purposes, Greater Hyderabad Municipal Corporation has been divided into many circles with each circle being homogeneous within and different from other circles. A random sampling procedure was adopted in the study and the sample required for the study was obtained using proportionate representation according to size.

Sample collection

Food samples were collected aseptically from retail markets, sweet shops, and households of Hyderabad. Samples were transported to the laboratory in the ice box and transferred to the refrigerator until further analysis. The interval between the sampling and the analysis will be less than one hour. A total of 420 food samples including khoa (a desiccated milk product), Kulfi (ice cream), paneer (a type of milk curd cheese used in Indian cooking), Dhal (a sauce made from lentils and spices, usually served with cooked rice), nonvegetarian curry (chicken meat and mutton), pineapple fruit juice, cooked rice, vegetarian curry and sapota/sapodilla juice including 30 households hand washings were collected for the analysis.

Isolation and identification of *S. aureus*

Isolation and identification of *S. aureus* were performed according to the US FDA bacteriological analytical manual. A 25 g of the food sample was collected aseptically and added to 225ml of buffered peptone water and the mixture was homogenized for 3-5mins. A 100 µl of the inoculum was taken and inoculated on Baird Parker Agar (HIMEDIA) with egg yolk tellurite emulsion and incubated at 35^{0}C for 24-48 hr and suspected colonies (a grey-black shiny convex colony with a narrow white margin surrounded by a zone of clearing) were confirmed by doing further biochemical tests like catalase test, coagulase production, anaerobic utilization of glucose and carbohydrate (mannitol) fermentation test.

Coagulase test

The coagulase test was done by taking 0.05ml of an overnight broth culture of *Staphylococci* or 2-3 pure colonies picked from an agar plate on a clean glass tube and then by adding 0.5ml of rehydrated plasma. Coagulase plasma (from rabbit) was procured from Himedia Laboratories Pvt. ltd. Both the solutions were mixed well and incubated at 37°C in the incubator for 4h. Agglutination or clumping of cocci within 4h was considered a positive result.

Preparation of bacterial culture for enterotoxin production

Pure *S. aureus* culture was pre-enriched in Brain Heart Infusion (BHI) broth. Centrifugation of the bacterial culture was done for 5 min at a minimum of 3500g/10^{0}C. Sterile filtration of the supernatant was done and 100 µl of the filtrate per well was used in the enzyme immunoassay

Enterotoxin detection

The coagulase-negative and positive *S. aureus* strains were selected for the detection of enterotoxin. RIDASCREEN SET total sandwich enzyme immunoassay kit, manufactured in Germany was used for the combined detection of *Staphylococcus* enterotoxins (SET) A, B, C, D, and E from bacterial cultures. All reagents required for the enzyme immunoassay were there in the test kit.

Statistical analysis

A proportion test has been done to see the differences in toxin production among coagulase-positive and coagulase-negative *Staphylococci* isolates.

Results and Discussion

The incidence of *S. aureus* (percentage of contamination) in food samples sold in various localities of Hyderabad is shown in Table 1. A total of 420 food samples including 30 hand washings were collected from retail markets, sweet shops, and households in Hyderabad. Among all the food samples analyzed, Sapota/ Sapodilla fruit juice was highly contaminated (91%) with *Staphylococcus*. Kulfi ice cream and paneer were less contaminated with *Staphylococcus* when compared to other food samples.

The results of coagulase and enterotoxin production by the *Staphylococcus* cultures are shown in Table 2. Among 143 (31.7%) cultures of *Staphylococci,* 106 (74.1%) showed coagulase enzyme production and 37 (25.9%) isolates were coagulase-negative. The number of *Staphylococcus-producing* coagulase was more than noncoagulase-producing Staphylococcus. Both coagulase-positive and negative cultures were able to produce enterotoxins. Only nine cultures (6.3%) showed positive results for enterotoxin production. It is known that >10^6 cfu/g of *S. aureus* is likely to produce enterotoxin, however, in the present study 17% of food samples have crossed the limit but very a small number of them were able to produce enterotoxin.

A study on the persistence and survival of *S. aureus* at different temperatures (4, 10, and 37°C) for different lengths of time (0-12 days) indicated that the *S. aureus* population varied with temperature and showed the highest population and viability at 37°C. On the 12th day differences in population were observed at lower and higher temperatures (Table 3). In the proportion test coagulase coagulase-positive isolates producing toxin was 6% (6/106) and coagulase-negative isolates producing toxin was 8% (3/37) which was not significant (P=0.620).

Table 1: Incidence of *S. aureus* (percentage of contamination) in food samples sold in various localities of Hyderabad

Food Samples	Samples positive for *S. aureus*	Mean±SD	Prevalence (%)
Rasmalai (n=60)	32	4.5±1.4	31.7
Khoa (n=60)	75	6.1±1.3	73.5
Paneer (n=60)	29	4.1±1.3	28.4
Kulfi (n=60)	22	4.1±1.3	22.0
Chiku/Sapota Juice (n=39)	36	3.0±1.1	91.8
Pineapple Juice (n=37	20	2.1±1.4	54

Table 2: Screening of coagulase and enterotoxin in Staphylococcal enterotoxin producing *S. aureus* from food sample

Type of foods	No. of *S.aureus* isolates	Coagulase +	Coagulase -	Enterotoxin (Coagulase +)	Enterotoxin (Coagulase -)
Dhal (30)	7	5	2	2	0
Khoa (60)	36	27	9	0	0
Kulfi(60)	5	4	1	0	0
Non veg(30)	12	5	7	1	1
Paneer(60)	7	6	1	0	0
Pine apple Fruit Juice (30)	2	2	0	0	0
Rasmalai(60)	22	20	2	1	0
Rice(30)	27	17	10	1	1
Sapota Fruit juice (30)	2	2	0	0	0
Veg curry(30)	16	12	4	1	1
Hand Washings (30)	7	6	1	0	0
Total (n=450)	143	106 (74.1%)	37 (25.9%)	6 (4.1%)	3* (2%)

* Out of 9 enterotoxin producing strains 3 are coagulase negative

Table 3: Population (log10cfu/g) of *S. aureus* on Rasmalai after storage at 4, 10 and 37 deg c for different length of time (0, 1,2,4,6,8,10 & 12d)

Mlik Product	Foodborne pathogen	Storage time (d)	Temperature		
			4	10	37
Rasmalai	*S. aureus*	0	2.61±0.00	2.72±0.02	2.72±0.02
		1	2.49±0.00	2.50±0.00	3.34±0.06
		2	2.45±0.01	2.53±0.04	3.32±0.02
		4	1.93±0.01	2.18±0.02	3.36±0.02
		6	1.23±0.09	2.14±0.00	2.75±0.02
		8	2.02±0.02	2.19±0.14	2.48±0.01
		10	1.77±0.04	2.79±0.02	2.92±0.03
		12	0.64±0.06	2.86±0.04	3.03±0.04

Food-borne diseases pose a threat to human health and the economy of individuals, families, and nations. In the Western hemisphere and in Europe, *Salmonella* serotype *Enteritidis* (SE) has become the predominant strain (WHO. 2011). A review of foodborne diseases in India indicated that the majority of the foodborne disease were caused due to vegetarian foods (Sudershan et al. 2011). Among the foods implicated in India, milk and milk products were predominantly involved in the foodborne disease outbreak (Sudershan et al. 2011).

Staphylococcus aureus food poisoning is an intoxication caused by the ingestion of food containing staphylococcal enterotoxins (SEs) and is one of the most common foodborne diseases in the world. The primary habitat of *S. aureus* is the nasal passage of humans and the skin and hair of warm-blooded animals (Kluytmans et al. 1997; Kuzma et al. 2003). *Staphylococcus aureus* produces a variety of extracellular products including the staphylococcal enterotoxins which have been implicated in human and animal diseases. The heat stability of *S.aureus* is one of the important properties of SEs in food safety (Le Loir et al. 2003). Contamination of foods by *S. aureus* may occur directly from infected food-producing animals or may result from poor hygiene during the production process or retail and storage of foods or from humans who will carry this microorganism (Doyle and Beuchat, 2007).

The present study showed that Staphylococcus contamination was higher in Sapodilla juice compared to other food samples indicating that they are hygienic of a low standard. Fruit juices sold on streets have been contaminated with various food and waterborne pathogens (Olorunjuwon et al. 2014; Lewis et al. 2006; Poonam, 2013; Tambekar et al. 2009; Mahale et al. 2008). The high contamination of Sapodilla fruit juice may be attributed to its neutral pH compared to other fruit juices which are of acidic pH.

In the present study, the number of coagulase-positive *Staphylococcus* cultures was more compared to coagulase-negative *Staphylococcus* (CNS). A study conducted by Cunha et al. on the detection of enterotoxin genes in coagulase-negative Staphylococci isolated from foods indicated that 22.7% were positive for CNS. Among them, four isolates were positive for enterotoxin genes (Cunha et al. 2006). A comparable study examining *S. aureus* isolates for the presence of classical enterotoxin genes revealed that none of the isolates were found to harbor any of these genes (Esemu et al. 2023). In a different study, a substantial prevalence (58.1%, 18/31) of classical staphylococcal enterotoxin genes was detected in meat samples collected in Zanjan, Iran. (Haghi et al. 2021)

In the present study, *S. aureus* population varied with temperature and showed the highest population and viability at 37^{o}C. A study on the Influence of holding temperature on the growth and survival of *Salmonella* spp. and *Staphylococcus aureus* and the production of staphylococcal enterotoxin in egg products indicated that Staphylococcal enterotoxin A and B are detected only in the egg products held at 37 or 22 degrees C. After holding at 37 degrees C for 36 h, scrambled egg inoculated with *S. aureus* contains the highest levels of SEA and SEB (Yang et al. 2001). In terms of Staphylococcal bacterial counts, winter emerged as the season carrying the highest risk, whereas concerning enterotoxin production, the peak risk was identified in autumn, particularly during October. The susceptibility to S. aureus contamination was most pronounced in dairy products (Bianchi et al. 2022). In India, there are many studies to show the prevalence of *Staphylococcus* in food samples but studies on its enterotoxin production are scanty.

There is a need to relook at the guidelines for microbiological requirements for different milk products which indicate that coagulase-positive *S. aureus* should be less than 100 per gram of the milk product. Although enterotoxins are produced mainly by coagulase-positive staphylococci, some coagulase-negative staphylococci are involved in a variety of human and animal infections (Cunha et al. 2006).

Conclusions

While assessing the risk of foodborne disease due to *S. aureus* contamination in foods enterotoxin production needs to be examined irrespective of its coagulase enzyme production. Further molecular characterization of classical and novel genes encoding different enterotoxins is necessary to find out different types of enterotoxins. The data will help set standards for the microbiological quality of foods.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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RESEARCH ARTICLE

Performance evaluation of strip based test for detection of urea adulteration in milk

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Abstract: Milk samples spiked with various concentrations of urea ranging from 0.02, 0.04, 0.06 and 0.08% was used to check the urea detection ability of the developed strip. The LoD in each type of milk viz. raw, pasteurized and boiled was found to be 0.07% urea. Further, 121 (nos.) milk samples (cow, buffalo and mixed milk) were analyzed with the MilkoScan FT120 and developed strip for urea content. The urea content of the milk sample obtained using MilkoScan was compared with the colorimetric result of the strips. Both these results were in alignment. Moreover, the data of urea content obtained from MilkoScan and strip were used to calculate sensitivity, specificity and overall accuracy of the strip which were found to be 90.90, 92.00 and 90.09%, respectively. The strip performance was also checked for its effectiveness spectrophotometrically using 10 mixed milk samples. The developed strips were found to be stable when stored at room temperature (30-40°C) and refrigeration temperature (7±2°C) up to six months.

Key words: Milk, urea, adulteration, strip, detection, performance

Introduction

Milk adulteration is one of the serious issue faced by the dairy industry, particularly in the developing countries. Milk is adulterated with many adulterants including urea. Urea is one of the common adulterants being reported in milk samples collected from various cities of India (Hanford et al. 2016; Roy et al. 2017). Urea is common adulterant found in milk to mask the developed acidity, to increase milk whiteness, to show apparent increase in protein content (Azad and Ahmed, 2016). The official wet chemistry based method for detecting urea in milk as recommended by FSSAI is labour-intensive, requiring specific glassware and reagents. The estimated average time of this analysis is about 15 min per sample. Wet chemistry methods are disadvantageous as it requires chemical preparation and hence becomes difficult to use at the field level. In contrary, dry chemistry based methods do not require chemicals and are easy to use at remote locations. IDF has also specified enzymatic method for determination of milk urea content using differential pH (ISO 14637, IDF 195: 2004). Several workers have recommended strip based methods for detection of urea in milk (Kumar et al. 2000; Panchal, 2013; Gautam, 2016; Luther et al. 2017). Thus, a low cost, sensitive urea detection strip can be a useful quality control tool for dairy industry.

There are several strip based tests available for detection of urea adulteration in milk, albeit with minimal published data on validation. Validation of the strips for urea detection is quite necessary as urea is one of the naturally occurring substance in milks of many species. The Limit of Detection (LoD) of the urea detection strip was determined using spectroscopic method, while sensitivity, specificity, overall accuracy was determined using FTIR method.

Savaliya et al. (2022) developed a strip-based test for detection of urea adulteration in milk. This study is contemplated to evaluate the performance of the developed strip-based test for the detection of urea adulteration in raw, pasteurized and boiled milk. The criteria were sensitivity, specificity and overall accuracy of the developed strip for detection of urea in milk. The effect of storage of urea added milk on the performance of the test was also evaluated. The FTIR method was selected as it is routinely used in the industry with the advantages of faster results, good sensitivity and simplicity. Further, if the strip performance is comparable to the results of the FTIR, it would be easy to convince the users to adopt a simple, rapid, sensitive and cheap alternative to high end instruments. Urea is natural constituent of milk,

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however a maximum limit for urea concentration in milk is set by FSSAI as 700 ppm (FSSR, 2011).

Materials and Methods

Milk samples

Pooled samples of cow and buffalo milk were procured respectively from the Livestock Research Station and Reproductive Biology Research Unit of Anand Agricultural University, Anand, Gujarat. Some of the milk samples were also procured from the local farmers for certain components of the study. Mixed milk samples were prepared by mixing cow and buffalo milk in equal proportions.

In order to study the effects of milk processing, the milk samples were pasteurized and boiled at the laboratory.

Total 121 milk samples of cow, buffalo and mixed milks were analyzed using both MilkoScan FT120 and developed strip.

Chemicals and instruments

The chemicals and reagents used were: β-mercaptoethanol (AR grade, Loba Chemie (P) Ltd., Mumbai, India); Ethanol (Shree Madhi Vibhag Udhyog Sahakari Manadali Ltd., Surat, India); Phenol red (AR Grade, Central Drug House Ltd., New Delhi); Urea (Allied Chemicals Corporation, Vadodara , India); Trichloroacetic acid (TCA)(Merck Specialities (P) Ltd., Mumbai, India); *p*- Dimethyl amino benzaldehyde (AR Grade, SRL Pvt. Ltd., Mumbai, India); Potassium dihydrogen orthophosphate (KH_2PO_4): (AR Grade, Qualigens Fine Chemicals, Mumbai, India); Dipotassium hydrogen phosphate (K_2HPO_4): (AR grade, Loba Chemie Pvt. Ltd., Mumbai, India) ; Chromatography paper (GE Healthcare, UK Ltd.); Soybean seeds (Anand Agricultural University, Anand); The UV-1800 model spectrophotometer from SHIMADZU, Japan were used for spectroscopic analysis and MilkoScan FT120 (FOSS), Denmark was used for rapid urea determination.

Methods for determination of urea in milk

The urea detection strips were prepared using the method as described by Savaliya et al. (2022).

In order to determine LoD of the developed strip, at first the natural urea content of the raw milk was determined spectrophotometrically using the DMAB method (FSSAI, 2016). The standard curve was prepared for the determination of urea content (Supplementary Fig. 1). The urea level in milk was then adjusted to 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1% (natural + spiked urea) using extraneous urea.
The quantitative results of urea in milks obtained using MilkoScan FT120 was used for comparison with strip results for sensitivity, specificity and overall accuracy of the developed strip for urea detection.
Simultaneously, the appearance of colour on the strip was also observed subjectively for positive or negative results of the strip test.
For determination of the response time, the colour developing on the strips was noted at different time interval of 1 to 10 min.

Results and Discussion

Determination of LoD of strip

For determination of LoD, 3 different types of milk samples viz. raw, pasteurized (heated at 75°C for 15 s) and boiled milk were used. The developed strips were dipped separately for 10-15 s in control and urea spiked milk samples. Subsequently, these strips were kept on a white paper sheet for observation of the developed colour.

In case of raw milk samples (Fig. 1), the strips dipped in the milk sample containing natural urea (control sample) turned yellow. The strips dipped in milk samples containing 0.05 and 0.06% urea produced different shades of orange colour. However, different shades of pink colour in the strips were observed for milk samples containing ≥0.07% urea. The change in colour of the strips used for control sample and milk containing extraneous urea could be differentiated at all levels, the minimum noticeable level to be differentiated being 0.07%. Thus, for raw milk, the LoD of the developed strips was considered to be 0.07%.

In case of pasteurized and boiled milk (Fig. 1), identical colour change in strips to that of raw milk samples (both control and urea spiked milk samples) were observed. Hence, for pasteurized and boiled milk samples, the LoD of the developed strips was considered to be 0.07%.

The results of the performance of the strip-based test indicated that the developed strip can be effectively used for detection urea in raw, pasteurized and boiled milk with the LoD of 0.07%.

Gautam (2016) and Panchal (2013) have indicated the LoD of their developed strips as 0.08 and 0.09% urea in milk respectively. Luther et al. (2017) have reported the LoD of paper card for urea as 0.07%. Panchal (2013) checked the effect of boiling of milk on the performance of urea detection strips using 0.25% externally added urea and did not observe significant effect on the colour intensity of the strips.

Determination of response time of strip

The developed strips were evaluated for determining the time required to obtain noticeable colour change between control and urea spiked milk. The total urea content in the pooled raw milk samples were adjusted to 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1% (natural + spiked urea) levels (Supplementary Fig. 2). The strips

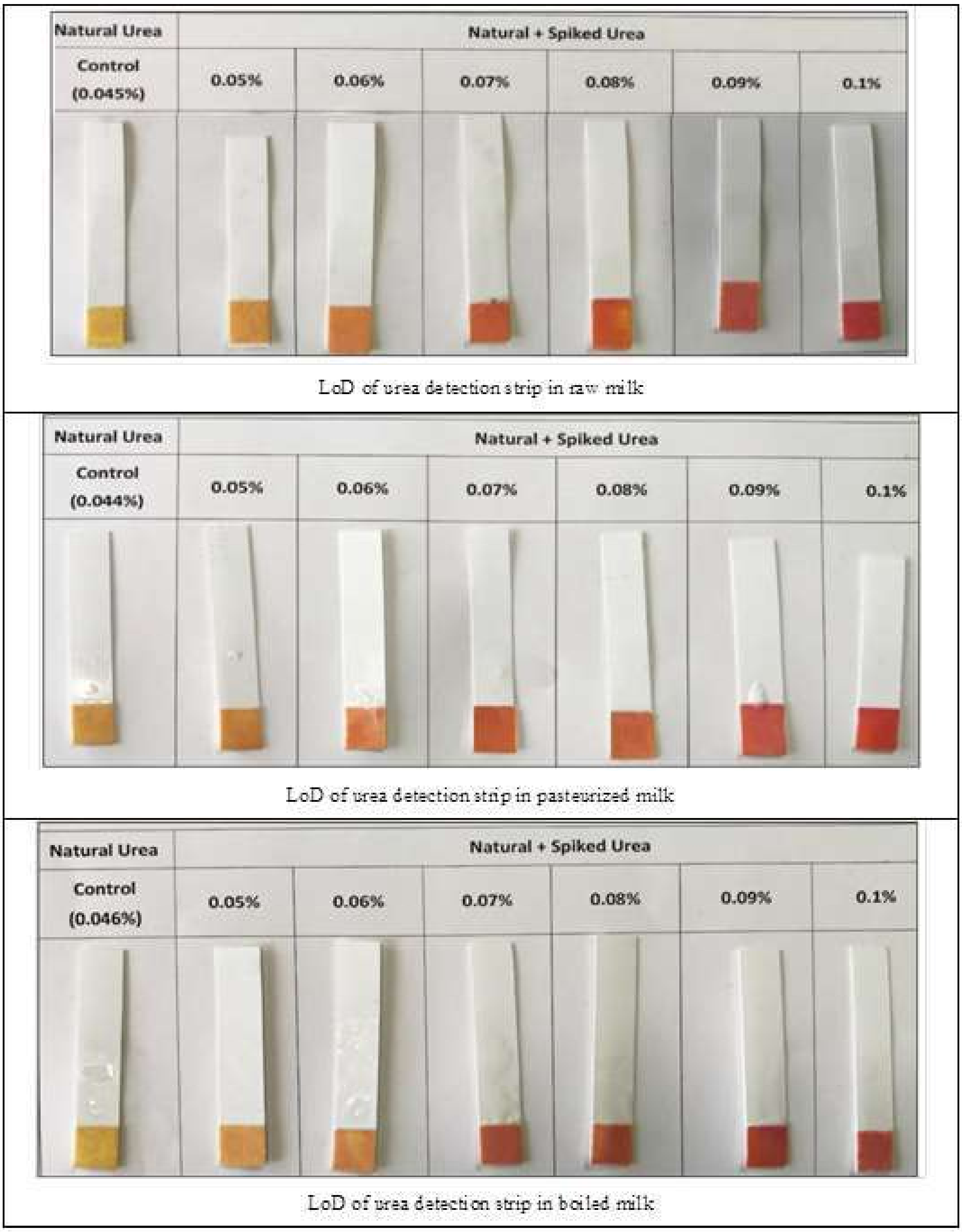

Fig. 1 LoD of urea detection strip in raw, pasteurized and boiled milk

were simultaneously dipped in all the samples of milk and kept for 10-15 s. Subsequently, the strips were kept on the white paper sheet and observed for colour development. The images of the treated strips were taken at the interval of 1 min. The change in colour development between strips used for control and urea spiked milk samples was observed. Up till 2 min. a visually noticeable colour change between control and urea spiked milk samples was not observed. Three minutes onwards the colour change developed between control and urea spiked samples was significantly clear till 5 min of time. Hence, the response time of the strip was considered as 3-5 min. It was also observed that after 6 min. the colour of the strips started to fade. This can lead to misinterpretation of the test result, so the result should be recorded within 3-5 min.

Repeatability of developed strip for urea detection

For checking the repeatability of the developed strip for urea detection in milk, 20 different mixed milk samples were analyzed. The natural urea content in these milk samples was estimated

Fig. 2 Repeatability of developed strip for urea detection

(DMAB method, FSSAI, 2016) and found to vary between 0.025 to 0.053%. Furthermore, these milk samples were spiked with external urea to adjust the total urea content to 0.07% (LoD). The milk samples without spiked urea (only natural urea) were used as a negative control.

As per Fig. 2, the milk sample no. 1-5, 10-15, 21-25 and 31-35 containing only natural urea gave negative test when checked using the developed urea detection strip, while, milk sample no. 6-10, 16-20, 26-30 and 36-40 (containing external urea to a level of 0.07%) gave positive test when checked using the developed urea detection strip. Based on these observations, it can be inferred that the developed strip can detect urea in all 20 milk samples containing 0.07% urea (i.e. pink colour). However, milk samples containing natural urea alone developed yellow to orange colour when checked using the strip. These observations ascertained the repeatability of the developed urea detection strip.

Reproducibility of the developed strip

The performance of the developed strip for urea detection was checked against FT120 milk analyzer and spectrophotometer in order to determine reproducibility, accuracy, sensitivity and specificity (Supplementary Fig. 3(A), 3(B), 3(C), 3(D)). For better understanding of the performance of the test, the results of the strip is portrayed in text of different colours (Supplementary Table 1).

Total 121 milk samples including cow, buffalo and mixed milk were analyzed using both MilkoScan FT120 and developed strip. However, 10 samples got soured and could not be tested on the MilkoScan FT120 but were analyzed with strips. The urea content

Fig. 3 Comparative performance of urea detection strip with spectroscopic method

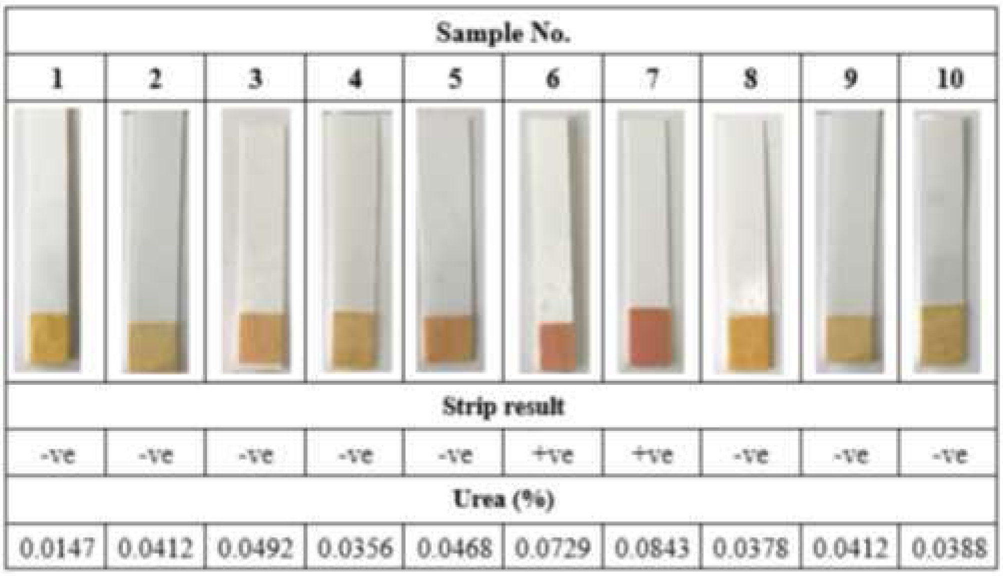

Table 1: Computation of sensitivity, specificity and overall accuracy of strip

Total number of samples analyzed: 111		
Strip	MilkoScan FT120 (Yes)	MilkoScan FT120 (No)
Positive test (yes)	10	8
Positive test (no)	1	92
a= True positive sample	10	
b= False positive sample	8	
c= False negative sample	1	
d= True negative sample	92	
Sensitivity of strip (%)	a / (a+c) =	90.90
Specificity of strip (%)	d / (b+d) =	92.00
Overall accuracy of strip (%)	(a+d) / (a+b+c+d)=	90.09

of the milk sample obtained using MilkoScan FT120 was compared with the colorimetric result of the strips.

The data obtained by analyzing the milk samples both using strip and MilkoScan FT120 were utilized for calculation of sensitivity, specificity and overall accuracy of the developed strip according to the formula given by Cleophas et al. (2012).

Accuracy can be defined as test's ability to show which milk is true positive or true negative with respect to parameters being tested. For the qualitative test the minimum level of desired accuracy is 50%, however as can be seen from the Table 1 the value of the overall accuracy obtained for the developed strip was 90.09%. Sensitivity is defined as the ability of test to detect true positive. In the developed strip value of sensitivity was found to be 90.90%. Specificity is defined as the ability to detect true negative sample. For the developed strip specificity was found to be 92.00%.

Performance of strip against spectrophotometric method

The strip performance was evaluated spectrophotometrically using 10 mixed milk samples. Milk samples were analyzed using the urea quantification method given by the FSSAI (2016). These samples were subsequently, analyzed using a developed urea detection strip. The results obtained by both methods were compared. The strips used for milk samples with urea content <0.07% did not give pink colour. Thus, the result was considered negative. Samples containing ≥0.07% urea showed pink colour on the developed strip, indicating positive results. The performance of the strip was validated by spectrophotometric method (Fig. 3).

Storage stability of strip at different storage temperatures

The developed strips for urea detection were packed in air tight plastic container with desiccant and stored at two different temperatures viz. room temperature (30-40°C) and refrigeration temperature (7±2°C). The strips were evaluated every 15 days for their performance with respect to their response time and LoD. Pooled mixed milk samples spiked with 0.07% urea were used to check the stability of the strips. At room temperature and refrigeration temperature, all the strips had the response time of 3-5 min with LoD values of 0.07%. The results indicated the stability of the strips and was found to be satisfactory both at room and refrigeration temperature, since, there was no change in response time and LoD.

Conclusion

Milk samples (raw, pasteurized and boiled) were spiked with extraneous urea (0.02, 0.04, 0.06 and 0.08%) for LoD determination of the developed strip using spectrophotometric method and was found to be 0.07% urea. Further, 121 (nos.) milk samples (cow, buffalo and mixed milk) were analyzed with the MilkoScan FT120 for urea content and the results were compared with that of the developed strip. Both the results were in alignment. These data were used to calculate sensitivity, specificity and overall accuracy and were found to be 90.90, 92.00 and 90.09%, respectively. The strip performance was also checked for its effectiveness spectrophotometrically using 10 mixed milk samples. The developed strips were found to be stable when stored at room temperature 30-40°C) and refrigeration temperature (7±2°C) for 120 days. The developed urea detection strip can be used conveniently at field, household and plant level to quickly detect adulteration urea in milk. This can be of great help in ensuring food safety and quality of milk. In general, it can be concluded that using inexpensive material and simple technology, we can produce milk urea detection strip with required accuracy, specificity and sensitivity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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RESEARCH ARTICLE

Microbiological, Biochemical, and Antibiotic susceptibility analysis of the lactic acid bacterial culture *Lactobacillus acidophilus* (MTCC No: 10307)

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Abstract: The link between health and nutrition has spurred a growing fascination with functional foods fortified with physiologically active constituents, delivering added advantages beyond fundamental nourishment. Probiotics, falling within the category of functional foods, are living microorganisms that impart health benefits when consumed in appropriate quantities. This research aimed to evaluate the susceptibility of *Lactobacillus acidophilus*, a widely used probiotic culture, to antibiotics. The probiotic culture's purity was examined via multiple microbiological examinations. The antibiotic susceptibility test was executed employing the standard disc diffusion assay based on the modified Kirby-Bauer technique. A total of thirty-six antibiotics underwent testing, and the outcomes were interpreted in line with recognized guidelines. Statistical analysis was carried out utilizing the One-way ANOVA approach. The results indicated the presence of innate resistance within certain antibiotic categories; however, this intrinsic resistance is typically non-transmissible and poses no significant risk. The findings of this investigation are set to enhance the comprehension of the safety and potential antibiotic resistance of lactic acid cultures utilized in various food products.

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Keywords: Lactobacillus, Probiotic, Safety, Antibiotic-susceptibility, Food

Introduction

There is an extensive focus on exploring the physiological benefits of various food items, which could potentially enhance overall well-being and mitigate the risk of chronic ailments. Recently, the connection between health and diet has sparked a surge of interest in nutritious foods. These functional foods are fortified with active components that include bioactive compounds containing phytochemicals, prebiotics, probiotics, vitamins, minerals, dietary fibers, fish oils, plant sterols, and oligosaccharides (Jan et al. 2023). According to the Food Safety and Standards Authority of India (Anonymous, 2017), functional foods, along with nutraceuticals, special dietary products, and health supplements, are defined as items that might contain plants, botanical extracts, as well as vitamins and minerals, either in their natural food form or in the form of powders, tablets, etc. (Tripathi and Giri, 2014). The discovery of the impact of probiotics is often attributed to Eli Metchnikoff, who correlated the robust physical health and longevity of Bulgarian peasants with their consumption of microorganisms found in yogurt, specifically *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. More contemporary definitions emphasize the preventive or therapeutic actions of probiotics. The Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) define probiotics as 'live microorganisms that, when administered in sufficient quantities, provide a health benefit to the host'. In 2017, the Food Safety and Standards Authority of India (FSSAI) issued guidelines (Anonymous, 2017) on functional foods, defining probiotic foods as 'foods containing live microorganisms beneficial to human health, which, when consumed in adequate numbers either as a single strain or a combination of cultures, confer one or more specified or demonstrated health benefits in human beings.'

A variety of bacterial strains, including those from the genera *Lactobacillus, Streptococcus, Enterococcus*, and *Bifidobacterium*, are harnessed to produce probiotic food products. Particularly, the Lactic Acid Bacteria (LAB) group is predominantly employed for this purpose. Typically, these bacteria ferment glucose, generating lactic acid, acetic acid, and hydrogen peroxide, along with diacetyl, low molecular weight antimicrobial substances, and bacteriocins. Acid production aids in reducing the pH of the gut, hindering the growth of pathogenic and putrefactive bacteria that generally thrive in an alkaline environment. Probiotics work to maintain a balanced intestinal flora, fortify the body against unwanted intruders, and safeguard against a leaky gut. Currently, probiotics are consumed globally through food, dietary supplements, or as active constituents of registered medications, and are available in diverse forms. However, it is imperative to ensure their safety, particularly concerning the potential spread of antibiotic resistance (ABR). Despite being a widely consumed bacterial group, the issue of antibiotic resistance in LAB has not received substantial attention compared to the growing concern over antibiotic resistance. Horizontal gene transfer between bacteria in nature and the subsequent dissemination of these resistant strains across populations is highly plausible (Arber, 2014). The past decade has witnessed a surge in reports documenting antibiotic resistance in LAB strains. While LAB are generally considered safe, there remains apprehension regarding the potential transference of resistance determinants to human and animal pathogenic and opportunistic bacteria. Some researchers acknowledge the presence of antibiotic resistance in LAB and endorse the possibility of their co-administration with antibiotic therapy, ensuring the restoration of a healthy gut flora, which is otherwise at risk (Kamath et al. 2023). Instances of resistance-coding genes and their transfer through plasmids and conjugative transposons have been documented in *Lactobacillus* species. Genes that confer resistance to various antimicrobials have been found on transferable genetic elements in several LABs (Kaszab et al. 2023). Consequently, there is a risk of transferring antibiotic resistance from probiotic strains to other bacteria, whether commensal or pathogenic, which could be detrimental. Reports have indicated resistance to current antibiotics in LAB from various commercially available dairy and food products. Therefore, it would be intriguing to assess the spectrum of resistance in cultivable microflora in human milk. Given that humans are routinely exposed to antibiotics, this exposure may influence the susceptibility/resistance profile of the human milk microflora, particularly the LAB group. The close contact of the native microbiota with the human intestine creates an ideal environment for the horizontal transfer of antimicrobial resistance genes facilitated by mobile genetic elements (Partridge et al. 2018). Consequently, routine antibiotic-resistance screening for starter and probiotic cultures is increasingly becoming a standard practice.

Materials and Methods

This study was conducted in the laboratories of the Department of Processing and Food Engineering, Punjab Agricultural University, Ludhiana, and the Department of Dairy Microbiology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Culture Procurement

The *Lactobacillus acidophilus* probiotic culture, identified as MTCC No: 10307, was obtained from the Institute of Microbial Technology (IMTECH) in Chandigarh. This probiotic culture has been designated a ‘Generally Regarded as Safe’ (GRAS) status.

Maintenance of culture

The freeze-dried form of the probiotic culture, *Lactobacillus acidophilus*, was sourced from the Institute of Microbial Technology (IMTECH) in Chandigarh. The culture was revived on de Man Rogosa and Sharpe (MRS) Media and cultivated for 24 hours at 37°C under aerobic conditions

Purity of probiotic culture

The probiotic culture’s purity underwent assessment through a series of tests including microscopic examination, colony morphology, gram staining, and various biochemical evaluations.

Catalase test:

A small amount of culture from a single, isolated colony was obtained with a sterile loop and placed in the center of a clean microscopic slide. The slide was then exposed to a 3-5% hydrogen peroxide solution. The presence of bubbles or effervescence indicated a positive reaction.

Indole test:

The probiotic culture, *Lactobacillus acidophilus*, was introduced into 5ml of tryptone broth and left to incubate for 24 hours. Following this, 0.2-0.3 ml of Kovacs reagent was added to the 24-hour culture, allowing the yellow reagent to settle on the surface. A deep red color on the surface layer indicated a positive reaction.

Hydrogen sulphide test:

A tube of Triple Sugar Iron (TSI) Agar was streaked and then incubated for 24-48 hours. The presence of blackening due to H_2S production was observed.

Methyl red reaction:

The methyl red (MR) indicator was dissolved in 300ml of alcohol and diluted with distilled water to 500ml. The test culture was incubated in MR-VP broth for 48 hours at 37°C. Following this, 5 or 6 drops of the reagent were added to the culture and observed for color changes. A red color was interpreted as MR positive, while a yellow color indicated MR negative.

Urease test:

The urease agar media was inoculated with the probiotic culture, *Lactobacillus acidophilus*, and incubated for 24 hours at 37°C. The presence of urease caused the indicator's color to change from yellow to pink due to the formation of ammonia.

Carbohydrate fermentation test:

Different carbohydrates (Sorbitol, Lactose, Glucose, Dextrose, Mannitol, and Sucrose) were incorporated into phenol red medium. The medium was then transferred to test tubes containing Durham's tubes and autoclaved at 15 psi pressure (121°C) for 15 minutes. After sterilization, 0.1 ml of the bacterial culture was added to each sugar tube separately and incubated at 37°C. Results were noted after 24 hours. Acid production was indicated by a color change from red to yellow in positive cases, while gas formation was observed through the presence of gas bubbles in the Durham's tubes.

Antibiotic Susceptibility Test of the given culture

To examine the susceptibility of the probiotic culture, *Lactobacillus acidophilus*, to commonly used antibiotics, an antibiogram test was conducted. A total of thirty-six (36) antibiotics were acquired from Hi-media Laboratories Pvt. Ltd. in Mumbai, India for the experiment. Details regarding the drug concentrations, antibiotic groups, and modes of action can be found in Table 1.

Table 1: The concentration of various drugs, antibiotic group, and mode of action

S. no.	Antibiotic Susceptibility Test				
	Name of drug		Concentration (µg)	Antibiotic group	Mode of action
1	OX-1	Oxacillin	1	β-Lactams	Inhibitors of the cell wall synthesis
2	MET-5	Methicillin	5		
3	FAR-3	Faropenem	3		
4	CMZ-30	Cefmatazole	30		
5	CEP-30	Cephalothin	30		
6	AMP-10	Ampicillin	10		
7	P-10	Penicillin-G	10 units		
8	MRP-10	Meropenem	10		
9	AMC-30	Amoxyclav	30		
10	IPM-10	Imipenem	10		
11	CX-30	Cefoxitin	30	Cephalosporins	
12	CF-30	Ce-Factor	30		
13	CTX-30	Cefotaxime	30		
14	TEI-30	Teiceplanin	30	Glycopeptides	
15	VA-30	Vanomycin	30		
16	CXM-30	Cefuroxime	30	Second generation	
17	CAZ-30	Ceftazidime	30	Third generation	
18	GEN-10	Gentamycin	10	Aminoglycosides	Inhibitors of protein synthesis
19	AK-30	Amikacin	30		
20	K-30	Kanamycin	30		
21	TOB-10	Tobramycin	10		
22	S-10	Streptomycin	10		
23	FC-10	Fusidic Acid	10	Fusidane	
24	TGC-15	Tigecycline	15	Glycylcycline	
25	CD-2	Clindamycin	2	Lincosamide	
26	AZM-15	Azithromycin	15	Macrolides	
27	E-15	Erythromycin	15		
28	TE-30	Tetracyclin	30	Tetracyclines	
29	NIT-300	Nitrofurantoin	300	Other	
30	C-30	Chloramphenicol	30		
31	GAT-5	Gatifloxanin	5	Quinolones	Inhibiting DNA replication and transcription
32	OF-5	Ofloxacin	5		
33	CIP-5	Ciprofloxacin	5		
34	NA-30	Nalidixic Acid	30		
35	COT-25	Co-trimoxazale	25	Other	Folic acid synthesis inhibitors
36	TR-5	Trimethoprin	5		

The standard disc diffusion assay, following the modified Kirby–Bauer method (Bauer et al. 1966), was utilized to assess the antibiotic susceptibility pattern. The testing of antibiotic susceptibility was conducted using MRS agar medium. A broth culture (100 µl, 0.5 McFarland, equivalent to 10^8 CFU/ml) of the *Lactobacillus acidophilus* probiotic culture under examination was combined with 8 ml of soft agar, which was then layered over a pre-solidified agar plate. Subsequently, antibiotic discs were meticulously placed equidistant to each other with sterile forceps.

The plates were pre-incubated at room temperature (25°C) for 1 hour to ensure proper diffusion. Following this, they were incubated overnight at 37°C. The interpretation of results was carried out according to the standards set by the Clinical and Laboratory Standards Institute (CLSI, 2015) guidelines. In this regard, isolates exhibiting a zone of inhibition equal to or less than 14 mm were classified as resistant (R), those with a diameter greater than 20 mm were categorized as susceptible (S), and those with a zone of inhibition ranging between 15 and 19 mm were considered intermediate (I).

Statistical Analysis

Tests were conducted three times to ensure precision. The collected data underwent statistical analysis using the CPCS-1 software to apply One-way Analysis of Variance (ANOVA).

Results and Discussion

Microbiological examination

Lactobacillus acidophilus probiotic culture was cultivated on MRS media. The colonies exhibited a moderate size and appeared creamish-white in color as shown in Figure 1. They were raised with a smooth texture and flat elevation, characterized by an entire margin as shown in Table 2.

Lactobacillus acidophilus is Gram-positive, Rod-shaped (Figure-2), non-motile, and exhibited negative results for catalase, citrate utilization, H_2S formation, indole production, oxidase test, urease activity, and Voges-Proskauer reaction. These observations align with the findings documented by Hawaz (2014).

Carbohydrate fermentation test

Seven sugars (sorbitol, lactose, maltose, mannitol, dextrose, glucose, and sucrose) were used to test the ability of *Lactobacillus acidophilus* to ferment the given sugars as explained in Table 3.

According to the results of the carbohydrate fermentation test, *Lactobacillus acidophilus* effectively fermented various sugars (such as lactose, maltose, glucose, sucrose, and dextrose), leading to acid production, which consequently altered the color from red to yellow. However, the specific probiotic culture, *Lactobacillus acidophilus*, was incapable of fermenting sorbitol and mannitol. Furthermore, no bubbling was observed in the Durham tubes, indicating the absence of gas production. These findings affirmed the homo-fermentative characteristic of *Lactobacillus acidophilus* (MTCC 10307).

Antibiotic susceptibility test of the probiotic culture, Lactobacillus acidophilus

The results of the antibiotic susceptibility test were organized in Table 04, indicating the response of *Lactobacillus acidophilus* (MTCC No: 10307) to various antibiotics, classified as resistant (R), intermediate (I), or susceptible (S). *Lactobacillus acidophilus* exhibited sensitivity to tetracycline, imipenem, chloramphenicol, clindamycin, amoxyclav, fusidic acid, tigecycline, and erythromycin (macrolide). Conversely, it displayed resistance to methicillin (β-lactams), oxacillin, cefuroxime, cefoxitin, cefatazimide, gentamicin, co-trimoxale, nalidixic acid, kanamycin, feropenem, and cefmatazole. A mixed response was observed for certain antibiotics: resistance–intermediate pattern for ceftazidime, streptomycin, tobramycin, nitrofurantoin, and cefotaxime; sensitive–intermediate pattern for ofloxacin, azithromycin, gatifloxacin, teicoplanin, vancomycin, penicillin, meropenem, and ciprofloxacin; and resistance–intermediate–sensitive pattern for

Table 2: Morphological and Biochemical characterization of the given culture, *Lactobacillus acidophilus* (MTCC 10307)

Morphological Characters		Biochemical Tests	
Gram Staining	Gram +ve	Catalase Test	-
Type Of Colony	Large	Indole Test	-
Color	Non-Shiny, Creamish white in Color	Methyl Red Test	+
Margin	Entire	Uraese Test	-
Elevation	Flat	Voge'sProskauer Test	-
Opacity	Opaque	Citrate Utilisation	-
Growth On MRS	High Growth	Hydrogen Sulphide Production	-
Endospore Formation	Non-Endospore Forming	+ indicates positive result	
Motility	Non-Motile	- Indicates negative result	

Fig. 1 Colony morphology of *L. acidophilus*

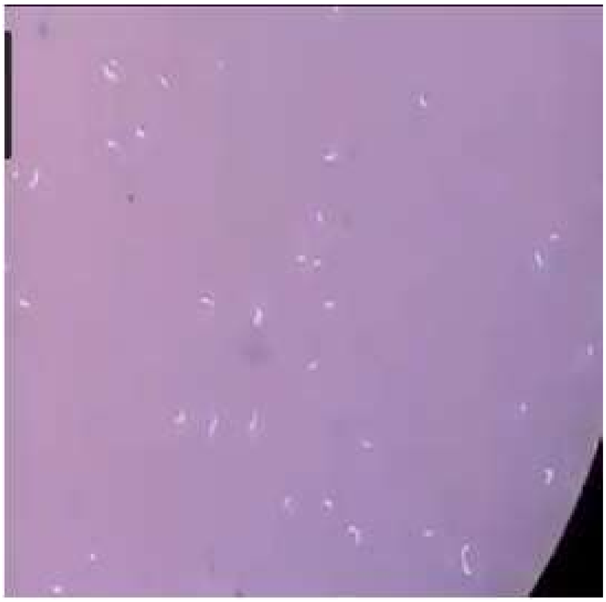

Fig. 2 Negative staining of *L. acidophilus* (40X magnification)

Table 3: Carbohydrate fermentation tests of the given culture, *Lactobacillus acidophilus* (MTCC 10307)

Sugar	Fermentation of Carbohydrates	
	Acid Formation	Gas Formation (Indicated by Bubbling in Durham Tube)
Sorbitol	-	-
Lactose	+	-
Maltose	+	-
Dextrose	+	-
Mannitol	-	-
Sucrose	+	-
Glucose	+	-

+ indicates positive result - Indicates negative result

ampicillin, trimethoprim, and cephalexin. *Lactobacillus acidophilus* demonstrated resistance to 16 out of 36 antibiotics, intermediate susceptibility to 11 out of 36 antibiotics, and susceptibility to 9 out of 36 antibiotics as shown in Figure 3.

A general resistance was noted in *Lactobacillus acidophilus* against the antibiotic discs containing cephalosporins. Additionally, *Lactobacillus acidophilus* showed intermediate susceptibility towards glycopeptides (teicoplanin, vancomycin) and quinolones (ciprofloxacin, ofloxacin). Notably, within the aminoglycoside group, streptomycin and tobramycin exhibited a moderate inhibitory effect on the growth of *Lactobacillus acidophilus*. On the other hand, *Lactobacillus acidophilus* demonstrated sensitivity to chloramphenicol, erythromycin, and tigecycline. A general susceptibility of the tested LAB to β-lactams was also observed. Overall, these findings are consistent with previous studies on *Lactobacillus acidophilus* species. Studies by Sharma et al. (2016) previously reported susceptibility of different LAB species to penicillin and ampicillin. They also documented high sensitivity of *L. acidophilus* to meropenem.

Resistance to β-lactams may be attributed to the presence of genes encoding for β-lactamases, which are known to transfer conjugally within different groups. Various studies have reported a high frequency of conjugation in different *Lactobacillus* species. Intermediate susceptibility to glycopeptides (teicoplanin, vancomycin) and quinolones (ciprofloxacin, ofloxacin) demonstrated by *Lactobacillus* in this study can be explained by the existence of intrinsic resistance mechanisms to both antibiotic families (Hawaz, 2014). Intrinsic resistance refers to the insensitivity of bacterial strains to approved drug doses, regulated by permeability barriers and active efflux. Such intrinsic resistance is typically non-transferable and poses no risk in LABs. Resistance to cephalosporins, a structural sub-type of β-lactam antibiotics, can be attributed to the presence of variants of broad-spectrum β-lactamases and the presence of efflux pumps associated with cell wall impermeability (Impey et al. 2020). Resistance to aminoglycosides can further be attributed to the absence of cytochrome-mediated electron transport, which enables antibiotic uptake. The susceptibility of *Lactobacillus* to both erythromycin and chloramphenicol has also been documented by Jiang et al. (2016). Similarly, Jiang et al. (2016) showed an intermediate pattern of human milk *Lactobacilli* against nitrofurantoin and susceptibility to tetracycline. Anisimova et al. (2022) observed that *Lactobacillus* strains were susceptible to chloramphenicol, erythromycin, tetracycline, and clindamycin.

Dhillon et al. (2021) and Natt and Katyal (2022) separately developed probiotic mango and guava juice enriched with *Lactobacillus acidophilus* MTCC 10307, respectively, each with varying shelf lives. Reddy et al. experimented with muskmelon juice enriched with four lactic acid bacterial cultures, including *Lactobacillus acidophilus* MTCC 10307, while Siddiqui et al. (2023) worked on bacterial nano-conjugates derived from *Lactobacillus acidophilus* MTCC 10307, demonstrating potent anti-oxidant, anti-bacterial, and cytotoxicity activities. Furthermore, Pattnaik et al. (2022) used *Lactobacillus acidophilus* MTCC 10307 to produce flavoring phenolic compounds from sugarcane baggage, as identified by high-performance thin-layer chromatography, while Bhukya and Bhukya (2021) studied *Pediococcus pentosaceus* for its probiotic efficiency, using *Lactobacillus acidophilus* MTCC 10307 as a reference strain.

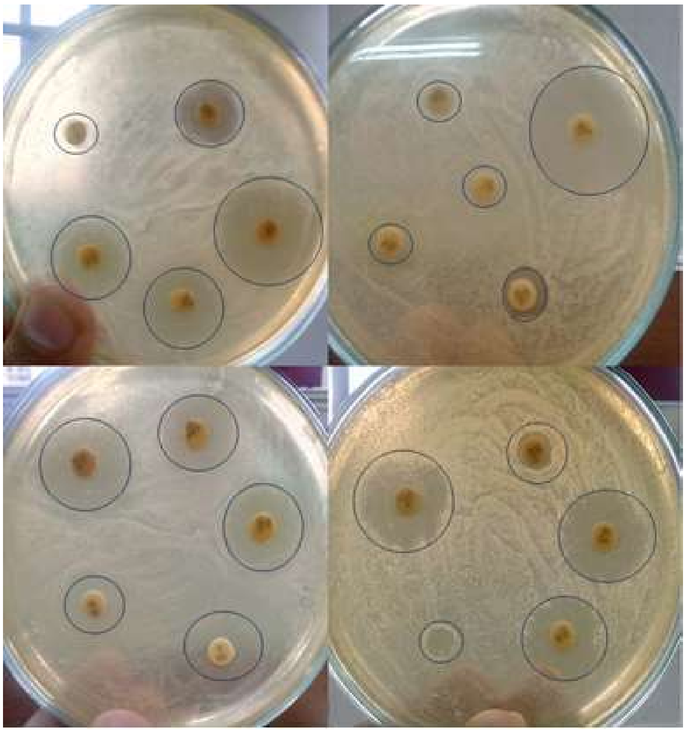

Fig. 3 Antibiotic discs showing zone of inhibition

Lactobacillus possesses a natural resistance to nucleic acid synthesis inhibitors, like trimethoprim and sulphonamides (co-trimoxazole). The combination of trimethoprim and co-trimoxazole has been widely used for various clinical conditions in humans since the late 1960s, mainly due to its cost-effectiveness, low toxicity, and high bactericidal activity, making it a preferable option, particularly in the developing world (Goldberg and Bishara 2012). The study emphasized the importance of inherent and non-transferrable resistance. Sharma et al. (2017) pointed out the

Table 4: Antibiotic susceptibility test of the probiotic culture, *Lactobacillus acidophilus* (MTCC No: 10307) in terms of resistant (R), intermediate (I) and susceptible (S)

S. no.		Name of drug	ZOI (Diameter in mm)	Interference
1	CAZ-30	Ceftazidime	11.3±1.15	R
2	OX-1	Oxacillin	10.3±0.58	R
3	CXM-30	Cefuroxime	10.7±0.58	R
4	CX-30	Cefoxitin	10±0	R
5	TR-5	Trimethoprin	11.7±2.89	R
6	GEN-10	Gentamycin	10.3±0.58	R
7	NA-30	Nalidixic Acid	10.3±0.58	R
8	MET-5	Methicillin	10.3±0.58	R
9	AK-30	Amikacin	10.7±1.15	R
10	K-30	Kanamycin	11±1	R
11	FAR-3	Faropenem	10.3±0.58	R
12	CMZ-30	Cefmatazole	12.3±2.08	R
13	COT-25	Co-trimoxazale	10.3±0.58	R
14	CF-30	Ce-Factor	10.7±1.15	R
15	TOB-10	Tobramycin	12±1	R
16	S-10	Streptomycin	12.7±0.58	R
17	NIT-300	Nitrofurantoin	16±3	I

18	CTX-30	Cefotaxime	16.3±3.21	I
19	MRP-10	Meropenem	16.7±2.08	I
20	CEP-30	Cephalothin	15.3±0.58	I
21	AMP-10	Ampicillin	15.3±0.58	I
22	AZM-15	Azithromycin	18.7±3.06	I
23	GAT-5	Gatifloxanin	16.7±1.15	I
24	OF-5	Ofloxacin	14.3±2.08	I
25	TEI-30	Teiceplanin	17.7±0.58	I
26	VA-30	Vanomycin	18±3.61	I
27	P-10	Penicillin-G	17±1.73	I
28	CIP-5	Ciprofloxacin	19.3±0.58	S
29	TGC-15	Tigecycline	19.3±2.08	S
30	FC-10	Fusidic Acid	23±1	S
31	AMC-30	Amoxyclav	21.3±2.08	S
32	C-30	Chloramphenicol	24.3±0.58	S
33	CD-2	Clindamycin	25±2.10	S
34	TE-30	Tetracyclin	25.3±1.15	S
35	E-15	Erythromycin	24.7±2.31	S
36	IPM-10	Imipenem	30.3±2.52	S

potential of the natural antimicrobial properties of LABs to work synergistically with antibiotic therapy in eliminating pathogenic strains.

Goldberg and Bishara (2012) examined the antimicrobial susceptibility of various *Lactobacillus acidophilus* strains from probiotics, nutritional foods, animals, and human sources, reporting susceptibility to certain antibiotics like penicillin, ampicillin, vanomycin, erythromycin, and clindamycin, while showing resistance to others including trimethoprim, gentamycin, fusidic acid, and chloramphenicol. Additionally, Anisimova et al. (2022) highlighted similar findings regarding the resistance and susceptibility of *Lactobacillus acidophilus* strains to specific antibiotics.

Conclusion

The freeze-dried form of the probiotic culture, *Lactobacillus acidophilus*, was acquired from IMTECH, Chandigarh. The maintenance of the culture was done on de Man Rogosa and Sharpe (MRS) media. Detailed analysis of the morphological and biochemical properties of the *Lactobacillus acidophilus* culture was performed.

On the MRS agar plate, the colonies exhibited moderate size, were creamish-white in color, raised, with an entire margin, slimy texture, and flat elevation. Biochemically, the culture displayed a positive methyl red test and exhibited acid formation from glucose. However, negative results were obtained for the catalase test, indole production test, urease test, Voges-Proskauer test, and citrate utilization test. In the carbohydrate fermentation tests, no bubbling was observed in Durham's tubes, indicating the homo-fermentative nature of *Lactobacillus acidophilus*. The culture was found capable of fermenting lactose, maltose, dextrose, and glucose, while no fermentation of sorbitol and mannitol was observed.

Antibiogram test was conducted to assess the susceptibility of the culture to commonly available antibiotics. Among all the antibiotic discs tested, imipenem, a β-lactam group antibiotic, demonstrated the most significant effectiveness. The results indicated the presence of intrinsic resistance within certain antibiotic families; however, this inherent resistance was found to be non-transferrable and posed no significant risk.

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RESEARCH ARTICLE

A study on the assessment of milking machine teat cup liner size fitting in indigenous dairy animals

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Abstract: Milking machines aim to efficiently harvest high-quality milk while ensuring animal comfort and protecting against mastitis. The present study focused on 128 and 125 teats of Sahiwal cows and Murrah buffaloes by measuring the teat morphometric parameters and comparing them with the dimensions of the teat cup liner size of the milking machine. The intra-mammary infection status was assessed using thermal imaging and the California Mastitis Test (CMT). Results showed that 37.50% of Sahiwal cows had the best fitting teat sizes for milking machine liners based on upper teat diameter (UTD), while 62.50% had a natural fitting. For Murrah buffaloes, 37.00% had the best-fitting teat size, and 63.00% had normal fitting teat size. In Sahiwal cows, 53.75 and 22.50% of teats were affected by subclinical (SCM) and clinical mastitis (CM) in normal-fitting UTD groups, whereas 43.75% and 18.75% were affected in the best-fitting UTD groups, respectively. In Murrah buffaloes, normal-fitting teat size related to UTD revealed 27.27 and 3.63% of SCM and CM-affected teats, and the mean values of UTD among the various groups based on the CMT score showed a significant difference ($p<0.05$). The mean UTD values for teats of the best-fitting size showed no mastitis incidences. The mean values of middle and lower teat diameters differed significantly ($P<0.05$) among both breeds' best and normal fitting groups. These findings present an exciting opportunity for improving milking efficiency and animal welfare in Sahiwal and Murrah breeds by addressing variations in teat size and optimizing teat cup liner fitting with milking machines.

Keywords: Murrah buffaloes, Sahiwal cows, teat diameter, teat liner, teat size

Introduction

Milking machines are designed to maximize milk yield efficiency and quality while prioritizing animal welfare and reducing mastitis risks (Kaskous and Pfaffl, 2023). They offer increased efficiency and consistency in milk extraction, thereby significantly reducing labour intensity and streamlining dairy farming operations. Maintaining udder health during milking is crucial for achieving high yields and milk quality. Improper machine milking settings can lead to adverse teat tissue changes (Stauffer et al. 2021), enabling pathogenic bacteria entry through the teat end's skin (Gasparik et al. 2018). This increases the risk of mastitis infections and elevates somatic cell counts (SCC) in the milk (Gayathri et al. 2024). Teat scoring via visual assessment is carried out to scale the color variations commonly. In addition to the conventional visual assessments and indirect estimation methods, sensor-based techniques have gained prominence for evaluating the teat-liner interface during milking. These methods are ultrasonography, infrared thermography (IRT) and pressure sensors (Tangorra et al. 2019, Rosca et al. 2017). IRT is a simple, effective, non-invasive and contactless method for measuring surface temperatures using specialized cameras that detect infrared radiation emitted from objects and is been utilized in assessing the dairy animal udder health status (Gayathri et al. 2023). Thus, ensuring udder and teat health during milking is essential for optimal milk production and animal welfare.

Selecting the right teat liner is a complex task with significant implications. It's crucial to consider the milking machine, vacuum settings, and the lactating animal's udder anatomy (Vermaak et al. 2022). The massaging function of teat cup liners in machine milking helps move blood and fluids toward the udder's base. However, excessively soft or stiff shafts can affect this function.

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Teat liners come in different shapes, with round liners being the most common worldwide. Studies have shown that liner type significantly impacts keratinization and the frequency of cracked teat ends, with round liners leading to higher keratinization scores compared to square liners (Holst et al. 2021). In practice, there often needs to be more flexibility in liner selection based on teat dimensions, than standard teat cup liners. While most milking equipment manufacturers strive to produce well-fitting teat liners, matching each lactating animal with a custom liner is challenging, resulting in some animals suffering from clinical or subclinical mastitis (Bobic et al. 2018). Thus, an ideal teat liner protects sensitive tissue, provides comfort during milking, adheres securely without causing noise, and helps prevent injuries, significantly improving animal welfare and health (Kaskous and Pfaffl, 2023). Owing to the scenario, the present study attempts to understand the effectiveness of the commercial standard teat liners used among indigenous breeds of Sahiwal cows and Murrah buffaloes. In addition, the present study aimed to assess udder health status via IRT while using standard milking machines' teat liners in varied teat shapes and sizes of Sahiwal cows and Murrah buffaloes.

Materials and methods

One hundred twenty-eight teat quarters of Sahiwal cows and one hundred twenty-five teat quarters of Murrah buffaloes belonging to different parity (1-6) were analysed separately for the present experiment during the autumn season. The investigation was carried out at the Livestock Research Centre (LRC), located in the ICAR-National Dairy Research Institute, Karnal, Haryana, India. The temperature-humidity index (THI) was calculated using the formula established by McDowell in 1972: $THI = 0.72(T_{db} + T_{wb}) + 40.6$ to assess the environmental conditions. In this formula, T_{db} represents the dry bulb temperature in ºC, and T_{wb} denotes the wet bulb temperature in ºC. The morphometric measurements of the teats were taken one hour before Sahiwal cows and Murrah buffaloes were milked. The teat diameter (upper, middle, lower) was measured using vernier callipers. The teat length, distance from the teat end to the floor and length of the teat cup liner were measured using measuring tape and the diameter of the teat cup with liner was measured with a vernier calliper. The cross-section of the teat cup with liner was taken as a model, and images of individual teats were captured against the cross-section of the teat cup. The attachment of the teat cup was assessed based on measurements and images captured using a Nikon camera. The teat size was measured in terms of teat diameter to the teat cup liner of the milking machine and classified the teats as the best fitting (Group 1) and normal fitting (Group 2) groups. The inner diameter of the metallic sheath of the milking machine's teat cup–teat cluster was 38.73 mm. The teat liner 1 (29 cm length, bore size 24 mm) used for Sahiwal cows had an upper, middle, and lower inner diameter (mm) of 28.65, 22.68, and 20.92, respectively. In Sahiwal cows, the teat size that falls in the range of 23.75 to 33.55 mm, 18.03 to 27.54 mm and 19.22 to 20.27 mm, respectively for upper, middle and lower teat diameters were considered as the best fitting group in the current study. In the case of Murrah buffaloes, teat liner 2 (30 cm length, bore size 20 mm) was used and had an upper, middle, and lower inner diameter (mm) of 26.65, 20.27, and 15.66, respectively. In Murrah buffaloes, the teat size that falls in the range of 21.11 to 31.64 mm, 18.27 to 25.53 mm and 17.48 to 18.54 mm, respectively for upper, middle and lower teat diameters were considered as the best fitting group in the current study. Those teat sizes that do not fall under the stipulated range for each breed were considered as normal fitting groups and observations were made accordingly. The healthy, subclinical mastitis and clinical mastitis were evaluated using the California Mastitis Test (CMT) and infrared thermography (IRT) via infrared thermal camera (Darvi DTL007 camera, TAK Technologies Pvt. Ltd., Noida, Uttar Pradesh, India) during the study period (Gayathri et al. 2023; 2024). The wet and dry bulb thermometer reading in the milking parlor was recorded during the study period.

Statistical analysis

In this study, data analysis was conducted using IBM SPSS Statistics version 26.0 (IBM Corporation, Armonk, New York, USA). Univariate linear models were used to analyze the data obtained from Sahiwal cows and Murrah buffaloes. For post hoc analysis, the Duncan Multiple Range Test (DMRT) with a significance level of $p< 0.05$ was utilized to compare the means of multiple groups and determine which pairs are significantly different from each other. Receiver Operating Characteristic (ROC) curves were used to assess thermograms' overall diagnostic performance in mastitis diagnosis. The effectiveness of thermograms in detecting mastitis was evaluated based on sensitivity and specificity. Additionally, the Area Under the Curve (AUC) was calculated to gauge the accuracy of the diagnostic tests. The ideal cut-off point was estimated using "Youden's J statistic," where J= Sensitivity + Specificity – 1 (Youden, 1950).

Results and Discussion

The average dry bulb and wet bulb thermometer readings were 26.25 °C and 24.00 °C, respectively, while the average relative humidity was 82.9 %. The calculated average THI was 76.78.

Sahiwal cows

Among the total teats measured in Sahiwal cows, the average teat diameter at upper, middle, and lower ends (mm) were 35.64 ± 0.81 (18.62 to 59.07), 29.15 ± 0.72 (15.06 to 51.88), and 21.24 ± 0.52 (11.52 to 39.54). The average teat length was 6.33 ± 0.14 cm (3.10 to 10.10). The average distance from the ground level (milking parlor) to the lower tip of the teat was 40.40 ± 0.56 cm, 87.50 % of which falls under 1SD, 95.31 % in 2SD. The average inner diameter covered by the teat cup liner (1) (bore size 24mm) over the upper end of the teat was 33.80 ± 0.81 mm (Figure 1, 2). The mean values of teat sizes to teat cup liner size in both the groups showed a significant difference ($p<0.05$) in UTD (upper teat diameter), MTD (middle teat diameter), LTD (lower teat diameter) of Sahiwal cows. The mean values of teat length of both the groups were comparable whereas teat tip distance from the ground showed a significant difference ($p<0.05$) between the groups (Table 1).

The results of Table 2 revealed a total of 80 and 48, 62 and 66, and 32 and 96 teats fall under group 1 and group 2, for UTD, MTD, and LTD, respectively. Among group 2 for Sahiwal cows, 53.75 and 22.50, 62.90 and 17.74, and 59.38 and 9.38 per cent of the teats were affected by SCM (sub-clinical mastitis) and CM (clinical mastitis), for UTD, MTD, and LTD, respectively. In group 1, 43.75 and 18.75, 37.87 and 24.24, and 46.87 and 25.00 per cent of teats were affected by SCM and CM, for UTD, MTD, and LTD, respectively. Compared to healthy teats, the UTD corresponding to the SCM and CM-affected teats showed a significant difference ($p<0.05$) among both the groups of Sahiwal cows. The MTD and LTD corresponding to the mastitis-affected teats compared to healthy teats were comparable within group 1 and showed a significant difference ($p<0.05$) within group 2 of Sahiwal cows.

The ROC analysis revealed a higher sensitivity, specificity, and accuracy for USST and TSST parameters for Sahiwal cows. The cut-off temperature with sensitivity, specificity, and accuracy (95% confidence interval) in the case of USST for SCM was ≥ 35.35 ºC, 0.98, 0.93, and 0.98 (0.97-0.99) and for CM, was ≥ 36.35 ºC, 0.98, 0.96, 0.99 (0.99-1.00) and that of TSST for SCM was ≥3 4.45 ºC, 0.97, 0.98, 0.99 (0.98-1) and for CM was ≥ 35.75 ºC, 0.97, 0.98, 0.99 (0.99-1), respectively. The statistical analysis of the thermograms revealed that the mean udder and teat surface temperature of SCM and CM-affected quarters compared to healthy quarters of Sahiwal cows showed a significant difference ($p < 0.01$) between the groups. In addition, the mean values of USST and TSST of SCM and CM-affected quarters compared to healthy quarters showed an increase of 1.00, 2.13 ºC, and 1.17, 2.42 ºC, respectively (Figure 3).

Murrah Buffaloes

The average teat diameter at the upper, middle, and lower ends (mm) was 30.19 ± 0.66 (21.11 to 31.64), 27.48 ± 0.55 (18.27 to 25.53), and 20.51 ± 0.31 (12.14 to 21.59). The average teat length was 7.36

Table 1 Mean ± S. E. of morphometric teat parameters of groups 1 and 2 - Sahiwal

Teat Parameter	Group 1	Group 2
UTD (mm)	$29.42^{a} \pm 0.41$	$39.37^{b} \pm 1.06$
MTD (mm)	$23.63^{a} \pm 0.31$	$35.02^{b} \pm 0.27$
LTD (mm)	$19.61^{a} \pm 1.02$	$26.81^{b} \pm 1.74$
TL (cm)	5.75 ± 0.26	6.19 ± 0.19
TTDG (cm)	$42.12^{b} \pm 0.73$	$39.37^{a} \pm 0.83$

Means bearing different superscripts differ significantly ($^{a,b}p<0.05$)
UTD- Upper teat diameter, MTD -Middle teat diameter, LTD- Lower teat diameter, TTDG- Teat tip distance from the ground, TL- Teat length.

Table 2 Mean ± S. E. values of UTD, MTD, LTD to the udder health status of groups 1 and 2 Sahiwal cow teat quarters

Health status	Upper teat diameter (UTD)	
	Group 1 (mm) (n)	Group 2 (mm) (n)
Healthy	$28.50^{ax} \pm 0.64$ (18)	$31.50^{ax} \pm 1.81$(19)
SCM	$30.58^{bx} \pm 0.51$ (21)	$42.88^{by} \pm 0.98$ (43)
CM	$28.55^{ax} \pm 0.95$ (9)	$42.73^{by} \pm 1.91$ (18)
	Middle teat diameter (MTD)	
	Group 1 (mm) (n)	Group 2 (mm) (n)
Healthy	$22.81^{x} \pm 0.61$ (25)	$27.97^{ay} \pm 2.55$ (12)
SCM	$24.06^{x} \pm 0.34$ (25)	$36.47^{by} \pm 1.18$ (39)
CM	$24.27^{x} \pm 0.57$ (16)	$35.78^{by} \pm 2.51$ (11)
	Lower teat diameter (LTD)	
	Group 1 (mm) (n)	Group 2 (mm) (n)
Healthy	$19.20^{x} \pm 0.48$ (27)	$15.04^{ay} \pm 1.32$ (10)
SCM	$20.22^{x} \pm 0.40$ (45)	$29.92^{by} \pm 1.61$ (19)
CM	$19.47^{x} \pm 0.49$ (24)	$34.68^{by} \pm 3.32$ (3)

Means bearing different superscripts differ significantly ($^{a,b,c}p < 0.05$) between rows; ($^{x,y}p < 0.05$) between columns). The parenthesis in each row indicates the number of observations. SCM- Subclinical mastitis, CM- clinical mastitis

Fig. 1 Mean ± S. E. values of thermograms in Sahiwal cows

Means bearing different superscripts differ significantly (p<0.01) ($^{A, B, C}$- USST; $^{P, Q, R}$-TSST) USST- Udder skin surface temperature, TSST- teat skin surface temperature

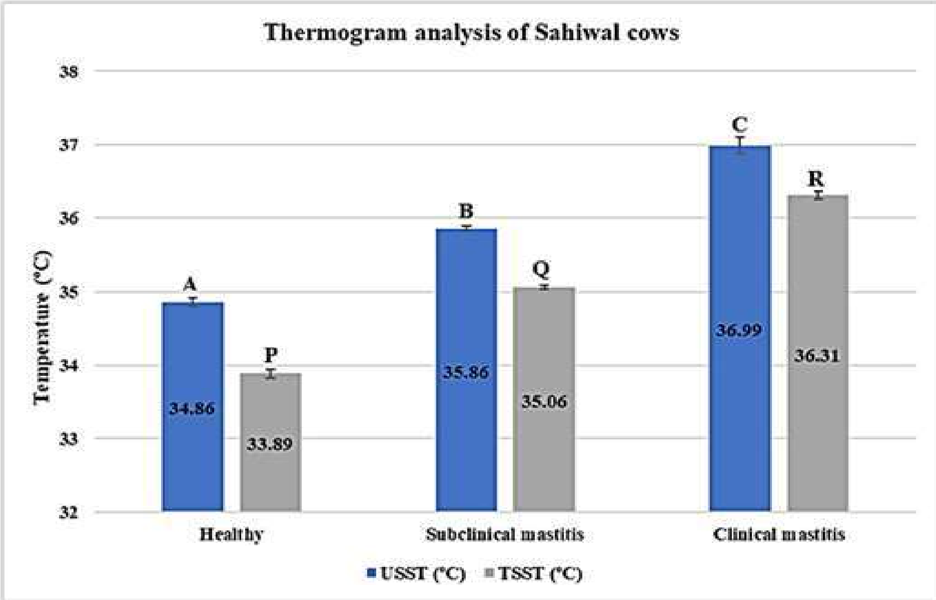
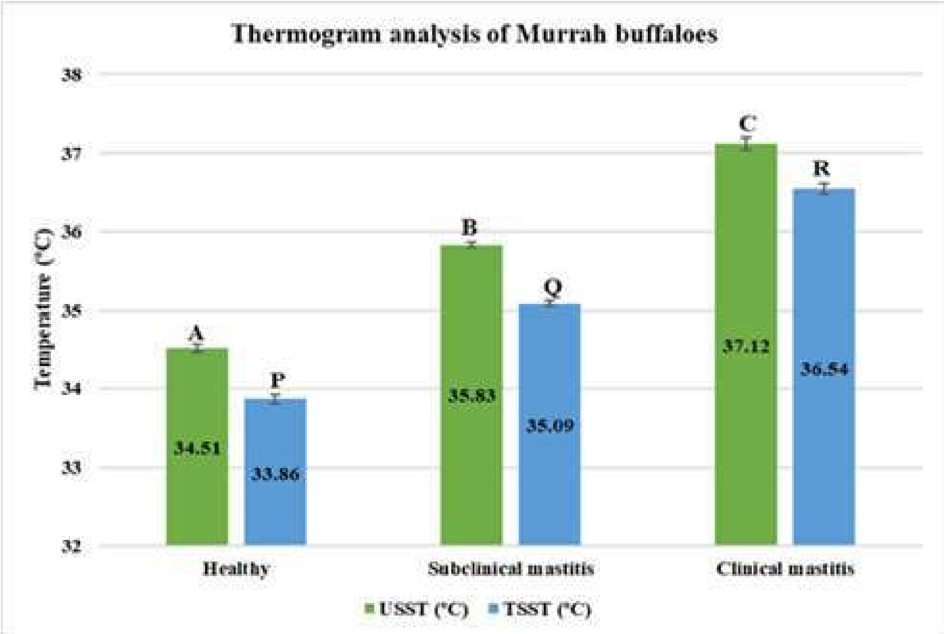


Fig. 2 Mean ± S. E. values of thermograms in Murrah Buffaloes

Means bearing different superscripts differ significantly (p<0.01) ($^{A, B, C}$- USST; $^{P, Q, R}$-TSST) USST- Udder skin surface temperature, TSST- teat skin surface temperature



± 0.17 cm (4.01 to 14.02). The mean values of desirable and undesirable teat lengths obtained were 7.18 ± 0.24 cm and 7.58 ± 0.3 cm, respectively. The average distance from the ground level (milking parlor) to the lower tip of the teat was 44.84 ± 0.57 cm, 87.20 % of which falls under 1SD, 96.8 % in 2SD. The average inner diameter covered by the teat cup liner (2) over the upper end of the teat was 30.34 ± 0.61 mm (Figure 4, 5). The mean values of teat size of both the groups to teat cup liner size showed significant differences (p<0.05) in the upper, middle and lower teat diameters of Murrah buffaloes. The mean values of teat length were comparable in both groups. The mean values of teat tip distance from the ground were similar between group 1 (44.24 ± 0.78 cm) and group 2 (45.59 ± 0.83 cm) Murrah buffaloes (Table 3).

Results of Table 4 revealed that 55 and 70, 78 and 47, and 58 and 67 teats fall under group 2 and group 1, for UTD, MTD, and LTD, respectively. A total of 27.27 and 3.63, 17.94 and 2.56, and 18.96 and 3.44 per cent of the teats were affected in group 2 by SCM and CM-affected quarters UTD, MTD, and LTD, respectively, in Murrah buffaloes. In group 1, all the teats were healthy for UTD and MTD, but for LTD, 5.97 per cent of the teats were affected with SCM. Compared to healthy teats, the upper, middle and lower teat diameters corresponding to the SCM, CM-affected teats showed a significant difference ($p < 0.05$) among group 2 of Murrah buffaloes.

The ROC analysis revealed a higher sensitivity, specificity, and accuracy for USST and TSST parameters for Murrah buffaloes. The cut-off temperature with sensitivity, specificity, and accuracy (95% confidence interval) in the case of USST for SCM was ≥ 35.35 ºC, 0.99, 0.96 and 0.99 (0.99-1) and for CM was ≥ 36.45 ºC, 0.98, 0.98, 0.99 (0.99-1) and that of TSST for SCM was ≥ 34.45ºC, 0.99, 0.96, 0.99 90.99-1) and for CM was ≥ 35.75 ºC, 0.98, 0.98, 0.99 (0.99-1), respectively. The mean udder and teat skin surface temperature of subclinical and clinical mastitis-affected quarters compared to healthy quarters of Murrah Buffaloes showed a significant diffidence ($p<0.05$) between the groups . Compared to healthy quarters, the mean USST and TSST of SCM and CM-affected quarters showed an increase of 1.32, 2.61 ºC, and 1.24, 2.69 ºC, respectively.

The microclimatic evaluation revealed that the Sahiwal cows and Murrah buffaloes were under mild heat stress conditions in the milking parlour (Dash et al. 2016). All the observations about teat morphometry and teat liner of the milking machine were taken in a similar environment of the milking parlour for the respective animals. Research findings have indicated that meteorological factors like humidity and ambient temperature can influence the performance of IRT (Almeida et al. 2022). Consequently, USST and TSST might also be impacted when thermal imaging is conducted in an unregulated environmental setting (Polat et al. 2010). However, in our current investigation, thermal imaging was conducted at morning milking hours within a milking parlour environment with mild heat stress conditions.

Sahiwal cows

The average teat diameter in Sahiwal cows at upper, middle, and lower ends (mm) were 35.64 ± 0.81, 29.15 ± 0.72, and 21.24 ± 0.52. Bello et al. (2023) reported that the average MTD (cm) of the fore teat and rear teat among 2nd, 3rd, and 4th parity of white Fulani cows were 2.11 and 2.01 cm, 2.11 and 1.91 cm, 2.09 and 1.90 cm, respectively. The results obtained regarding MTD were not in line with the observations of Bello et al. 2023. This difference in average MTD may be due to the breed difference. The average teat length observed in the current study was 6.33 ± 0.14 cm, but

Table 3 Mean ± S. E. of morphometric teat parameters of groups 1 and 2 in Murrah buffaloes

Teat Parameter	Group 1	Group 2
UTD (mm)	$26.91^{a} \pm 0.33$	$34.59^{b} \pm 1.19$
MTD (mm)	$22.41^{a} \pm 0.27$	$30.69^{b} \pm 0.62$
LTD (mm)	$17.71^{a} \pm 0.24$	$23.21^{b} \pm 0.28$
TL (cm)	7.18 ± 0.24	7.58 ± 0.23
TTDG (cm)	44.24 ± 0.78	45.59 ± 0.83

Means bearing different superscripts differ significantly ($^{a,b}p<0.05$) between the groups. UTD-Upper teat diameter, MTD -Middle teat diameter, LTD- Lower teat diameter, TTDG- Teat tip distance from the ground, TL- Teat length

Table 4 Mean ± S. E. values of UTD, MTD, LTD to the udder health status of groups 1 and 2 Murrah buffalo teat quarters

Health status	Upper teat diameter (UTD)	
	Group 1 (mm) (n)	Group 2 (mm) (n)
Healthy	$26.76^{x} \pm 0.34$ (70)	$32.76^{ay} \pm 1.18$ (38)
SCM	--	$37.39^{ab} \pm 2.79$(15)
CM	--	$46.97^{b} \pm 8.31$ (2)
	Middle teat diameter (MTD)	
	Group 1 (mm) (n)	Group 2 (mm) (n)
Healthy	$22.39^{x} \pm 0.28$ (47)	$29.74^{ay} \pm 0.58$ (62)
SCM	-- (0)	$32.91^{ab} \pm 2.33$ (14)
CM	-- (0)	$39.23^{b} \pm 3.95$ (2)
	Lower teat diameter (LTD)	
	Group 1 (mm) (n)	Group 2 (mm) (n)
Healthy	$18.06^{x} \pm 0.26$ (63)	$23.13^{y} \pm 0.26$ (45)
SCM	$17.2^{x} \pm 1.65$ (4)	$24.06^{y} \pm 1.17$ (11)
CM	--	25.63 ± 0.26 (2)

Means bearing different superscripts differ significantly ($^{a,b,c}p < 0.05$) between rows; ($^{x,y}p < 0.05$) between columns). The parenthesis in each row indicates the number of observations. SCM- Subclinical mastitis, CM- clinical mastitis

Oshin et al. (2022) reported the average teat length of Sahiwal cows in their experiment was 7.82 ± 0.19 cm. This may be due to the individual animal difference in the teat length. Similar to the observations of the present study, Sinha et al. (2022) reported the average teat length of fore and rear teat among the healthy and mastitis-affected groups as 6.75 and 7.26 cm; 5.21 and 8.93 cm among the Sahiwal cows. Also, the authors reported the average teat diameter of healthy and mastitis-affected groups as 2.58 and 2.69 cm, respectively. The average shortest distance from the fore teat end to the floor for healthy and mastitic groups was 42.38 and 41.83 cm, respectively, and that of the rear teat was 43.51 and 41.98 cm, respectively, in Sahiwal cows. These findings were in line with the present study's observations.

In the current study, the variability in UTD relating to teat morphometry in group 2 is due to the different teat shapes among the Sahiwal cows. The teat diameter influences the incidence of SCM and CM (Gleeson et al. 2004). Danish et al. (2018) reported several teat shapes in Sahiwal cows such as cylindrical, funnel, bottle, and pear-type with the highest frequency of teat shapes being funnel-shaped. Authors also reported that the shorter-sized teats are more prone to mastitis than the lengthier ones because the short teat canal enables the quicker movement of the microbes than the larger ones. Saleh et al. (2023) reported the average MTD of Friesian cows as 2.74 cm which was lower than the average MTD of Sahiwal cows in the present study. The variation in the teat diameters in the present study in comparison to other reports may be due to the variations in experimental animals in terms of breed status, milk flow rate and teat wall thickness. Sunder et al. (2022) evaluated teat measurement parameters such as overall teat diameter (OTD), diameter at the level of Furstenberg (FTD), teat canal length (TCL), teat wall thickness (TWT), and cistern diameter (CD). Results in their study revealed that OTD in mastitis-affected quarters was significantly more than in healthy quarters. A significant correlation ($p<0.01$) of OTD was manifested with FTD and TWT. The most probable causes of teat end trauma during the milking process are poorly fitting cup liners, excessive temporary vacuum losses, lack of vacuum reserve, hasty removal of the milking unit without closing the vacuum, and disproportionate milking vacuum. Thus, an increased teat-end callosity paves the way for a surface to which bacteria can easily adhere. The present study did not focus on the teat wall thickness and its associated effect on udder health status. However, these findings are also applicable to the present study as the intramammary infection (IMI) status was higher among group 2 rather than group 1.

In group 1, 43.75 and 18.75 per cent of udder were affected by SCM and CM, respectively, in the present study. However, these values were less than the group 2 teats of Sahiwal cows. This could be due to the variation in the teat end shape, steak canal, and teat orifice along with the smooth muscle sphincter and length of the central ligament. The Sahiwal breed, widely acknowledged as an indigenous dairy cattle breed in tropical and subtropical regions, exhibits a remarkable genetic capacity for sustained milk production throughout its productive lifespan. This breed can thrive in significant temperature variations and demonstrates resistance to parasites (Ilatsia et al. 2012). Despite the inherent lower susceptibility of indigenous cows to mastitis, their intensive selection for increased milk yield has rendered them more vulnerable to mastitis. In addition, machine milking blips induce changes in the teat dimensions and cause changes in the teat tissues, such as hyperkeratosis and congestion, which could be another reason for IMI.

In accordance with a research investigation carried out by Polat et al. in 2010, the sensitivity and specificity values of USST were reported as 95.6 % and 93.6 %, respectively, yielding an accuracy rate of 98.5 %. These outcomes were achieved by implementing a threshold of >34.7 ºC in combination with SCC exceeding 400 x 10^3. Similarly, Gayathri et al. (2023) reported sensitivity, specificity and accuracy values of pre-milking TSST as 0.96, 0.95 and 0.99 at a cut-off value of ≥36.5 ºC for CM, respectively and that of SCM was 0.95, 0.92 and 0.98 at a cut-off value of ≥35.8 ºC, respectively. These findings were in line with the results of the current study, thereby further substantiating the potential efficacy of thermographic parameters in the identification of udder health status in Sahiwal cows. The degree of increment in CM cases of USST in the present study was in line with the observation reported by Metzner et al. (2014) in dairy cows (2.06°C) by *E. coli* infusion in the udder. Reports have also revealed an increment in USST during SCM to the tune of 0.8°C in KF cows by Sathiyabarathi et al. (2018), which were concurrent to the present study.

Murrah Buffaloes

The average teat diameter at the upper, middle, and lower ends (mm) were 30.19 ± 0.66, 27.48 ± 0.55, and 20.51 ± 0.31. The average teat length of Murrah buffaloes for the present study was 7.36 ± 0.17 cm, but Poudel et al. (2022) reported 8.46 ± 0.17 cm among the Murrah buffaloes of Chitwan district, Nepal. Also, the authors reported the mean teat diameter of Murrah buffaloes as 3.59 ± 0.17 cm, which was higher than the present study. Similarly, Chandrasekar et al. (2016) reported the average teat length of Nili-Ravi buffaloes for left fore quarter, left rear quarter, right fore quarter, and right rear quarter as 3.89 ± 0.11, 4.03 ± 0.17, 3.93 ± 0.17, 4.13 ± 0.30.19 cm, respectively, which was lower than the present study. In line with the present study, Prasad et al. (2010) reported that the average teat diameter for Murrah buffaloes was 2.76cm. The authors reported a significant variation in the mean teat diameters of bowl, globular, goaty, and pendulous shapes with respect to the udder of Murrah buffaloes as 2.76, 2.75, 2.60 and 2.93 cm, respectively. The average teat length of the bowl-shaped teat concerning the udder was 7.33 cm, which was in line with the current study rather than with globular (7.80 cm), goaty (8.98 cm), pendulous (2.84 cm) teats of Murrah buffaloes.

In the current study, all the teats were healthy in group 1 of UTD and MTD but LTD showed SCM (5.97 %), indicating that liner size is optimum in Murrah buffaloes. In group 2, irrespective of the region of the teat diameter, the IMI status was higher compared to group 1. The variation in the diameter of these teats could be due to the difference in teat shape and thickness, leading to the risk of IMI. The various shapes found in Murrah buffaloes are cylindrical, funnel, bottle, conical and pear-type teat shapes, with the cylindrical shape being the most frequently observed (Danish et al. 2018). In addition, the variation in liner size of the milking machine could impede the proper function of the vacuum, disrupting blood and lymph circulation. It can result in unnatural strain on the teat tissue, causing pain and triggering immune reactions in lactating animals. These artificial physiological and pathological changes in the circulatory system can harm the teat ends, reduce the effectiveness of the udder's defence mechanisms (Gleeson et al. 2004), and potentially lead to mastitis. These changes in teat condition can be categorized into short-term, medium-term, and long-term. Short-term changes caused by circulatory disorders include teats becoming discoloured and swollen and exhibiting openings in the teat canal, as reported by Vermaak et al. (2022). These short-term changes can be reversed from one milking session to another or within a few hours. Recent findings from Holst et al. (2021) demonstrated that round liners outperformed triangular ones regarding higher milk flow and shorter milking time. It indicates that round liners are better for the health and condition of the teats. In the present study, round liners were used in the milking machines for dairy animals. Also, the best-fitting group of Murrah buffaloes with respect to teat liners were free of mastitis in the current investigation. So, an effective teat cup liner seals both ends of the shell completely airtight and provides a mouthpiece and shaft that are appropriately sized to accommodate a range of teat shapes and sizes, minimizing liner slippage, cluster dropping, and potential damage that could lead to mastitis (Kaskous and Pfaffl, 2023). An efficient teat cup liner makes milking as quick and thorough as possible, reducing congested teats, discomfort, and potential injury (Bobic et al. 2018). It is easy to clean and maintain its integrity over time without significant changes. Therefore, the design of teat cup liners must be tailored to the specific characteristics of each breed.

In line with the current study, Gayathri et al. (2024) observed the sensitivity, specificity and accuracy of pre-milking USST for SCM as 0.95, 0.92 and 0.98, respectively, at a cut-off value of ≥35.4°C and for CM, it was 0.95, 0.91 and 0.94, respectively, at a cut-off value of ≥36.4°C in Murrah buffaloes. The authors also reported the sensitivity, specificity and accuracy of pre-milking TSST for SCM were 0.93, 0.92 and 0.97, respectively, at a cut-off value of ≥35.1°C and for CM, it was 0.88, 0.87 and 0.95, respectively, at a cut-off value of ≥36.2°C in Murrah buffaloes. In addition, the increment in temperature observed in thermogram analysis of the current study in Murrah buffaloes was in line with the observations reported by Zaninelli et al. (2018) in Holstein Friesian cows to the tune of 1.89 °C for CM cases and that for SCM cases, the results were following the reports of Bolanos et al. (2021) in Holstein cows to the tune of 0.4-1.1°C.

The present study indicates that the best-fitting group have less IMI compared to the normal group of Sahiwal cows and Murrah buffaloes, reflecting the highest milking efficiency. Thus, the current study assesses the teat size fitting to the teat liners of milking machines for indigenous dairy breeds of Sahiwal cows and Murrah buffaloes maintained in an organized dairy farm.

Conclusion

The milk production and productivity enhancement in the dairy sector is technology-driven. The milking machine is a game-changer device applied in the dairy sector to boost profitability. The present study indicates the variability in the teat size, regarding the upper, middle and lower teat diameters of the Sahiwal cows and Murrah buffaloes. The intramammary health status of the best-fitting group in terms of teat size compared to the teat cup liner of the milking machine was better than the normal groups. Thus, addressing variations in teat size and optimizing teat cup liner fitting with milking machines, offers promising prospects for enhancing milking efficiency and promoting better animal welfare standards in Sahiwal cows and Murrah buffaloes.

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Conflict of Interest

The authors have no conflict of interest.

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RESEARCH ARTICLE

Mineral status of soil, water, fodder, buffalo's milk and blood of Meerut District in Western Uttar Pradesh

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Abstract: A total of 140 feed samples, 120 fodder samples, 105 water samples, 58 milk samples, 51 blood samples and 29 soil samples were collected randomly from all the 12 blocks of Meerut districts in western Uttar Pradesh. After processing, the samples were analysed for different macro and micro elements by Inductively Coupled Plasma (ICP) instrument (instrument name MY2015CQ05 of Agilent Technologies). Among the feeds, wheat bran was found deficient in copper by 15%. Both copper and zinc content in Mustard oil cake was found marginal. Copper was deficient in bajra fodder, berseem fodder and jowar fodder by 7.5%, 30% and 29.3%, respectively. Sugarcane top, bajra fodder, berseem fodder and jowar fodder were found deficient in zinc by 7.3%, 14.67%, 5% and 2%, respectively. Copper and zinc were also found deficient in blood sample by 15.38% and 1.25%, respectively whereas animal milk samples were deficient in copper by 66.67%. It was found that most of the feed, fodders, blood, milk and soil samples were found deficient in copper and zinc content. Macro minerals were not found deficient.

Keywords: Buffalo, Mineral, Meerut, Sample

Introduction

The macro-minerals include calcium, phosphorus, sodium and chloride, while the micro-elements include iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, fluoride, chromium, selenium and sulphur. (Soetan et al. 2010)

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The ultra-trace elements include silicon, boron, arsenic and nickel which are found in animals and are believed to be essential for these animals (Soetan et al. 2010). The presence of mineral elements in animal feed is significant for the animal's metabolic processes Mineral deficiencies or imbalances in soils and forages report to some extent for low animal production and reproductive problems. Plants withdraw essential elements from the soil solution in quantities to satisfy their own requirement as well as satisfy many of the requirements of grazing livestock (Fadlalla, 2022) The trace elements are key constituents of enzyme systems, hence, deficiencies of mineral elements have intense effects on metabolism and tissue structure (Soeton, et al. 2010). Minerals play a crucial role in nutrient utilization and a number of other biochemical functions concerning production and reproduction. The availability of minerals to cattle depends upon the production system, feeding practices and environment (Gupta et al. 2017). These resources do not always supply all the needed mineral requirements of dairy animals (Shekhar et al. 2020). The studies have been conducted in different parts of the country for ASMM preparation (Hedge et al. 2016 and Hedge et al. 2016). In the Indian context, there is widespread deficiency of Zn and Cu (Datt and Chhabra, 2005) In Meerut district, no such work has been conducted earlier. So, a project was taken up to formulate the area specific mineral mixture for Meerut district of Uttar Pradesh.

Materials and Methods

Samples of animal feed, fodder, water, milk, blood and soils were collected randomly from all the 12 blocks of Meerut districts in western Uttar Pradesh (Table 1).

Processing and storage of feed samples

Digestion of all kinds of feed samples (concentrate ingredients, fodder, concentrate mix etc.) was done by following process of digestion. About 1 g of previously ground and stored feed sample was taken in Kjeldahl digestion flasks and 15 mL of tri-acid mixture (HNO3: H2SO4: HClO3 = 3: 1: 1) was added to it and the contents were allowed to stay overnight. After that, flasks were heated on digestion bench. Indication of the end of digestion was disappearance of white fumes of perchloric acid and disappearance of black particles. The digested samples were filtered through Whatman filter paper No. 1. Repeated washing of digestion vessel and filter was done by triple distilled water. The digested samples were diluted to 25 mL final volume.

Processing and storage of soil samples

Soil samples were collected from 15 cm depth by using auger. Approximately 50 gm of soil was collected. Available phosphorus, available potassium and cationic micronutrients (Fe, Mn, Cu and Zn) in soil samples were extracted with a diethylene 30 triamine pentaacetate (DTPA) solution (0.005 M) DTPA+0.01 M $CaCl_2$ +0.1 Mtriethanolamine, pH 7.3 (Lindsay and Norvell, 1978). The final volume of filtrate was made to 50 mL by triple distilled water.

Processing and storage of milk, water and blood samples

Samples of blood and milk were collected from both organized and unorganized dairy farms. Samples of water were collected from tube well, hand pump, borings, canal, ponds and river using plastic bottles of 100 mL capacity. Milk (20 mL) was measured by an acid washed measuring cylinder. Digestion was done by above mentioned process. The final volume was made to 40 mL by triple distilled water. For blood sample, 5 mL of plasma sample was digested by adding equal volume of acid. Milk and blood samples are to be digested as soon as possible before spoiling.

Estimation of minerals

Important macro and micro minerals were estimated by Inductively Coupled Plasma (ICP) instrument (instrument name MY2015CQ05 of Agilent Technologies). Conditions of instrument were Read time (s) 5, RF power (KW) 1.2, Stabilization time (s) 10, viewing mode axial viewing height (mm) 8, nebulizer flow (L/min) 0.7, plasma flow (L/min) 12, aux flow (L/min) 12, aux flow (L/min)1, Makeup flow (L/min) 0, Oxygen (%) 0

Statistical analysis

Data were analyzed as per the standard statistical procedure statistically (Snedecor and Cochran, 1994) using IBM Statistical Package for the Social Sciences (IBM SPSS, version 20). Significant differences ($P<0.05$) among treatment means and separation of homogenous subset was determined as per Duncan's multiple range test (Duncan, 1955). The analyzed data were considered to be significant at $P<0.05$ and declared as trend at $0.05 <P \leq 0.10$.

Results and Discussion

Mineral profile of feed stuffs in Meerut district of Uttar Pradesh

Mineral profile of different locally available feed stuffs samples i.e., fodders (green and dry), concentrate ingredients, compound feed (locally prepared and commercially available) collected in polythene bags from the owners of animals has been presented in Table 2. Wheat straw was found deficient of P, Cu, K, Mg and Zn. Whereas wheat bran was found deficient in Cu and Mo. Bajra fodder was deficient in Cu and Zn. Sugarcane top was deficient to the extent of 3.3% in Ca, 56% in P, 35% in Mg,7.3% in Zn, 14% in Mn, 69.34% in Mo, 46.67% in Se, respectively. Berseem fodder was found to be deficient in Na, Cu and Zn to a level of 66.67, 30 and 5%, respectively. Jowar fodder was found deficient in Mg, Cu and Zn @ 5, 29.3 and 2%, respectively. The values were compared with recommended critical level given by researcher (Liu et al. 2021). Deficiency of Zn in wheat straw may be due to long term cropping and increased crop yield

Table 1: Details of samples collected from different blocks of Meerut district

Block	Number of samples					
	Feed	Fodder	Water	Milk	Blood	Soil
Daurala	12	08	07	04	04	02
Hastinapur	10	09	08	05	05	03
Jaani	13	10	09	07	06	02
Kharkhoda	11	07	10	03	04	03
Machhara	09	11	11	02	03	02
Mawana	13	12	07	06	04	02
Meerut	14	13	09	07	05	03
Parikshitgarh	10	10	09	07	03	02
Rajpura	13	11	10	06	04	03
Rohta	10	08	10	04	06	02
Sardhana	11	11	07	03	03	03
Saroorpur	14	10	08	04	04	02
Total	140	120	105	58	51	29

Table 2. Mineral profile of feeds and fodders in Meerut district of western Uttar Pradesh

Ingredients	No. of samples	Ca%	P %	Na %	Mg %	K%	S %	Cu ppm	Zn ppm	Feppm	Mn ppm	Co ppm	Cr ppm	Mo ppm	Se ppm
Critical level*		0.30	0.25	0.06	0.20	0.60	0.20	8.00	30.0	50.0	40.0	0.10	1.00	0.75	0.15
Wheat Bran	52	0.48± 0.01	0.63 ± 0.01	0.09± 0.01	0.30± 0.01	0.71± 0.02	1.29± 0.02	6.80± 0.06	34.9± 0.15	251.1±0.64	60.9± 0.26	0.14± 0.01	1.20± 0.02	0.53± 0.01	0.21±0.01
MOC	55	0.48±0.01	1.09±0.02	0.59±0.02	0.22±0.01	1.11±0.02	2.06±0.03	8.10±0.07	32.3±0.16	196.8±0.58	40.0±0.15	0.29±0.01	1.90±0.02	0.60±0.01	0.55±0.01
Concentrate/ Pellet	33	0.60±0.01	1.37±0.02	0.89±0.03	0.30±0.01	1.08±0.02	0.38±0.01	22.30±0.12	143±0.49	422.9±2.18	94.7±0.35	0.36±0.01	8.47±0.19	0.96±0.02	0.56±0.01
Wheat straw	24	0.33±0.02	0.16±0.01	0.15±0.01	0.12±0.01	0.15±0.01	1.13±0.09	3.70±0.14	11.5±0.21	175.0±0.51	65.0±0.24	0.13±0.01	0.31±0.03	1.03±0.09	0.23±0.04
Jowar fodder	26	0.48±0.04	0.51±0.04	0.35±0.03	0.19±0.02	1.73±0.08	3.36±0.11	5.66±0.14	29.4±0.18	478.1±1.95	41.7±0.25	0.18±0.02	3.68±0.09	0.90±0.05	0.80±0.04
Sugarcane Top	22	0.29±0.01	0.11±0.01	0.08±0.01	0.13±0.01	1.04±0.02	3.17±0.03	8.50±0.06	27.8±0.16	495.7±2.15	34.4±0.21	0.15±0.01	3.78±0.04	0.23±0.02	0.08±0.01
Bajra fodder	22	0.69±0.02	0.25±0.01	0.09±0.01	0.32±0.01	1.13±0.02	3.19±0.15	7.40±0.21	25.6±0.19	543.0±3.21	40.6±0.26	0.18±0.01	4.36±0.15	1.25±0.02	0.42±0.01
Berseem fodder	26	1.26±0.02	0.40±0.01	0.02±0.01	0.39±0.01	4.69±0.17	2.28±0.15	5.60±0.21	28.5±0.15	412.0±1.98	52.0±0.29	0.16±0.01	4.39±0.18	0.98±0.02	0.74±0.01

Table 3. Mineral profile of soils, blood, milk and water in Meerut district of Uttar Pradesh

Item	No. of samples	Ca	P	Na	Mg	K	S	Cu	Zn	Fe	Mn
Blood	51										
Unit		mg/ dl	mg/ dl	Mmol/L	mg/ dl	Mmol/L	g/dl	mg/L	mg/L	mg/L	mg/L
critical value		8.0	4.5	120	2.0	3.0	0.09	0.65	0.80	1.10	0.20
Observed value		10.75± 0.12	6.3± 0.11	134± 0. 35	4.1± 0.09	5.25± 0.08	0.11± 0.01	0.55± 0.02	0.79± 0.02	2.76± 0.06	1.08± 0.05
Milk	58										
Unit		g/L	g/L	g/L	g/L	g/L	g/L	mg/L	mg/L	mg/L	mg/L
Critical value		1.2	1.0	0.5	0.1	1.5	0.2	0.15	4.0	0.5	0.03
Observed value		1.89± 0.05	0.97± 0.03	0.41± 0.01	0.09± 0.01	0.12± 0.01	0.21± 0.01	0.05± 0.01	4.8± 0.09	1.25± 0.05	0.13± 0.01
Water	105										
Unit		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L
Observed value		12.32± 0.20	5.25± 0.69	16.89± 0.28	11.8±0.21	4.25± 0.16	3.01±0.11	550± 0.71	2560± 4.5	276± 0.45	156± 0.25
Soil	29										
Unit		ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Observed value		63.5± 4.01	7.89± 3.25	15.02± 9.25	15.8± 5.69	0.85± 1.65	7.33± 0.58	0.67±0.01	1.97± 0.04	34.7± 0.18	16.67± 0.28

(Naliyapara et al. 2023). Cu deficiency in feeds and fodder was reported by Das et al. (2003) in West Bengal. Similarly, Sharma and Joshi (2004) found Cu deficiency in feeds and fodder in hilly region of Garhwal division of Uttarakhand. Cu deficiency in wheat straw (Kalwani et al. 2023) and wheat grain (Kalwani et al. 2023; Chatterjee et al. 2011) were also observed. In many geographical zones of India, Zn is found to be deficient (Demir et al. 2023). Similarly, in our experiment, wheat straw was found low in Zn content. The concentration of Fe was found high in all analyzed feedstuffs. Similar findings were also reported in other parts of country like Karnataka (Shekhar et al. 2020), Haryana (Panwar et al. 2022) and Haridwar (UK) (Tiwary et al. 2007). Bhagat et al. (2017) found deficiency of Zn in feeds and fodder in Sindhudurg district of Maharashtra. The average Cu content was observed low in straws, green fodders and concentrate ingredients in Bharatpur district of Rajasthan (Garg et al. 2008). They also observed deficiency of Zn in most of the feedstuffs and suggested supplementation in the ration of animals for proper productive and reproductive functions. Gupta et al. (2016) also observed that wheat straw, wheat grain, barley and mustered cake were deficient in Cu and feeds contained more Fe than critical level Hedgg et al. (2016) concluded that soils were deficient in Zn and plants were deficient in Zn, Cu and Co while cattle were deficient in all the minerals. Hedge et al. (2018) found that the soils were deficient in Zn and plants were deficient in Zn, Cu and Co while cattle were deficient in all the minerals. Hedge et al. (2018) found that soil, plants and dairy cattle were deficient for majority of macro minerals.

Mineral profile of soil, blood, milk and water in Meerut district of Uttar Pradesh

Mineral profile of soils, blood, milk and water has been presented in Table 3. Level of major and trace mineral indicated deficiency of 3% P, 18% Na, 10% Mg, 92% K and 66.67% Cu in milk and 15.38% Cu, 1.25% Zn in blood, respectively. The levels of Ca, P, Mg and S water samples were 12.32 ± 0.20, 5.25 ± 0.69, 11.89 ± 0.21 and 3.01 ± 0.11 mg/L, respectively in water samples collected from different locations of Meerut district. Cu, Zn, Fe and Mn content in water samples were found to be 550 ± 0.71, 2560 ± 4.5, 276 ± 0.45 and 156 ± 0.25 µg/L, respectively while soil samples contained Ca, P, Mg and S at levels of63.59 ± 4.01, 7.89 ± 3.25, 15.89 ± 5.69, 7.33 ± 0.58 ppm, respectively. Cu, Zn, Fe and Mn content in soil samples were found to be0.67 ± 0.01, 1.97 ± 0.04, 34.76 ± 0.18, 16.67 ± 0.28 ppm, respectively. The values were compared with recommended critical level (Gupta et al. 2019). Blood plasma levels of different minerals were found above the critical level except in case of Cu and Zn. Low dietary Cu in feed stuffs than requirement of animals leads to low Cu concentration in blood serum (Demir et al. 2023). Hypocuprosis in animals might be attributed to copper deficiency in feedstuffs. Shekhar et al. (2020) observed lower Cu concentration in blood plasma in anoestrous cows. Present finding is in accordance with Udar et al. (2003), who reported Cu deficiency in cattle of Vidharbha region of Maharashtra. High Fe content in fodder samples could be another possible reason for low level of Cu in blood plasma as Fe interferes in copper metabolism in the body. Fe deficiency is rarely observed in animals because Fe is quite abundant in all feeds (Demir et al. 2023). The average concentrations of plasma Fe (ppm) in animals' plasma samples were found above the critical limit of 1.1 mg/L as suggested by Gupta et al. (2019). Panda et al. (2015) observed that cattle blood serums were severely deficient in Ca, Zn, and Cu and marginally deficient in P and Mn. Our present study showed 66.67% Cu deficiency in milk samples. Kumar et al. (2011) conducted a survey to find the status of copper, zinc and iron in milk and hair of animals of all the districts of Haryana state where deficiency of zinc and copper were observed in milk samples. Critical concentration of Ca, Mg, P, Cu, Zn and Iron was reported as 71, 9.10, 5, 1, 1.50 and 20 ppm, respectively (Liu et al. 2021). In our present experiment, we found deficiency of Cu in soil. There was marginal deficiency of Ca. Fe content was found higher than the critical concentration. Similar to our observation, Bhagat et al. (2017) found deficiency of Ca and Cu, whereas P, Mg, Zn and Fe contents were found to be adequate in non-irrigated and irrigated region of Sindhudurg district of Maharashtra. Gupta et al. (2019) indicated that P, K, Ca, Zn and Mg were moderate in the soils, where Fe and Cu levels in all the soils were adequate in Banswara district of Rajasthan. Yatoo et al. (2011) showed that soil has adequate Cu, Fe and Co but deficient in Zn. Panda et al. (2015) found all the macro and micro minerals of the soil was below the critical level except Fe at Kashmir valley. Sarma et al. (2015) investigated that the soil Ca, P and Mg were found to be below critical levels; but Cu, Zn and Fe were optimum in all studied locations at Mizoram.

Conclusion

Most of the feed, fodders, blood, milk and soil samples were found deficient in copper and zinc content. Macro minerals was not found deficient.

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RESEARCH ARTICLE

Growth performance and economics of feeding Soymilk in Murrah buffalo calves

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Abstract: This research was designed to evaluate the effect of Soymilk on growth and economics of feeding in Murrah buffalo calves. Total 15 buffalo calves were randomly divided into three treatment groups. Whole milk/dam's milk fed experimental calves were taken in control group. The experimental groups consist of Whole milk and Soymilk in 75:25 and 50:50 ratio. Standard feeding and management practices were followed throughout the experimental period. To assess the efficacy of Soymilk feeding on the growth performance, body weight and body measurements (body height, heart girth, abdominal girth and body length) were recorded at the start of the experiment and thereafter at fortnightly interval. The results of the study indicated that body weight parameters (average body weight, body weight gain, cumulative body weight gain and average daily body weight gain) and body measurements parameters (body height, cumulative body height gain, heart girth, cumulative heart girth gain, abdominal girth and cumulative body length gain) did not differ significantly among the control group and Soymilk fed treatment groups. The economics of feeding calculated at the end of experiment revealed that net profit in total feeding cost/kg weight gain was Rs. 106.92 and 130.35 for 25 percent and 50 percent Soymilk replacement group, respectively. It can be concluded that Soymilk has similar effect on growth performance of calves as whole milk and more economical than whole milk feeding, therefore Soymilk may possibly replace whole milk up to 50 percent level.

Keywords: body measurements, growth performance, economics, Soymilk, whole milk.

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Introduction

The largest contributor to the country's agriculture Gross Domestic Product (GDP) is dairy industry (Singh, 2015). Calves are the herd's future and the economical upbringing of calves is essential to the dairy industry's success (Sorathiya et al. 2019). Feeding and management of calves during the early stages of life have a significant impact on their future output. The first three to four month of a calf's life is the most crucial period and adequate balanced feeding is required for optimum growth during this phase. Feeding of milk to calves is crucial because it provides the necessary nutrients for tissue development (Kertz et al. 2017).

It is expensive to provide whole milk to calves for Indian farmers and feel burden to do so because they must sell their milk to people for a living (Shakya et al. 2017). This causes the calves to develop slowly, take longer to mature and have a dismal future in terms of productivity. For availability of milk for sale, milk replacer finds a very important role for dairy farmers. According to Khan et al. (2012), milk replacers are any feed ingredients or a combination of such elements that can be used to replace whole milk in a calf's diet. Milk replacer (MR) is a constituted feed consisting almost similar nutritional value as that of whole milk and cheaper than milk so that it gives economic benefit too (ICAR, 2013). There are many factors associated while choosing a milk replacer such as form, composition and cost effectiveness of milk replacer.

Soybean is the best source of plant protein containing about 40% of crude protein (dry basis) and rich in nutritive minerals and dietary fiber (Giri and Mangaraj, 2012). Soymilk is a white emulsion which resembles buffalo milk in both appearance and consistency (Yadav et al. 2018) and is prepared by soybean (*Glycine max*) seed. When compared with whole milk, it is a cheap source of protein and calories that can be utilized to effectively replace whole milk in developing nations to address the issue of malnutrition (Mazumder and Begum, 2016). Soymilk is very nutritious and a great source of high-quality proteins, B vitamins, and isoflavones and it is lactose-free (Fahmida, 2018) and also rich in iron, unsaturated fatty acids, and niacin, but low amounts of fat, carbohydrates, and calcium as compared with cow milk (Mazumder and Begum, 2016). Soybean protein is comparable to milk protein in terms of suckling calves' growth performance when used as a protein source for milk replacers (Huang et al. 2015). The partial or total replacement of milk proteins with soy proteins in milk replacers does not impair calf performance and significantly improves the economic efficiency of calf diets (Ansia and Drackley, 2020). Various studies revealed that replacing soymilk for whole milk while feeding calves led to improved growth performance (Bartlett et al. 2006; Ghorbani et al. 2007; Masum et al. 2009; Roy et al. 2016 and Fahmida, 2018).

Therefore, the present study is envisaged to assess the efficacy of Soymilk feeding on growth performance and economics of feeding in Murrah buffalo calves.

Materials and Methods

The present investigation was carried out with prior approval by the Institutional Animal Ethics Committee (IAEC) Vide No.: VCC/IAEC/2022/1624-51, Dated: 10-05-2022 at buffalo farm of Department, Lala Lajpat Rai University of Veterinary and Animals Sciences, Hisar for a period of three months.

Experimental Design

For this experiment, a total 15 Murrah buffalo calves at the age of 5 days were randomly divided into three treatment groups, having 5 buffalo calves in each treatment on the basis of similar body weight and sex. In T_1 treatment group (control group) calves were reared on Whole milk while in T_2 and T_3 groups calves were reared on Whole milk and Soymilk at 75:25 and 50:50 ratio, respectively. The experimental animals were kept under loose housing pens with proper space in open and covered area. Feeding and general management practices remain the same in all the treatment groups except milk feeding. Milk feeding of the calves was done at around 5 am in the morning and at around 5 pm in the evening by using the milk feeding bottles. Whole milk was fed to T_1 group while in T_2 and T_3 group Whole milk and Soymilk was given at 75:25 and 50:50 ratio, respectively. During the entire study period, the calves were given balanced diet in form of green fodder, dry roughage and concentrate mixture to meet their dietary requirements for growth following standard feeding management. Buffalo calves were given ad lib fresh water throughout the experimental period. Before formulation of rations, the feed ingredients used in the diet formulations were analyzed for the proximate composition by adopting method of AOAC (2012).

Preparation of Soymilk

For this study, good quality soybean seeds were procured by the department and extraneous materials in soybean seeds were manually removed which were later analyzed for proximate composition. Then Soybean seeds were cleaned by washing with fresh water and after washing were soaked in water 4-5 times of their weight for 24 hours and water was changed after every 12 hours. Soaked soybean seeds were dehulled manually by rubbing between both palms and soaked and dehulled soybeans were dried overnight in hot air oven at 60^{O}C temperature. Dried soybeans were grinded in mill and then procured grinded soybeans were sieved by using 2mm sieve to obtain soypowder. Soypowder was stored in airtight container in hygienic condition. For preparation of one litre soymilk, 150 gram soypowder was dissolved in 1000 ml of luke warm water. With the help of stirrer, continuous stirring was done to mix soypowder properly with water and later it was strained with fine muslin cloth. Thereafter, this Soymilk was mixed with the Whole milk as per the treatment groups.

Parameters studied

The body weight of buffalo calves was taken at beginning of the experiment and thereafter at fortnightly intervals. The body weight was recorded in the morning by using digital weighing balance. Average body weight gain, average daily body weight gain and cumulative body weight gain over the experimental period were calculated for each treatment. Body measurements (body height, heart girth, abdominal girth and body length) of the experimental buffalo calves were recorded at the beginning and thereafter at 15 days intervals during the experiment with the help of measuring tape/rod on inch scale. The cumulative body measurements were calculated for each treatment through the course of experimental period. Cost of whole milk, feed ingredients (including price of dry roughage, green fodder, concentrate and soybean), prevailing at the time of purchase were collected from the university.

The means of data obtained from the study were compared by one way analysis of variance (ANOVA) as per the methods described by Snedecor and Cochran (1994). The data was analyzed using "SPSS" software (version-23). The mean differences among different treatments were separated by Duncan's (1955) multiple range tests. Consequently, a level of significant ($P<0.05$) was used as the criterion for statistical significance.

Results and Discussion

Body weight

The results of Soymilk feeding on body weight parameters (average body weight, average body weight gain, cumulative body weight gain and average daily body weight gain) of experimental calves have been shown in Table 5, 2, 6 and 3. The perusal of tables revealed that the body weight parameters i.e., average body weight, average body weight gain, cumulative body weight gain and average daily body weight gain of all the experimental buffalo calves were observed to be statistically non-significant among Whole milk and Soymilk fed treatment groups through the course of the experiment. The findings of body weight indicated that Soymilk feeding did not compromise the growth of experimental animals over the experimental period.

Analogous to our findings, Masum et al. (2009) reported statistically similar total average body weight gain and average daily weight gain of calves fed on cow milk, strained Soymilk and unstrained Soymilk. Masum et al. (2011) observed non-significantly different total weight gain of calves fed on whole milk and vitamin- mineral fortified Soymilk. Similar trend was also reported by Roy et al. (2012) and Huang et al. (2015) for body weights and average daily gain. The present study can be compared with Sarker et al. (2015) who reported that Black Bengal kids fed on whole milk alone and Soymilk at 25 and 50 percent level in milk replacers had similar total live weight gain. Shakya et al. (2017) revealed that the final body weight and average daily body weight gain of buffalo calves fed on whole milk and a milk replacer in which whole milk was replaced with Soymilk at 20 percent level did not differ significantly. Similarly, Yadav et al. (2018) found statistically similar fortnightly body weight (kg) and average daily weight gain (g/d) in the calves fed whole milk and fortified Soymilk. Fahmida (2018) also observed increased but statistically non-significant body weight and average daily gain in calves among groups fed Soymilk at 25 and 50 percent levels and whole milk fed treatment group. Toukourou and Moubarack (2021) concluded that use of Soymilk as a dietary supplement during the pre-weaning period has proved to be a promising strategy to improve the weight performance.

Table 1: Proximate composition (% DM Basis) of feed ingredients fed to experimental buffalo calves

Ingredients	DM%	CP%	CF%	EE%	Ash%	OM%
Wheat straw	90.00	3.00	35.46	1.02	12.97	87.03
Green fodder	27.43	6.12	23.50	6.50	6.50	93.50
Maize	88.08	9.01	2.80	3.56	1.75	98.25
Ground Nut cake (GNC)	91.47	42.70	9.15	7.68	7.80	92.20
Wheat	88.61	10.89	3.77	2.15	2.23	97.77
Barley	88.60	11.00	4.99	2.51	2.50	97.50
Soybean meal	88.60	45.00	8.00	1.75	8.64	91.36
Mustard cake	91.46	35.62	8.33	6.25	8.83	91.17
Concentrate	83.95	22.87	5.38	3.71	4.74	95.26

Table 2 Effect of Soymilk feeding on body weight gain (kg) of Murrah buffalo calves

Period of experiment (days)	Treatments		
	T_1	T_2	T_3
0-15	4.85 ± 0.56	4.40 ± 0.83	2.90 ± 0.40
15-30	4.27 ± 1.19	3.60 ± 0.91	3.24 ± 0.58
30-45	5.92 ± 1.96	6.65 ± 1.18	5.56 ± 1.22
45-60	5.80 ± 1.11	5.59 ± 1.20	5.26 ± 0.59
60-75	5.52 ± 0.65	7.82 ± 0.72	6.72 ± 0.72
75-90	6.64 ± 0.57	7.62 ± 0.41	6.72 ± 0.64

Values are means ± standard errors

Table 3 Effect of Soymilk feeding on average daily body weight gain (kg/day) of Murrah buffalo calves

Period of experiment (days)	Treatments		
	T_1	T_2	T_3
0-15	0.32 ± 0.04	0.29 ± 0.01	0.19 ± 0.03
15-30	0.28 ± 0.08	0.24 ± 0.06	0.22 ± 0.04
30-45	0.39 ± 0.13	0.44 ± 0.08	0.37 ± 0.08
45-60	0.39 ± 0.07	0.37 ± 0.08	0.35 ± 0.04
60-75	0.37 ± 0.04	0.52 ± 0.05	0.45 ± 0.05
75-90	0.44 ± 0.04	0.51 ± 0.03	0.45 ± 0.04
0-90	0.37 ± 0.03	0.40 ± 0.01	0.34 ± 0.02

Values are means ± standard errors

In contrary to our findings, Shukla (2014) found a significantly lower growth rate for calves fed on formulated soy based milk replacer than the whole milk fed group, respectively. This study also revealed that average daily gain (ADG) of calves fed whole milk was significantly higher than commercial and formulated milk replacer. Gadzama et al. (2017) unveiled that feeding of calves with Soymilk:cow milk (25:75) improved live weight gain and average daily gain than calves fed with cow milk alone. Similarly, Alam et al. (2021) also reported that significantly ($P<0.05$) higher average final live weight in kids fed soybean based milk replacer in comparison to naturally milk suckled kids.

Body measurements

The results of body measurements are presented in Table 5 and 6. The outcomes of present study revealed that body measurements (body height, cumulative body height gain, heart girth, cumulative heart girth gain, abdominal girth, cumulative abdominal girth gain, body length and cumulative body length gain) did not differ significantly during the entire period of experiment in Murrah buffalo calves under different treatments, except cumulative heart girth gain. There was significant ($P<0.05$) higher value observed in cumulative heart girth gain during the period of 0 to 45 days in which cumulative heart girth gain was significantly higher in T_2 (WM and SM in 75:25 ratio) group in comparison to T_1 (WM) and T_3 (WM and SM in 50:50 ratio) treatment groups, although it was statistically similar between T_1 and T_3 group.

Present findings are corroborated with Masum et al. (2009) who reported that non-significant differences were seen for increment in wither height, body length and heart girth in calves fed cow milk, strained Soymilk and unstrained Soymilk; Masum et al. (2011) who reported non-significantly increment in wither height, body length and heart girth between groups in which calves reared by cow milk and reared by combination of cow milk and fortified Soymilk (50:50) and Sarker et al. (2015) who reported that Black Bengal kids fed on whole milk alone and Soymilk at 25 and 50 percent levels had similar body measurements. The present results of body measurements agreed with Yadav (2016) who concluded that increase in the body height was similar fortnightly among whole milk and fortified Soymilk fed group. Similarly, Shakya et al. (2017) found statistically similar body length, heart girth and wither height in Murrah buffalo calves fed on whole milk and whole milk plus 20 percent Soymilk. Fahmida (2018) also revealed that no significant difference was found in body length, heart girth and body height of calves among whole milk feeding group and Soymilk feeding group (replacement up to 50%).

In contrast to this study, Gadzama (2017) reported significantly ($P<0.05$) improvement in body length, heart girth and body height of calves in Soymilk fed treatment groups than cow milk fed treatment group. Alam et al. (2021) observed that body height, heart girth and body length were significantly ($P<0.05$) higher in soybean based milk replacer fed kids than naturally suckled kids.

Economics of feeding

Major outcome of research was in terms of economics of Soymilk feeding which is presented in Table 4. The pursual of data indicated that profit in total feeding cost per kg weight gain of calves was Rs. 106.92 and 130.35 for Soymilk feeding group at 25% and 50% level, respectively than control group, suggesting that Soymilk feeding is more economical than Whole milk feeding without compromising the growth of calves.

Table 4 Economics of feeding Soymilk under different treatment groups

Variables	T_1	T_2	T_3
Cost of feeding dry fodder {cost/calf (Rs.)} @ Rs.11/kg	198.79	189.49	173.65
Cost of feeding green fodder {cost/calf (Rs.)} @ Rs.1.6/kg	145.54	138.77	128.42
Cost of feeding concentrate {cost/calf (Rs.)} @ Rs.27.5/kg	1235.32	1202.17	1143.88
Cost of feeding Soymilk {cost/calf (Rs.)} @ Rs.70/kg	0	833.805	1667.61
Whole milk feeding Cost {cost/calf (Rs.)} @ Rs.50/kg	13500	10125	6750
Total milk feeding cost/calf (Rs.)	13500	10958.80	8417.61
Total feeding cost/calf (Rs.)	15079.65	12489.23	9863.56
Profit in milk feeding cost/calf (Rs.)	0	(+) 2541.19	(+) 5082.39
Profit in total feeding cost/calf (Rs.)	0	(+) 2590.42	(+) 5216.09
Average body weight gain/calf (kg)	33	35.68	30.2
Total feeding cost/kg weight gain {cost/calf (Rs.)}	456.96	350.03	326.61
Profit in total feeding cost/kg weight gain {cost/calf (Rs.)}	0	(+) 106.92	(+) 130.35

Table 5 Effect of Soymilk feeding on average body weight (kg), body height (inch), heart girth (inch), abdominal girth (inch) and body length (inch) of Murrah buffalo calves

Days of experiment	Body weight (kg)			Body height (inch)			Heart girth (inch)			Abdominal girth (inch)			Body length (inch)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
0th	43.80 ± 3.44	43.40 ± 2.06	43.40 ± 2.56	31.10 ± 1.00	30.60 ± 0.51	30.00 ± 0.32	33.00 ± 0.71	32.00 ± 0.63	32.00 ± 0.71	33.00 ± 0.71	33.60 ± 0.24	33.40 ± 1.25	26.00 ± 0.84	26.40 ± 0.51	26.20 ± 0.49
15th	48.65 ± 3.48	47.80 ± 1.97	46.30 ± 2.71	32.30 ± 0.70	31.20 ± 0.37	31.60 ± 0.68	33.60 ± 0.68	33.40 ± 0.59	33.20 ± 0.80	34.80 ± 0.73	34.40 ± 0.98	36.00 ± 1.05	27.30 ± 0.97	27.60 ± 0.24	27.60 ± 0.24
30th	52.92 ± 4.05	51.40 ± 1.56	49.54 ± 2.22	32.80 ± 0.86	32.30 ± 0.49	32.50 ± 0.45	35.00 ± 1.05	34.50 ± 0.58	34.20 ± 0.66	36.60 ± 1.03	36.60 ± 1.08	36.40 ± 0.60	29.20 ± 0.97	28.30 ± 0.39	28.40 ± 0.40
45th	58.84 ± 5.44	58.05 ± 2.44	55.10 ± 3.18	33.40 ± 1.08	32.60 ± 0.48	33.00 ± 0.63	36.20 ± 1.07	36.50 ± 0.59	35.00 ± 0.63	38.10 ± 0.78	38.90 ± 0.51	37.20 ± 0.58	30.00 ± 0.95	29.00 ± 0.32	29.80 ± 0.37
60th	64.64 ± 5.70	63.64 ± 1.77	60.16 ± 3.23	34.00 ± 0.71	33.30 ± 0.37	33.40 ± 0.43	37.40 ± 1.13	37.30 ± 0.44	36.60 ± 0.66	40.70 ± 1.80	39.80 ± 1.07	38.50 ± 0.45	30.80 ± 1.07	29.90 ± 0.33	30.20 ± 0.37
75th	70.16 ± 5.30	71.46 ± 1.46	66.88 ± 3.71	34.80 ± 0.72	34.10 ± 0.40	34.40 ± 0.60	39.20 ± 0.82	39.00 ± 0.32	38.00 ± 0.95	41.60 ± 1.21	41.40 ± 0.75	40.80 ± 0.58	31.60 ± 0.75	31.20 ± 0.41	31.40 ± 0.68
90th	76.80 ± 5.72	79.08 ± 1.30	73.60 ± 4.22	35.80 ± 0.98	35.20 ± 0.34	35.10 ± 0.78	40.80 ± 1.16	40.60 ± 0.29	39.10 ± 1.14	43.00 ± 1.22	43.50 ± 0.74	43.10 ± 1.08	32.50 ± 1.00	32.50 ± 0.32	32.60 ± 0.40
Overall	59.40 ± 4.49	59.26 ± 4.90	56.43 ± 4.20	33.46 ± 0.60	32.76 ± 0.60	32.86 ± 0.65	36.46± 1.08	36.19 ± 1.16	35.44 ± 0.97	38.26 ± 1.40	38.31 ± 1.37	37.91 ± 1.22	29.63 ± 0.88	29.27 ± 0.80	29.46 ± 0.84

Values are means ± standard errors

Table 6 Effect of Soymilk feeding on cumulative body weight gain (kg), cumulative body height gain (inch), cumulative heart girth gain (inch), cumulative abdominal girth gain (inch) and cumulative body length gain (inch) of Murrah buffalo calves

Period of experiment (days)	cumulative body weight gain (kg)			cumulative body height gain (inch)			cumulative heart girth gain (inch)			cumulative abdominal girth gain (inch)			cumulative body length gain (inch)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
0-15	4.85 ± 0.56	4.40 ± 0.83	2.90 ± 0.40	1.20 ± 0.41	0.60 ± 0.25	1.60 ± 0.51	0.60 ± 0.24	1.40 ± 0.51	1.20 ± 0.37	1.80 ± 1.24	0.80 ± 0.86	2.60 ± 0.40	1.30 ± 0.37	1.20 ± 0.37	1.40 ± 0.40
0-30	9.12 ± 0.95	8.00 ± 1.19	6.14 ± 0.63	1.70 ± 0.20	1.70 ± 0.54	2.50 ± 0.32	2.00 ± 0.55	2.50± 0.45	2.20 ± 0.20	3.60 ± 1.60	3.00 ± 1.14	3.00 ± 0.71	3.20 ± 0.58	1.90 ± 0.51	2.20 ± 0.49
0-45	15.04 ± 2.14	14.65 ± 0.87	11.70 ± 0.90	2.30 ± 0.20	2.00 ± 0.35	3.00 ± 0.45	3.20^{a} ± 0.37	4.50^{b} ± 0.45	3.00^{a} ± 0.32	5.10 ± 1.08	5.30 ± 0.37	3.80 ± 0.86	4.00 ± 0.45	2.60 ± 0.24	3.60 ± 0.68
0-60	20.84 ± 2.71	20.24 ± 1.11	16.76 ± 1.04	2.90 ± 0.33	2.70 ± 0.30	3.40 ± 0.49	4.40 ± 0.53	5.30 ± 0.80	4.60 ± 0.19	7.70 ± 2.24	6.20 ± 1.02	5.10 ± 0.93	4.80 ± 0.58	3.50 ± 0.45	4.00 ± 00.45
0-75	26.36 ± 2.57	28.06 ± 1.14	23.48 ± 1.68	3.70 ± 0.46	3.50 ± 0.22	4.40 ± 0.40	6.20 ± 0.34	7.00 ± 0.55	6.00 ± 0.32	8.60 ± 1.63	7.80 ± 0.73	7.40 ± 0.75	5.60 ± 0.51	4.80 ± 0.46	5.20 ± 0.80
0-90	33.00 ± 3.12	35.68 ± 1.27	30.20 ± 2.22	4.70 ± 0.41	4.60 ± 0.37	5.10 ± 0.56	7.80 ± 0.58	8.60 ± 0.62	7.10 ± 0.66	10.00 ± 1.70	9.90 ± 0.81	9.70 ± 0.61	6.50 ± 0.77	6.10 ± 0.40	6.40 ± 0.75

Values are means ± standard errors
Mean values with different superscripts in a row differ significantly (P<0.05)

These results of lower cost per kg gain in calves fed on Soymilk as compared to those fed on whole milk were in accordance with the findings of Kamble et al. (2003) who observed that cost per kg body weight gain was significantly higher in the treatment T_1 (whole milk) than T_2 (30 per cent Soymilk + 70 per cent whole milk) and T_3 (40 per cent Soymilk + 60 per cent whole milk) treatments respectively; Matter et al. (2005) who reported that values of feed costs for Soymilk fed group were significantly lower than those fed other groups and Ghorbani et al. (2007) who concluded that feed related weaning costs were lower for Soymilk fed group than whole milk fed group. Similar findings were observed by Masum et al. (2011) who revealed that the cost per kg gain in calves was lower for a combination of Soymilk and cow milk as against whole milk alone. Roy et al. (2012) also reported that the feeding cost per calf per day as 21.27 and 107.77 Taka for the soy and whole milk fed groups, respectively and Shukla (2014) reported that the feed cost per kg gain was lower for calves fed on soy based milk replacer than those fed on whole milk alone. In the same line, Shakya et al. (2016) found significantly lower recurring cost of rearing of the buffalo calves in Soymilk fed groups than whole milk fed group. Similarly, Yadav et al. (2018) revealed that total feeding cost (Rs/kg weight gain) was form to reduce by Rs. 176.42, 236.32 and 237.61 in T_2, T_3 and T_4 groups, respectively as compared to control group and also, Fahmida (2018) reported that the cost per kg gain was lowest in calves fed on Soymilk at 50 per cent Soymilk level followed by those fed on Soymilk at 25 percent level.

Conclusion

It can be concluded that Soymilk feeding up to 50% level with Whole milk had similar effect on growth as Whole milk without compromising the performance of calves and found to be more economical in comparison to Whole milk feeding; therefore Soymilk may possibly replace whole milk up to 50 percent level.

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RESEARCH ARTICLE

Resource use efficiency in milk production in different dairy-integrated farming systems in Terai Region of West Bengal

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Abstract: The study was undertaken in the Terai region of West Bengal with the objective to assess the resource use efficiency in milk production in different dairy-integrated farming systems. The study covered a total of 180 dairy farmers from different farming systems. The results revealed that milk production in Dairy (D), Dairy + Crop + Goat (D + C + G), Dairy + Crop + Mushroom (D + C + M) and Dairy + Crop + Fishery (D + C + F) farming systems could be increased by feeding more amount of green fodder. Similarly, dry fodder had a positive effect on milk production in Dairy + Crop (D + C), Dairy + Crop + Goat (D + C + G) and Dairy + Crop + Poultry (D + C + P) farming system whereas in Dairy (D) farming system it had a negative impact on milk production. The study also revealed that green fodder was under-utilised and concentrate was over-utilised in Dairy + Crop + Mushroom (D + C + M) farming system. In Dairy + Crop + Fishery (D + C + F) farming system concentrate was over-utilised. In overall farming system, concentrate was over-utilised in indigenous cattle in the study area, resulting in a higher per litre cost of milk production.

Keywords: Cobb-Douglas Production Function, Marginal value product, Resource use efficiency

Introduction

One of the integral parts of India's agriculture sector is the livestock sector which grew at a CAGR of 7.9% during 2014-15 to 2020-21 (at constant prices), and its contribution to total agriculture GVA (at constant prices) has increased from 24.3% in 2014-15 to 30.1% in 2020-21 (Economic Survey 2022-23). Livestock population has increased between 2012 to 2019 from 30.35 million to 37.40 million in the state of West Bengal, registering a positive growth of 23.22 % in the total number of animals of various species (20th Livestock Census, 2019). Though livestock density is higher (730 per square km) in the Terai region compared to another agro-climatic region in West Bengal, milk productivity is comparatively low as this region is mainly dominated by indigenous and non-descriptive cattle. Therefore, it is not possible for the farmers in this region to depend only on cattle rearing for their livelihood. The prominent dairy breeds found in this region were North Bengal Grey cattle, Jersey, some non-descriptive cattle etc. Some farmers were also found rearing Holstein Friesian and Sahiwal breeds. But it must say these breeds were not that much prevalent in the region.

Income from dairy production supports on average 40% of total households in the East, 32% in the North, 21% in the South and 34% in the West zone of the country (Ray et al. 2012). Several genetic (types of breeds and ability for milk secretion by individual animals) and non-genetic variables (types, quantity and quality of feeds and fodders fed, order of lactation, stage of lactation, herd size, labour use, climatic conditions etc) have a significant impact on milk production. Therefore, choosing appropriate factors to evaluate milk production is crucial. The best possible use of the numerous inputs utilised by milk producers should be a top priority. It is crucial to understand whether or not milk producers are using their inputs effectively. Planning, predicting, and developing dairy development plans in a specific area require an empirical analysis of the factors that affect milk production and the effective use of resources. Different inputs are consumed by various species of animals in varying amounts, including green fodder, dry fodder, concentrate, labour, etc., which either directly or indirectly affect milk production. It is important to know whether the resources owned by milk producers are under-utilised or over-utilised for milk production. Therefore, the knowledge of the best use of scarce resources for milk production is essential for making dairy farming a profitable enterprise. Keeping in view the above facts, the present investigation was carried out in the Terai region of West Bengal and an attempt has been made to

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know resource use efficiency in milk production in different dairy-farming systems in the area.

Materials and Methods

Study area and sampling

The study pertains to the state of West Bengal. A multistage random sampling design was adopted, which included selection of state, agro-climatic zone, districts, blocks, cluster of villages and respondents. Out of the 6 Agro-climatic zones in West Bengal, Terai region was selected purposively on the basis of highest livestock density (730/sq. m). Then out of four districts of that region two districts, i.e., Cooch Behar and Jalpaiguri were selected randomly. From each district three blocks (Cooch Behar – Mathabhanga II, Cooch Behar II, Sitalkhuchi and Jalpaiguri – Dhupguri, Mal, Jalpaiguri) were selected and from each block one cluster of villages was selected randomly. Thus, a total of 6 clusters of villages were selected and from each cluster of villages 30 households were selected as respondents. In all, a total of 180 respondents were selected from the region. A complete enumeration of the cluster of villages (with respect to their components of farming systems) was carried out after selecting the clusters of villages randomly. The farmers having dairy farming as one of the components in their farming system and as well as having at least two lactating animals were considered as respondents for the study. The data were collected with the help of a well-structured interview schedule by personal interview/ enquiry method during the months of February-March, 2022.

Milk production function

Milk production is a complex variable, which is influenced by several explanatory variables. In this research, production function analysis is employed to estimate resource use efficiency in milk production in the Terai region of West Bengal.

The choice of a specific functional form was based on economic and the statistical criteria and co-efficient of multiple determination (R^2). The output and input in the above analysis is measured in monetary terms rather than their physical quantities because of the variability in the quality of feed and fodder. The Cobb-Douglas production function was the best fit for the data in the present study. Hence, Cobb-Douglas production function is used for further analysis.

The functional form of Cobb-Douglas production function is: -

$$Y = AX_1^{b_1}X_2^{b_2}X_3^{b_3}X_4^{b_4}X_5^{b_5}e^{u}$$

In logarithms, the function is of the following form: -

$$\log Y = \log A + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 + b_4 \log X_4 + b_5 \log X_5$$

where,

Y = Income from milk per animal per day (^{1})

X_1 = Expenditure on green fodder per animal per day (^{1})

X_2 = Expenditure on dry fodder per animal per day (^{1})

X_3 = Expenditure on concentrates per animal per day (^{1})

X_4 = Value of labour used per animal per day (^{1})

X_5 = Expenditure on veterinary services per animal per day (^{1})

Elasticities b_1, b_2, $b_3 \ldots b_5$ indicate the percentage change in the output due to 1% change in the particular input, while all other inputs are kept constant.

The production function was fitted to the data aggregated for all categories of animal species due to small number of observations in different farming systems. At last, production function for overall farming system was also estimated by combining all the farming systems together.

Estimation of Marginal value product (MVP) and resource use efficiency

Marginal value product (MVP) of inputs from the most appropriate milk production function i.e., Cobb-Douglas production function is worked out as: -

$$MVP_i = b_i \frac{\bar{Y}}{\bar{X}_i}$$

Where, $\bar{Y}$ and $\bar{X}_i$ are the geometric means of Y and i^{th} input, respectively, and b_i is the estimated regression coefficients of i^{th} input.

Resource use efficiency of inputs measures whether or not the inputs are used efficiently. They are used optimally if the MVP_i of the input is equal to its unit price (P_i), i.e.,

$$MVP_i = P_i$$

In order to examine the resource use efficiency, the MVP of various inputs is worked out for significant estimated parameters in the estimated milk production function. Any deviation of MVP of input from its unit price indicates as that the resource is not used efficiently. A higher value of MVP than its unit price implies under-utilisation and lower value of MVP than its unit price implies over-utilisation of the resource. Further, t-test is used to test the statistical significance of the difference between MVP of an input and its unit price.

$$\text{Calculated } t = \frac{MVP_{X_i} - P_{X_i}}{S.E.(MVP_{X_i})}$$

Where, SE = Standard Error of MVP. It is calculated as follows: -

$$SE(MVP_i) = SE(b_i)\frac{\bar{Y}}{\bar{X}_i}$$

Where, and are the geometric means of Y and i^{th} input respectively and b_i is the regression coefficient of i^{th} input.

Results and Discussion

The study area was completely enumerated and the most prevalent farming systems with dairy are identified as follows: - (Table -1)

Most prominent farming system in the study area was Dairy + Crop (D + C) farming system followed by Dairy + Crop + Goat (D + C + G) farming system.

To examine the resource use efficiency in milk production, the Cobb-Douglas production function is fitted separately for different farming systems and also for overall farming system. The estimated production function coefficients along with their standard error for each of the identified farming systems are presented in Table 2. The table reveals that 76%, 82%, 70%, 68%, 66% and 67% of variability in milk production in D, D + C, D + C+ G, D + C+ P, D + C + M and D + C+ F farming system, respectively, can be explained by the variables included in the model, i.e., green fodder, dry fodder, concentrate, labour value and veterinary charges. The regression coefficients for green fodder are positive and significant in D, D + C + G, D + C + M and D + C + F farming system implying milk production in these farming systems could be significantly increased through efficient feeding of green

Table 1: Description of different identified farming systems

Sl No.	Name of Farming System	Percentage of total sample	Average Land holding size (ha)	Average animal holding size (lactating animals)
FS – I	Dairy (D)	9.44	0.28	2.29
FS – II	Dairy + Crop (D +C)	24.44	0.48	2.12
FS – III	Dairy + Crop + Goat (D + C + G)	21.11	0.32	2.16
FS – IV	Dairy + Crop + Poultry (D + C + P)	18.33	0.45	2.03
FS – V	Dairy + Crop + Mushroom (D + C + M)	18.33	0.43	2.15
FS – VI	Dairy + Crop + Fish (D + C + F)	8.33	0.31	2.13

Table 2: Estimated milk production function for different farming systems

Variables	D	D + C	D + C + G	D + C + P	D + C + M	D + C + F
Intercept	6.219***	0.881	1.223	2.190	-0.507	1.135
	(1.920)	(1.058)	(0.847)	(1.351)	(2.998)	(2.602)
Green Fodder	0.724***	0.099	0.288***	-0.165	1.216***	0.992**
	(0.210)	(0.180)	(0.106)	(0.134)	(0.288)	(0.473)
Dry Fodder	-0.899***	0.461**	0.862***	0.696*	0.674	0.126
	(0.236)	(0.211)	(0.230)	(0.398)	(0.808)	(0.461)
Concentrate	0.058	0.701***	0.124	0.571***	0.327***	0.505***
	(0.117)	(0.075)	(0.132)	(0.135)	(0.158)	(0.182)
Labour	-0.170	0.209	0.271	-0.144	0.019	-0.352
	(0.731)	(0.243)	(0.163)	(0.214)	(0.419)	(0.411)
Veterinary Charge	-0.466	0.344	0.364	0.150	0.106	0.521
	(0.274)	(0.343)	(0.189)	(0.298)	(0.332)	(0.404)
Coefficient of Determination (R2) Value	0.76	0.82	0.70	0.68	0.66	0.67
Total no. of Observations	17	44	38	33	33	15

*Significant at 10% level (P <0.1), ** significant at 5% level (P<0.05). ***significant at 1% level (P <0.01), Figures in the parentheses indicate the standard error of estimated parameters fodder. Regression coefficients of dry fodder are positive and significant in D + C, D + C + G and D + C + P farming system whereas in D farming system the coefficient is negative and significant. This implies milk production can be increased by feeding more dry fodder in D + C, D + C + G and D + C + P farming system. Positive and significant coefficients of dry fodder in milk production function is also observed by Mehra et al. (2018) in the study conducted in Hilly areas of Kumaon region of Uttarakhand. The negative impact of dry fodder on milk production in D farming system implies increase in dry fodder feeding does not increase milk production. It could be due to use of poor-quality dry fodder in dairy enterprise. Coefficients of concentrate are positive and significant in D + C, D + C + P, D + C + M and D + C + F farming system. This indicates use of more concentrate in dairy in these farming systems could enhance milk production. The findings are in line with the findings of Singh et al. (2012) who reported that milk production of buffaloes could be significantly increased through the efficient feeding of concentrates in Varanasi district of Uttar Pradesh. In another study conducted by Sharma et al. (2014), the partial regression coefficients of expenditure on concentrates and green fodder are positive and significant for all types of dairy cattle. However, the regression coefficients of labour and veterinary charges are non-significant in all farming systems implying that labour and veterinary services do not have significant impact on milk production in the area.

Marginal value product (MVP) of all the significant inputs is computed at their geometric mean level for different farming systems. The results are presented in Table 3 along with their unit prices. Since all the inputs are expressed in monetary terms in the production function, the acquisition cost of the inputs is taken as ₹ 1. The estimated marginal value product is, therefore, compared with unity to examine the resource use efficiency. The table reveals that in D, D + C, D + C + G and D + C + P farming

Table 3: Marginal Value Product (MVP) of inputs along with unit price in different farming systems

Farming Systems	D		D + C		D + C + G		D + C + P		D + C + M		D + C + F	
Variables	Green Fodder	Dry Fodder	Dry Fodder	Concentrate	Green Fodder	Dry Fodder	Dry Fodder	Concentrate	Green Fodder	Concentrate	Green Fodder	Concentrate
Marginal Value Product (MVP)	1.563	1.646	0.808	0.962	0.926	1.748	1.271	0.847	3.016	0.474	1.886	0.527
Unit Price of Input	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Difference	0.564	0.647	-0.192	-0.038	-0.074	0.748	0.271	-0.153	2.016**	-0.526*	0.886	-0.473**
Standard Error (MVP)	0.454	0.432	0.316	0.103	0.341	0.466	0.727	0.200	0.714	0.229	0.899	0.189
t Value	1.243	1.496	-0.608	-0.369	-0.218	1.603	0.372	-0.763	2.822	-2.299	0.985	-2.489

*Significant at 5% level (P<0.05), **significant at 1% level (P <0.01)

Table 4: Estimated milk production function for different animal species in overall farming system

Variables	Indigenous Cow		Crossbred Cow	
	Coefficient	Standard Error	Coefficient	Standard Error
Intercept	2.193**	0.521	0.891	1.939
Green Fodder	0.054	0.101	0.502*	0.202
Dry Fodder	0.507**	0.120	0.496*	0.253
Concentrate	0.486**	0.081	0.429	0.464
Labour	-0.093	0.105	0.097	0.195
Veterinary Charge	0.049	0.118	0.031	0.175
Coefficient of Determination (R2) Value	0.62		0.30	
Total no. of Observations	118		62	

*Significant at 10% level (P <0.1), ** significant at 5% level (P<0.05). ***significant at 1% level (P <0.01),

Table 5: Marginal Value Product (MVP) of inputs along with unit price for in different animal species in overall farming systems

Particulars	Indigenous Cow		Crossbred Cow	
	Dry Fodder	Concentrate	Green Fodder	Dry Fodder
Marginal Value Product (MVP)	0.869	0.712	1.199	1.022
Unit Price of Input	1.000	1.000	1.000	1.000
Difference	-0.131	-0.288**	0.199	0.022
Standard Error (MVP)	0.206	0.119	0.483	0.521
t Value	-0.637	-2.429	0.413	0.041

** significant at 5% level (P<0.05)

system, the difference between the marginal value product of estimated significant inputs and unit price is non-significant. This implies that in D and D + C + G farming system, green fodder and dry fodder; and in D + C and D + C + P farming system, dry fodder and concentrate are used efficiently. Whereas, in D + C + M farming system, the difference is positive and significant for green fodder; negative and significant for concentrate. This implies that green fodder is under-utilised and concentrate is over-utilised. Similarly, Table 2 reveals that in D + C + F farming system, concentrate is over-utilised, which indicates excessive expenditure made on concentrate by the milk producers. Over-utilization could be due to a mindset of the producers that more concentrate feeding may enhance milk production.

Table 4 presents estimated regression coefficients for overall farming system in the Terai region of West Bengal. It can be seen from the table that the coefficients for dry fodder and concentrate in case of indigenous cattle; green fodder and dry fodder in the case of crossbred cattle are positive and significant. This implies that by increasing amount of feed of these inputs could enhance milk production in the study area. The coefficient of multiple determination reveals that 62% and 30% of the variability in milk production can be explained by the variables included in the model in the case of indigenous cattle and crossbred cattle, respectively. The result of the positive and significant effect of concentrate on indigenous cattle is similar to the results found in the studies conducted by Kumar et al. (2013) and Meena et al. (2019) on resource use efficiency in milk production in Haryana and Rajasthan, respectively.

The calculated marginal value product of the significant variables along with their unit price is presented in Table 5. The table reveals that in the case of crossbred cattle, green fodder and dry fodder are used efficiently. On the other hand, in case of indigenous cattle, the difference between marginal value product and unit price of dry fodder is non-significant but the difference is significant in case of concentrate. This implies that dry fodder is used efficiently for indigenous cattle. The marginal value product of concentrate is less than its unit price indicating over-utilisation of concentrate for indigenous cattle in the study area.

Conclusion

Based on the foregoing discussion, it can be concluded that there is wide range of variation in resource use efficiency in different types of farming systems in Terai region of West Bengal. The cost for maintaining milch animals and for milk production can be rationalized by appropriately feeding the animals. In overall farming system, it is found that dry fodder and concentrate in case of indigenous cattle; and green fodder and dry fodder in case of crossbred cattle have positive and significant influence on milk production. From the field experience, it can be said that more than 50 percent the milk producers were not fully aware of the productivity of the inputs used in the production of milk. In overall farming system, concentrate is over-utilised in case of indigenous cattle. Therefore, use of concentrate should be reduced in indigenous cattle in order to get the optimum return from milk production. Finally, it can be suggested that through extension programmes on animal husbandry like demonstrations, field visits, *etc*., it is necessary to inform and inspire livestock rearers about the scientific feeding of concentrate (quantity, time, proportion of mixture) in different lactation stages. It is also necessary to carry out livestock breeding programmes like upgradation of non-descriptive cattle with improved indigenous breeds and production of those cattle so that farmers can purchase the breeds of dairy animals at a reasonable price. Besides these, veterinary facility can be made available to the farmers as and when required in every village for easy accessibility.

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RESEARCH ARTICLE

Economic impact of milk price incentive scheme on dairy farming in Karnataka

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Abstract : The present study attempts to examine the economic impact of milk price incentive scheme announced by Government of Karnataka on production, productivity and profitability of milk production in Karnataka. To assess the impact of scheme on production and productivity of milk, time series data on number of milch cows and buffaloes, production and productivity were collected from livestock census reports from 1997 to 2019. To examine its impact on profitability, pertinent information was elicited from 60 sample dairy farmers coming under the jurisdiction of SHIMUL, Karnataka. Exponential growth models, production function considering production/productivity as dependent variable and share of cross bred in total milch bovine population, dummy variable to capture the influence of scheme as independent variables and enterprise budgeting to examine profitability were employed. The results indicated that number of indigenous milch cows and buffaloes have decelerated while that of cross bred cows have accelerated at a greater pace. Substantial and significant Milk production in case of cross bred cows was due to extensive production while in case of indigenous bovines it was mainly due to intensive production. The milk price incentive of Rs.5 per litre announced by Government of Karnataka in 2016 has increased milk production by 79.90 percent and productivity by 115 percent compared to milk production and productivity without milk incentive (prior to 2008). The share of cross bred cows has almost doubled after the implementation of the scheme. In terms of profitability, farmers were under loss of Rs. 1.43 per litre of milk in the absence of incentive while they realized profit of Rs. 3.57 per litre with Rs. 5 per litre as incentive. Delay in the payment of incentive and its inability to keep the rising pace of growth in input prices are considered as the major lacuna of the scheme. Farmers have suggested for doubling the existing incentive to Rs. 10 per litre to absorb the shock of rising prices of inputs and to obtain sustained profit.

Keywords: Incentive, SHIMUL, Exponential growth models, Economic impact

Introduction

In order to encourage the rural farmers to involve themselves in dairy farming, a lucrative subsidiary occupation, Government of Karnataka in the Year 2008 launched a flagship scheme entitled "Milk incentive Scheme" to provide an incentive of Rs. 2 per litre for those dairy farmers who sell their milk to Milk Producers cooperative societies situated in the rural areas. Later, the Government of Karnataka realized the marked impact of the milk incentive scheme of 2008 in terms of increased productivity and production of milk forcing it to revise the incentives from Rs. 2 per litre to Rs. 4 per litre. The revised incentive of Rs. 4 per litre was brought into letter and spirit from 2013. The process of revision did not halt, rather it gained momentum and again a revision of incentive price from Rs. 4 per litre to Rs. 5 per litre was announced in the year 2016. The objective of the scheme was to make dairying a profitable enterprise especially for marginal and small farmers, landless labourers, individuals belonging to the weaker sections and women folk. The other objective is to increase milk production to impart food security, improve socio-economic condition of dairy farmers, encourage cooperatives in dairy sector and to attract rural youth to dairying thereby their migration could be avoided. In this context, it becomes imperative to probe into the impact assessment of milk incentive scheme on dairy farming.

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Methodology

The study aimed at assessment of economic impact of milk incentive scheme on milk production and productivity, profitability of dairy farming in Karnataka state. The study was carried out hypothesizing that provision of incentive prices to dairy farmers enables them to exhibit transition in bovine composition. It was also hypothesized that due to change in bovine composition, milk productivity and production changes and have bearing on profitability of milk production. The scheme was even hypothesized to have positive bearing on expansion of cooperatives in dairy sector. The hypotheses were formulated to validate the functioning of the scheme keeping its specific objectives as reference.

Data

To accomplish the specific objectives of the study, secondary data on number of milch bovines' viz., indigenous cows, crossbred cows and buffaloes were collected from the published livestock census reports. State wise statistics on number of milch animals was available in the Livestock Census reports of 1997, 2003, 2007, 2012 and 2019 while district wise statistics was available in all other census reports excepting for 2019. From the same reports, data on milk productivity and production of different milch bovines (indigenous cows, crossbred cows and buffaloes) was also collected. The temporal data on milk production available for the Karnataka state from 1997 to 2019 was also collected. To ascertain the impact of the scheme on the expansion of cooperatives in dairy sector, secondary data on number of dairy cooperative societies, number of cooperative members, milk procurement per day, payment made to the dairy farmers and total turnover of the KMF were elicited from the annual report of the KMF. The impact of scheme on bovine composition and in-turn bovine composition's bearing on milk productivity and production was ascertained for the districts coming under the jurisdiction of SHIMUL ie., Chitradurga, Davangere and Shivamogga.

The perusal of temporal data on number of indigenous milch animals and cross bred animals and their relative share in the total milch bovine population in districts coming under SHIMUL jurisdiction clearly indicated the inclination shown by the dairy farmers towards cross bred cows. Accordingly, to assess the impact of milk incentive price given by the government of Karnataka to the dairy farmers to boost the production of milk, purposively dairy farmers rearing more of cross bred cows were chosen. The intention behind selection of farmers rearing more of cross bred cows was to examine how the scheme has made impact on the profitability these farmers. Working out of economics provides the researcher a hunch on the cost incurred per litre of milk production, average returns or price realized per litre with and without incentive price. How with the escalating prices of inputs, dairy farmers rearing more of cross bred cows are managing dairy enterprise could be ascertained. Around 60 sample dairy farmers coming under the jurisdiction of Shivamogga Milk Union Limited (SHIMUL) was selected. The primary data on the capital investment made on the dairy unit, type of bovines reared, experience in dairy farming, number of milch cows reared, expenditure made on variable resources like human labour, feed such as dry fodder, concentrates and green fodder, medicines and vaccines, output realized in the form of milk, cowdung, calves etc, price at which milk, cowdung, calves in case if sold was elicited. In addition, since from when, the benefits of incentive prices were received, opinion about milk incentive scheme and suggestions for improvement in the implementation of scheme was also elicited.

The temporal growth in milk production, milk productivity and number of milch bovines over census was estimated using exponential growth model.

$Y_t = ab^t.e^t$

Y_t = Milk production/Milk productivity/Number of milch bovines in Karnataka state or of respective districts viz., Shivamogga, Davangere and Chitradurga coming under the jurisdiction of SHIMUL

a= Y- intercept

b= slope coefficient

e = Stochastic error term

t= time period (census)

The estimable form of the exponential model was arrived at by taking logarithmic transformation

$Ln\ Y_t = Ln\ a + t\ Ln\ b$

The compound rate of growth over census period was estimated using

CGR (%) = (antilog b -1) *100

Impact of milk incentive price on milk production in Karnataka

The impact of milk incentive scheme on milk production in Karnataka was examined by estimating milk production function. Milk production function establishes functional relationship between milk production and variables capturing the influence of milk incentive scheme (institutional intervention) and share of milch crossbred cows in total milch bovines. Institutional intervention in the form of milk incentive scheme was announced by Government of Karnataka in three phases announcing incentives of Rs. 2 per litre from 2008, Rs. 4per litre from 2013 and Rs. 5 per litre from 2016. Since, it is a qualitative variable, its influence was captured using dummy variables. As there were four categories, three dummy variables were used viz., D_1, D_2 and D_3. It was hypothesized that both the independent variables ie., institutional intervention and share of cross bred cows in total milch bovine population bears positive influence on the dependent variable ie., milk production.

Milk Production = $a + b_1D_1 + b_2D_2 + b_3D_3 + b_4X + e$

D_1 is a dummy variable taking value 1, 0, 0 to represent milk incentive of Rs. 2 per litre announced during 2008

D_2 is a dummy variable taking value 0, 1, 0 to represent milk incentive of Rs. 4 per litre announced during 2013

D_3 is a dummy variable taking value 0, 0, 1 to represent milk incentive of Rs. 5 per litre announced during 2016

X – ratio of number of milch crossbred cows in total number of milch bovines

e- stochastic error term

a indicates Y-intercept

b_1, b_2, b_3, b_4 indicates partial regression coefficients

Impact of milk incentive price on milk productivity in Karnataka

The milk productivity in Karnataka was hypothesized to be influenced by the institutional intervention of the Government in the form of announcement of milk incentive scheme. The very aim of the scheme was to motivate farmers in rural areas to undertake dairying to increase milk production. Increased milk production may be due to increase in the number of high milk yielding bovines or through change in bovine composition ie., shift from indigenous cows/buffaloes to cross bred cows (Khalandar *et.al*, 2019). The provision of incentives may be considered as key for motivating farmers to increase milk production through switching over to high yielding crossbred cows. Hence, it becomes imperative to decipher the influence of milk incentive scheme and change in the bovine composition captured via share of milch crossbred cows in total milch bovines on milk productivity. Linear relationship was established between independent and dependent variables. Institutional intervention was observed in three phases since, it is a qualitative variable, its influence was captured using three dummy variables. The functional form is presented below

Milk Productivity = $a + b_1D_1 + b_2D_2 + b_3D_3 + b_4X + e$

D_1 is a dummy variable taking value 1, 0, 0 to represent milk incentive of Rs. 2 per litre announced during 2008

D_2 is a dummy variable taking value 0, 1, 0 to represent milk incentive of Rs. 4 per litre announced during 2013

D_3 is a dummy variable taking value 0, 0, 1 to represent milk incentive of Rs. 5 per litre announced during 2016

X – ratio of number of milch crossbred cows in total number of milch bovines

e- stochastic error term

a indicates Y-intercept

b_1, b_2, b_3, b_4 indicates partial regression coefficients

Results and Discussion

Transition in milch bovine population in Karnataka

Number of indigenous milch cows and buffaloes exhibited negative growth in Karnataka state over the livestock census at the rate of 14.04 and 9.02 percent, respectively. The scenario was not that different in case of districts coming under the jurisdiction of SHIMUL ie., Shivamogga, Chitradurga and Davangere. The negative growth of 1.26 and 10.85 percent in Shivamogga, 2.03 and 2.79 percent in Chitradurga and 12.94 and 7.22 percent in Davangere was observed. With respect to crossbred cows, the rate of growth in their number was positive in case of Karnataka, Shivamogga, Chitradurga and Davangere at 35.76, 42.41, 60.75 and 39.85 percent respectively (Table 1). The percent change indicates the relative changes or deviation in relative terms occurred in the number of milch bovines. The percentage change was negative at 49.97 and 32.68 percent with respect to indigenous cows and buffaloes in case of Karnataka state while it was positive at 233.04 percent in case of crossbred cows. It reiterates the results of exponential growth model. Similar pattern of deviation was observed in the districts coming under the jurisdiction of SHIMUL. Number of respective category of milch bovine has exhibited a declining trend over the census compared to the base period (1997). The growth rate provides an insight about the transition occurred in the bovine composition among the dairy farmers. Farmers have shown an inclination towards rearing of crossbred cows which are input responsive and high yielding compared to indigenous milch cows and buffaloes. Though, from food security point of view it is a welcoming sign but contrarily on sustainability front, it could be regarded as a forewarning.

Growth in milk production in Karnataka

The temporal progress in milk production could be ascertained through estimation of growth rates. Increased production may be due to increase in number of milch animals (extensive production) or due to increased productivity (intensive production). Karnataka state as a whole exhibited positive but a meager growth of 0.9 and 2.81percent in milk production of indigenous milch cows and buffaloes while the rate of growth in milk production among crossbred cows stood at 37.46 percent. Increased milk production in case of indigenous milch cows and buffaloes was due to increased productivity and could be inferred as intensive production while in case of crossbred cows, increased production was due to increase in the number of milch animals and hence inferred as extensive production.

The rate of growth in milk production of crossbred milch cows in case of Shivamogga, Chitradurga and Davangere was positive and substantial at 42.95,49.61 and 46.74 percent, respectively. The milk production of indigenous milch cows exhibited positive growth of 8.13 percent and 12.82 percent in Shivamogga and Davangere districts, respectively while it was negative at 7.08 percent in case of Chitradurga. Negative rate of growth of 8.81 percent and 13.19 percent was observed in case of buffalo's milk production in Shivamogga and Chitradurga districts, respectively while it was positive in case of Davangere at 9.25 percent. The negative growth in milk production of indigenous milch cows/ buffaloes was due to the deceleration in their actual numbers which superseded the influence of their positive rate of growth in productivity.

Table 1: Transition in milch bovine composition in Karnataka state and districts coming under SHIMUL

	Milch animals ('000)				
Livestock census	Indigenous cows ('000)	Crossbred cows ('000)	Buffaloes ('000)	Milch cattle ('000)	Milch bovines ('000)
		Karnataka			
1997	2978	690	2335	3668	6003
2003	2500	903	2215	3403	5618
2007	2656	1259	2374	3915	6289
2012	2201	1732	1898	3933	5831
2019	1490	2298	1572	3788	5360
CGR (%)	-14.04	35.76	-9.02	2.11	-1.88
Percent change	-49.97	233.04	-32.68	3.27	-10.71
		Shivamogga			
1997	163	21	103	184	287
2003	150	30	94	180	274
2007	163	42	91	205	296
2012	152	61	71	213	284
CGR (%)	-1.26	42.41	-10.85	5.86	0.46
Percent change	-6.75	190.48	-31.07	15.76	-1.05
		Chitradurga			
1997	76	5	93	81	174
2003	73	9	100	82	182
2007	83	14	106	97	203
2012	68	21	83	89	172
CGR (%)	-2.03	60.75	-2.79	4.61	0.75
Percent change	-11.76	76.19	-12.05	8.99	-1.16
		Davanagere			
1997	92	25	120	117	237
2003	77	39	122	116	238
2007	81	58	120	139	259
2012	57	67	94	124	218
CGR (%)	-12.94	39.85	-7.22	3.62	-1.65
Percent change	-38.04	168.00	-21.67	5.98	-8.02

Growth in milk productivity in Karnataka

Karnataka state as a whole experienced positive and substantial rate of growth of 9.82 and 8.64 percent in terms of milk productivity of indigenous milch cows and buffaloes, respectively while the rate of growth was a meager 0.89 percent in case of crossbred cows. Shivamogga and Davanagere also experienced similar trend in terms of milk productivity of indigenous cows, buffaloes and crossbred cows. The trend was exactly opposite in case of Chitradurga district wherein negative growth was observed in terms of milk productivity across indigenous milch cows, buffaloes and crossbred cows.

Impact of 'milk incentive scheme' on milk production in Karnataka

The estimated production function was found to be a good fit with significant 'F' statistic (32.24) and adjusted coefficient of multiple determination of 0.85 indicating that the included independent variables could explain variation in the dependent variable to the tune of 85 percent. The estimates of the production function were found to be statistically significant at less than one percent alpha for all the variables excepting the variable indicating share of cross bred cows in total milch bovine population. Though, it was not significant but it was economically substantial. The estimated milk production in Karnataka state could be deciphered for different situations viz., without price incentive and with price incentive. The milk production was estimated inserting the average value of independent variable (share of cross bred cows in total milch bovine population) in the

Table 2: Growth in Milk production in Karnataka and districts coming under SHIMUL

	Milk Production ('000MT)				
Livestock census	Indigenous cows	Crossbred cows	Buffaloes	Milch cattle	Milch bovines
		Karnataka			
1997	1427	920	1601	2347	3948
2003	1070	1398	1350	2468	3818
2007	1229	1648	1387	2877	4264
2012	1404	2515	1740	3919	5659
CGR (%)	0.90	37.46	2.81	18.43	12.64
Percent change	-1.61	173.37	8.68	66.98	43.34
		Shivamogga			
1997	65	26	81	91	172
2003	60	42	41	102	143
2007	73	46	55	119	174
2012	79	83	54	162	216
CGR (%)	8.13	42.95	-8.81	20.74	9.19
Percent change	21.54	219.23	-33.33	78.02	25.58
		Chitradurga			
1997	60	9	112	69	181
2003	29	12	43	41	84
2007	33	15	56	48	104
2012	45	32	64	77	141
CGR (%)	-7.08	49.61	-13.19	4.99	-5.22
Percent change	-33.33	71.88	-75.00	10.39	-28.37
		Davanagere			
1997	32	32	58	64	122
2003	32	49	58	81	139
2007	36	59	65	95	160
2012	46	108	75	154	229
CGR (%)	12.82	46.74	9.25	32.23	22.50
Percent change	43.75	237.50	29.31	140.63	87.70

estimated milk production function. Noteworthy observation is that the share of crossbred cows in total bovines has increased from 0.23 prior to implementation of milk incentive scheme to 0.40 with milk incentive of Rs. 5 per litre. The estimated milk production function deciphered using estimated milk production function is depicted in Table 5. The estimates of milk production in '000 tonnes without incentive came to 4204 which rose to 7564 after revision of incentive to Rs. 5 per litre. The percent rise in milk production after the implementation of incentive scheme with

Table 3: Growth in Milk productivity in Karnataka and districts coming under SHIMUL (Litres per cow per lactation)

Livestock census	Indigenous cows	Crossbred cows	Buffaloes	Milch cattle	Milch bovines
		Karnataka			
1997	479.18	1333.33	685.65	639.86	657.67
2003	428.00	1548.17	609.48	725.24	679.60
2007	462.73	1308.98	584.25	734.87	678.01
2012	637.89	1452.08	916.75	996.44	970.50
CGR(%)	9.82	0.89	8.64	14.36	12.36
Percent change	33.12	8.91	33.71	55.73	47.57
		Shivamogga			
1997	398.77	1238.10	786.41	494.57	599.30
2003	400.00	1400.00	436.17	566.67	521.90
2007	447.85	1095.24	604.40	580.49	587.84
2012	519.74	1360.66	760.56	760.56	760.56
CGR(%)	9.50	0.38	2.29	14.06	8.70
Percent change	30.33	9.90	-3.29	53.78	26.91
		Chitradurga			
1997	789.47	1800.00	1204.30	851.85	1040.23
2003	397.26	1333.33	430.00	500.00	461.54
2007	397.59	1071.43	528.30	494.85	512.32
2012	661.76	1523.81	771.08	865.17	819.77
CGR(%)	-5.15	-6.93	-10.70	0.36	-5.92
Percent change	-16.18	-15.34	-35.97	1.56	-21.19
		Davanagere			
1997	347.83	1280.00	483.33	547.01	514.77
2003	415.58	1256.41	475.41	698.28	584.03
2007	444.44	1017.24	541.67	683.45	617.76
2012	807.02	1611.94	797.87	1241.94	1050.46
CGR (%)	29.59	4.92	17.75	27.61	24.56
Percent change	132.02	25.93	65.08	127.04	104.06

Table 4: Estimates of milk production function

Independent variables	Estimates (t- Value)
Ratio of milch crossbred cows in total milch bovines	2724(1.14)
D1- Dummy variable to capture the influence of Milk incentive of Rs. 2 per litre announced during 2008	793 (2.75)
D2 Dummy variable to capture the influence of Milk incentive of Rs. 4 announced per litre during 2013	1605(3.87)
D3 Dummy variable to capture the influence of Milk incentive of Rs. 5 announced per litre during 2016	2896(5.81)
Intercept	3578 (6.14)

Dependent variable – Milk Production ('000 tonnes) incentive of Rs. 2 per litre was 20.80 from the milk production observed during control period (4204 '000 tonnes). It rose to 44.65 percent on revising incentive price to Rs. 4 per litre and 79.90 percent on revising incentive to Rs. 5 per litre from the control.

Table 5: Estimated Milk production in Karnataka as influenced by milk incentive scheme and ratio of crossbred to total milch bovine population

Particulars	Year of announcement of incentive price	Average value of Share of milch crossbred cows in total milch bovines (X)	Estimated milk production function	Estimated milk production ('000 tonnes)
Without Price incentive	Prior to 2008	0.23	Milk production = 3578 + 2724 X	4204.52
With price incentive of Rs. 2 per litre	2008	0.26	Milk production = 3578 + 793D_1 + 2724 X	5079.24
With price incentive of Rs. 4 per litre	2013	0.33	Milk production = 3578 + 1605D_2+2724 X	6082.00
With price incentive of Rs. 5 per litre	2016	0.40	Milk production = 3578 + 2896 D_3+ 2724 X	7564.00

Table 6: Estimates of milk productivity function

Independent variables	Estimates (t- Value)
Ratio of milch crossbred cows in total milch bovines	0.19 (0.37)
D1- Dummy variable to capture the influence of Milk incentive of Rs. 2 per litre announced during 2008	0.19 (3.21)
D2 Dummy variable to capture the influence of Milk incentive of Rs. 4 announced per litre during 2013	0.40 (4.66)
D3 Dummy variable to capture the influence of Milk incentive of Rs. 5 announced per litre during 2016	0.71 (6.82)
Intercept	0.60 (4.97)

Table 7: Estimated Milk productivity in Karnataka as influenced by milk incentive price scheme and ratio of crossbred to total milch bovine population

Particulars	Year of announcement of incentive price	Average value of Share of milch crossbred cows in total milch bovines (X)	Estimated milk productivity function	Estimated milk productivity (litres per cow per lactation)
Without Price incentive	Prior to 2008	0.23	Milk productivity = 0.60 + 0.19 X	643.70
With price incentive of Rs. 2 per litre	2008	0.26	Milk productivity = 0.60 + 0.19D_1 + 0.19 X	839.40
With price incentive of Rs. 4 per litre	2013	0.33	Milk productivity = 0.60 + 0.40D_2+0.19 X	1063.00
With price incentive of Rs. 5 per litre	2016	0.40	Milk productivity = 0.60 + 0.71 D_3+ 0.19 X	1386.00

Estimated Milk production = $3578 + 793D_1 + 1605D_2 + 2896\,D_3 + 2724\,X$

Impact of milk incentive scheme on milk productivity in Karnataka

The same analogy was used to estimate the milk productivity function hypothesizing that productivity is influenced by the breed composition reflected in the share of cross bred cows in the total bovine population and institutional intervention in the form of milk incentive scheme. The estimated productivity

Table 8: Temporal growth in coverage of dairy farmers and turnover of KMF in Karnataka

Particulars	1976	2009	2013	2021	CGR % between 1976 to 2009	CGR % between 2009 to 2022
Dairy Cooperative societies (no.)	416	11542	13889	16997	10.59	3.02
Milk producer members (lakh no.)	0.37	20.19	22.47	25.82	12.88	1.91
Milk procurement (lakh kg per day)	0.5	36.68	51.65	81.63	13.90	6.35
Daily payment to farmers (crores)	0.09	4.49	12.02	22.74	12.58	13.29
Total turnover of KMF (crores)	8.82	3135	9089	18032	19.48	14.41

Table 9: Impact of milk incentive price on the profitability of dairy farming

(n=60, Average herd size = 5 milch cows)

Particulars	Qty	Rate	Value	Share (%)	Cost/ Returns per litre
Variable cost					
Human labour (mandays)					
Men	258.00	322.13	83108.25	20.48	5.00
Women	302.00	157.63	47602.75	11.73	2.87
Inputs					
Feed (tonnes)					
Adult cows					
Dry fodder	9.93	4020.00	39928.65	9.84	2.40
Green fodder	29.29	520.00	15231.45	3.75	0.92
Concentrates	5.74	20000.00	114760.00	28.29	6.91
Calves					
Dry fodder	1.66	4020.00	6662.40	1.64	0.40
Green fodder	4.50	520.00	2339.61	0.58	0.14
Concentrates	0.25	20000.00	4960.00	1.22	0.30
Milk (litres)	159.38	25.60	4080.00	1.01	0.25
Medicines			2755.00	0.68	0.17
Artificial insemination			215.63	0.05	0.01
Electricity			2531.25	0.62	0.15
Insurance premium			3082.50	0.76	0.19
Interest on working capital			11454.01	2.82	0.69
Total variable cost			338711.49	83.48	20.40
Fixed costs					
Rental value of land			217.00	0.05	0.01

Interest on fixed capital			39807.24	9.81	2.40
Depreciation			26980.39	6.65	1.62
Total fixed cost			67004.62	16.52	4.03
Total cost			405716.11	100.00	24.43
Returns					
Milk (without price incentive)	16605.50	23.00	381926.50		
Milk (with price incentive of Rs. 5 per litre)	16605.50	28.00	464954.00		
Cowdung (Tractor loads)	4.70	9890.00	46483.00		
Sale of female calf (no.)	2.00	9000.00	18000.00		
Appreciation of adult cows	5.00	5750.00	28750.00		
Gross returns (without price incentive)			475159.50		28.61
Net returns (without price incentive)			69443.39		4.18
Gross returns (with price incentive)			558187.00		33.61
Net returns (with price incentive)			152470.89		9.18

Table 10: Opinion of farmers about milk incentive price scheme

Particulars	Frequency (%)
Incentive price is the root cause for transition in breed composition	43 (71.67)
Incentive price of Rs. 5 per litre announced for milk is not sufficient	60 (100.00)
Undue delay is very common in the payment of incentive prices to dairy farmers	60 (100.00)
Incentive prices given not covers the rising prices of inputs such as dry fodder, concentrates and human labour in dairy farming	53 (88.33)
Payment of incentive prices should be done regularly on monthly basis	46 (76.67)
Increase in milk price or provision of additional incentive of Rs. 5 per litre totaling to Rs. 10 per litre will benefit dairy farmers in the eve of escalating input prices	60 (100.00)

function was found to be a good fit with significant ‘F’ statistic (36.09) and adjusted coefficient of multiple determination of 0.86 indicating that the included independent variables could explain variation in the dependent variable to the tune of 86 percent. The estimates of the productivity function were found to be statistically significant at less than one percent alpha for all the variables excepting the variable reflecting breed composition ie., share of cross bred cows in total milch bovine population. Though, it was not significant but it was economically substantial. The milk productivity was estimated inserting the average value of share of cross bred cows in total milch bovine population in the estimated milk productivity function. The estimated milk productivity function deciphered using estimated milk productivity function is depicted in Table 7. The estimates of milk productivity in litres without incentive came to 644 which rose to 1386 after revision of incentive to Rs. 5 per litre. The percent rise in milk production after the implementation of incentive scheme with incentive of Rs. 2 per litre was 30.34 from the milk productivity observed during control period (644 litres). It rose to 65.06 percent on revising incentive price to Rs. 4 per litre and 115.22 percent on revising incentive to Rs. 5 per litre from the control.

Estimated Milk productivity = $0.60 + 0.19D_1 + 0.40D_2 + 0.71\ D_3 + 0.19X$

Impact of milk incentive scheme on growth of Karnataka Cooperative Milk Producers’ Federation limited

Karnataka Cooperative Milk Producers’ Federation Limited (KMF) is the apex body working in the dairy sector in Karnataka state. It is considered as the second largest dairy cooperatives in the country. In Southern India, it stands first in terms of milk procurement and sales. KMF is one among few federations in the country, which has converted dairying from a subsidiary occupation to an industry. It works in line with that of AMUL and started functioning in letter and spirit from 1974-75. One of the objectives of milk incentive scheme was to bring in enormous growth in cooperatives relating to dairy sector i.e., KMF.

The perusal of Table 8 indicates the progress of KMF from 1976 to 2021 in terms of coverage of dairy members and its turnover. To ascertain the influence of milk incentive scheme on its growth, factors such as number of dairy cooperative societies, milk producer members, daily procurement of milk, daily payment made to dairy farmers and annual turnover of KMF was considered. Growth was assessed through computation of compound growth rate using exponential model. It was estimated for two time periods ie., prior to implementation of milk incentive scheme (1976 -2009) and after the implementation of scheme (2009-2021). The rate of growth in number of dairy cooperative societies was around 10.59 percent over the span of almost three decades prior to implementation of scheme while it was 3 percent in a span of decade after the implementation of scheme. In terms of membership (coverage of dairy farmers), the rate of growth was 12.88 percent prior to the scheme while it was 1.91 percent after the implementation of scheme. The noteworthy observation in Table 8 is all about the average daily procurement of milk which was 36.68 lakk kg per day during 2009 which got almost doubled in a decade during 2021 (81.63 lakh kg per day). In terms of payment made to dairy farmers, the scenario was almost the same as above wherein payment made to farmers was around Rs. 4.49 crores which increased by almost five times to Rs. 22.74 crores during 2021. The economic performance of KMF captured in terms of its total turnover exhibited the same scenario wherein the total turnover was Rs. 3135 crores which rose to Rs. 18032 by 2021. The rate of growth in turnover was around 19.48 percent over the span of three decades while it was 14.41 percent for a span of decade signals the extent of business growth of KMF. The rate of growth exhibited by the average daily milk procurement was 13.90 percent over a span of three decades while 6.35 percent of growth was witnessed in a span of one decade after the implementation of milk incentive scheme. From the preceding discussion, it could be inferred that implementation of milk incentive scheme by Government of Karnataka has paved a way for enormous physical and economic growth of KMF ie., cooperative sector in dairy industry.

Impact of milk incentive scheme on profitability of dairy farming

The economics of milk production has been worked out considering the average herd size of five milch animals having breed composition of four cross bred cows and one indigenous cow. The economics comprised of three sub parts viz., variable costs, fixed costs and returns (Sunil et al. 2016 and Revappa et al. 2022). The total cost incurred towards production of 16605.50 litres of milk came to Rs. 405716.11. The variable costs formed 83.48 percent of the total cost at Rs. 338711.49 while the remaining amount was shared by fixed cost at Rs. 67004.62. Of the variable cost, the expenditure made on feed and human labour cornered major chunk at Rs. 187962.1 and Rs. 130711. The opportunity cost of working capital estimated at 7 percent rate of interest apportioned for 6 months came to Rs. 11454.01was the next major variable cost item. Depreciation of assets and interest on fixed capital cornered major share in the total fixed costs. Returns were realized from the sale of milk, calves, cowdung and also from the appreciation in the value of lactating animals (Sadananda et. al. 2022). The gross returns and net returns without milk incentive came to Rs. 475159.50 and Rs. 69443.39**.** The gross returns and net returns after considering incentive of Rs. 5 per litre came to Rs. 558187.00 and 152470.89, respectively (Table 9).

The per litre cost of production of milk was estimated to be Rs. 24.43 of which Rs. 20.40 was on variable costs and Rs. 4.03 on fixed costs. Among the total cost per litre of milk, feed for adult milch animals and calves formed Rs.11.42 followed by human labour required for maintenance of dairy unit at Rs. 7.87. The price paid by the Milk union to the dairy farmers per litre was Rs. 23 exclusive of incentive. A cursory look at the disparity in cost incurred and price realized per litre of milk is the prima facie indicator of rising input prices in dairy industry and the inability of milk prices to keep the rising pace of the input prices. To make dairy industry a profitable venture, provision of incentive is a sine-qua-non for the government. The price realized per litre by the farmers after giving due consideration for the returns realized from the sale of by-products came to Rs. 28.61 leaving the net returns of Rs. 4.18 per lite. The returns from by-product is not certain hence, returns from After considering the incentive of Rs. 5 per litre, the realized price per litre inclusive of returns from the sale of by-products came to Rs. 33.61 leaving the net returns per litre of Rs. 9.18. From the preceding results, it could be inferred that on providing incentive of Rs. 5 per litre, dairy farmers could able to reap 120 percent extra profit per litre (Table 9).

Opinion of farmers regarding milk incentive scheme

Undue delay in the payment of incentive prices, insufficiency of incentive price to cover rising prices of inputs such as concentrates, dry fodder and human labour are the major impediments encountered by the dairy farmers with respect to milk incentive scheme. Cent percent of the farmers are of the opinion that incentive prices should be hiked to Rs. 10 per litre or the prices paid per litre of milk should be increased by Rs. 5 from the existing payment (Rs. 23 per litre) to enable them to absorb the brunt of rising input prices. Vast majority (76.67 percent) of the dairy farmers expressed that payment of incentive prices should be made on regular basis at an monthly interval (Table 10).

The study indicated deceleration in the number of indigenous cows and buffaloes in the Karnataka state which is a cause of concern while cross bred cows have accelerated over the time period. Increased milk production in cross bred cows was due to increase in their actual number and not because of increase in productivity while indigenous cows and buffaloes exhibited increased production due to increase in productivity is a welcoming sign. The incentive price of Rs. 5 announced by the Government of Karnataka has resulted in increased milk production and productivity to the tune of 79.90 and 115.22 percent, respectively compared to the years without incentive price. Dairy farmers opined that delay in the payment of incentive prices and its insufficiency are the major flipsides of the scheme.

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RESEARCH ARTICLE

Knowledge level of dairy farmers about bovine tuberculosis as neglected zoonosis

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Abstract: The study entitled "consciousness of dairy farmers about Bovine Tuberculosis as neglected zoonosis" was carried out between the periods of August to October 2020. A total of 180 dairy farmers from 36 villages of 6 talukas of Panchmahals and Mahisagar districts of Gujarat state (India) were randomly selected. Most dairy farmers (95.00%) had high to very high consciousness about bovine tuberculosis. The majority of dairy farmers had a medium to high level of knowledge (71.11%), a very high level of sensitivity (77.78%), and a favorable to most favourable attitude (95.55%) towards the control of bovine tuberculosis. Path analysis shows the maximum positive direct effect exerted by scientific orientation, mass media exposure, and education. Economic motivation, extension participation, and training exerted maximum indirect positive effects. Dairy farmers can enhance their consciousness about bovine tuberculosis through formal education and participation in social-extension activities using mass media, the internet, and other psychological variables. The research findings serve as a guideline for training institutions, researchers, planners, extension agencies, and concerned organizations to form effective & realistic training programmes to impart consciousness amongst dairy farmers about tuberculosis and other zoonoses.

Keywords: Attitude, Consciousness, Dairy farmers, Knowledge, Tuberculosis, Sensitivity, Zoonosis

Introduction

Tuberculosis (TB) is one of the zoonotic & re-emerging diseases which cause multi-billion-rupees loss and human death annually worldwide. The disease affects humans and animals caused by a group of bacteria called *Mycobacterium tuberculosis* complex of different species, including *Mycobacterium tuberculosis* and *Mycobacterium bovis* (Thoen et al. 2009). Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis,* which is a highly prevalent infectious disease of cattle, buffaloes, and many wild species worldwide (Ameni et al. 2007; Le Roex et al. 2013). Bovine tuberculosis causes a significant economic impact accounting 10 to 25 per cent loss in productive effi-ciency in dairy cattle due to its chronic and progressive nature, often resulting in high morbid-ity in animals. The World Health Organization has classified bovine tuberculosis as one of the seven neglected zoonotic diseases having the potential to infect human beings, either by consuming raw milk, meat, and their products (Malama et al. 2013) or by direct contact or inhaling infective droplets of infected animals. There is a higher risk to dairy farmers who live in rural areas and keep animals. A direct correlation between *M. bovis* infection in cattle and the disease in the human population has been well documented. Srinivasan et al. (2018) revealed a pooled prevalence estimate of 7.3 per cent based on a random-effects meta-regression model, indicating that there may be an estimated 21.8 million infected cattle in India. In addition to being a threat to public health, bovine tuberculosis is also a significant economic concern, costing an estimated US $3 billion worldwide annually due to losses from reduced cattle productivity, culling and movement and trade restrictions (Waters et al. 2012). The annual costs to farmers only from loss in milk production in cows and buffaloes in India are estimated to range between US $375 and 544 million (Srinivasan et al. 2018). Rahman and Samad (2009) showed a 17% decrease in cow milk yield in Bangladesh. Boland et al. (2010) showed a significant decrease in milk production in BTB reactors in Irish dairy farms ranging from 120 to 573 kg milk loss per lactation. This implies that dairy farmers must be educated to acquire a higher level of consciousness and technical skills to prevent and control tuberculosis to avoid economic losses and animal and human health hazards.

Materials and Methods

Study area

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The study was conducted in the Panchmahals and Mahisagar districts of Gujarat state (India) from August to October 2020. Panchmahals district has seven talukas (Jambughoda, Shehera, Godhra, Halol, Kalol, Morwa (Hadaf), and Ghoghamba). Mahisagar district consists of six talukas (Kadana, Virpur, Santrampur, Balasinor, Lunawada, and Khanpur). The cattle and buffalo populations are 3,35,220 and 4,34,464 in Panchmahals and 3,08,108 and 4,06,003 in the Mahisagar district, respectively (20th Livestock census, 2019).

Study design

Three talukas were selected from the Panchmahals and Mahisagar districts based on the higher bovine population. Six villages from each taluka were selected randomly. Five dairy farmers were selected from each village randomly.

Survey instruments

The independent variables of this research study were measured with the help of appropriate scales used by other researchers with due modification. To measure the consciousness of dairy farmers, a standardized scale was developed by Jadav and Patel, (2021), which is used. Consciousness means that the state of being awake, aware of what is around, and able to think. Consciousness is a term used to describe the awareness of one's physical and mental experience (Tononi and Koch, 2015). Knowledge about bTB and attitude towards control of bTB were measured with the help of a scale developed by the researcher, and sensitivity towards bTB was measured by the teacher-made scale.

Knowledge, attitude and sensitivity score

In the knowledge score aspect, a weightage of 1 was assigned to the correct answer, and a weightage of "0" was given to an incorrect answer. The possibility of getting the knowledge scores ranged between 0 and 15 for an individual. Concerning sensitivity scores, 1 and 0 were assigned for correct and wrong responses attributed by a dairy farmer, respectively. There were a total of ten sensitivity statements for bTB. For the attitude score aspect, out of the 12 selected statements, eleven were positive and had indicators of a favourable attitude, and one was negative and had an unfavourable attitude. Items related to attitude were measured on a Likert scale ranging from 1 to 5 (1 = strongly disagree to 5 = strongly agree), with higher scores indicating the most desired attitude. The possibility of getting the attitude scores ranged from 12 to 60 for an individual.

Data analysis

The arbitrary method categorizes respondents into very low, low, medium, high and very high categories. The coefficient of correlation (r) was calculated to know the relationship between each of independent variables and dependent variable. Path coefficient analysis was used to know the direct and indirect effect of independent factors on the consciousness of dairy farmers about bTB (Wright, 1921). The primary data were collected with the help of a pre-tested Gujarati version of the interview schedule by personal interview, and then after it was compiled, tabulated, and analyzed to get a proper answer for the specific objectives of the study with the help of various appropriate statistical tools. SPSS 21 (IBM, USA) processed the collected data.

Ethics statement

Researchers had verbal consent with respondents for the right of confidentiality of information that they provided us.

Results and Discussion

Knowledge of dairy farmers about bovine tuberculosis, including the general part of disease, transmission route, symptoms in animals, symptoms in humans, and prevention & control aspects, was studied. The mean percentage score for knowledge was 62.00 (SD: 17.00). It can be observed in Table 1 that more than half (56.67%) of the dairy farmers knew that zebu cattle are relatively more resistant to bovine tuberculosis than exotic cattle. Humped cattle are considered to be more genetically resistant to bTB than non-humped cattle (Murray et al. 2013). The majority of dairy farmers (83.89%) knew that common feeding & drinking troughs are important sources of tuberculosis infection. Almost half of the dairy farmers (47.22%) knew tuberculosis could transmit through infected animals' faeces & urine. The majority of the dairy farmers (78.33%) had knowledge that poor hygiene and poor ventilation can contribute to the entry and establishment of tuberculosis. More than one-third of dairy farmers (40.56%) knew that TB-infected animal milk was unsafe for human consumption. A similar finding was obtained by Bihon et al. (2021), who reported that 42.9 per cent of cattle owners knew that ingesting raw animal products (milk and meat) was the mode of transmission of bovine TB. Many participants (66.9%) disagreed that pasteurization of milk before consumption prevents tuberculosis, as reported by Hailu et al. (2022). The remaining dairy farmers still believed that raw milk from TB-infected animals was safe for human consumption. This shows a need for more information about the transmission of bTB as neglected zoonosis among farmers, especially those who directly or indirectly contact livestock, and to put more emphasis on the consumption of pasteurised milk. They drink the raw milk of animals as they believe in the good taste of milk. There was 71.11 per cent of them knew that TB can be transmitted via the inhalation route. A contrast finding was reported by Bihon et al. (2021), who conducted a study in Ethiopia and revealed that only 7.1 per cent of cattle owners knew that bovine TB could be transmitted through inhalation. Almost half of the dairy farmers did not know that human TB could infect cattle or vice-versa. So, half of the dairy farmers still did not know TB as zoonosis. A similar finding was reported by Hailu et al. (2022) that 75.6 per cent of respondents disagreed with the idea that tuberculosis can be transmitted from animals to humans and vice versa. More than two-thirds of them (72.22%) had known that bovine tuberculosis could be transmitted to other cattle animals. Addo et al. (2011) reported that only 33 per cent of the herdsmen knew about the mode of transmission of TB. Almost half of the dairy farmers knew about tuberculosis symptoms in animals, like loss of body weight, dry & painful cough, dyspnea, abortion in late pregnancy, milk secretion gradually diminished, and infertility. Haemoptysis, cough, and loss of body weight are the symptoms of tuberculosis in humans which had knowledge by the majority of dairy farmers (73.33%). Addo et al. (2011) reported that coughing was mentioned by 77 per cent of the herdsmen as a symptom of TB in cattle, while 54 per cent noted it

Table 1 Knowledge, sensitivity and attitude level of dairy farmers about tuberculosis (n=180)

Sr.No.	Knowledge statements	No. (%) of respondents
1	Zebu cattle are relatively more resistant to bovine tuberculosis than exotic cattle.	102 (56.67)
	Transmission route	
2	Common feeding and drinking troughs are important sources of tuberculosis infection.	151 (83.89)
3	Tuberculosis can transmit through infected animal's feaces & urine.	85 (47.22)
4	Poor hygiene and poor ventilation can contribute to the entry and establishment of tuberculosis.	141 (78.33)
5	TB infected animal's milk is not safe for human consumption.	73 (40.56)
6	TB can be transmitted via inhalation route.	128 (71.11)
7	Human TB is able to infect cattle.	90 (50.00)
8	Bovine tuberculosis can be transmitted to other cattle animals.	130 (72.22)
9	Bovine tuberculosis is able to transmit humans.	85 (47.22)
	Symptoms in animal (Suffering from tuberculosis)	
10	Lose body weight, dry & painful cough and dyspnoea are the symptoms of bovine tuberculosis.	89 (49.44)
11	Abortion in late pregnancy, milk secretion gradually diminished and infertility are the symptoms of bovine tuberculosis.	88 (48.89)
	Symptoms in human (Suffering from tuberculosis)	
12	Haemoptysis, cough and lose body weight are the symptoms of tuberculosis in human.	132 (73.33)
	Prevention & control aspects	
13	Tuberculosis can prevent through cleaning of feed and water troughs.	163 (90.56)
14	Calves can be prevented from tuberculosis through providing pasteurized milk.	123 (68.33)
15	Human TB is able to prevent by vaccination.	94 (52.22)
Sr.No.	Sensitivity statements	No. (%) of respondents
1	Sensitive regarding the effect of tuberculosis on human health.	163 (90.56)
2	Sensitive to wash your hands with soap before & after handling animals.	166 (92.22)
3	Sensitive to avoid children handling diseased animals.	144 (80.00)
4	Sensitive to avoid pregnant women to handle diseased animals.	135 (75.00)
5	Sensitive to avoid handling the dung of animals with bare hands.	171 (95.00)
6	Sensitive regarding cleaning and sanitizing the equipment used for handling milk.	172 (95.56)
7	Sensitive to apply a face mask, hand glove, and use boot while handling animals.	166 (92.22)
8	Sensitive to keep your animal sheds well ventilated & lighted.	168 (93.33)
9	Sensitive to prevent overcrowding in animal sheds.	165 (91.67)
10	Sensitive to keep animals suffering from tuberculosis away from the rest of the herd.	168 (93.33)
Sr. No.	Attitude statement	Mean Percent Score
1.	I believe that tuberculosis can be eradicated from our country. (+)	80.44
2.	I feel that tuberculosis is hazardous to human health. (+)	84.44

3.	There is a lot of propaganda about the tuberculosis, but it is not so in a real situation. (+)	73.56
4.	I would like to purchase tuberculosis -free animals for my farm. (+)	79.89
5.	I believe that tuberculosis is the current burning issue to handle seriously in dairy farming. (+)	73.00
6.	I believe that the sale of animals infected with tuberculosis is ethical. (-)	64.22
7.	I feel that constant monitoring is needed to control tuberculosis. (+)	79.89
8.	Tuberculosis -infected animals affect badly dairy farm income. (+)	81.78
9.	I believe that tuberculosis can be successfully controlled through managemental practices. (+)	80.22
10.	I believe that vaccination is the right way to prevent tuberculosis. (+)	82.33
11.	I feel that control of tuberculosis should be a prime goal of veterinary public health. (+)	80.00
12.	I believe that public awareness regarding tuberculosis is the need of hours. (+)	81.44

(-) indicate the negative question asked to respondents.

Table 2 Knowledge, Sensitivity & Attitude level of the dairy farmers about tuberculosis (n=180)

Sr.No.	Knowledge category	Frequency	Per cent
1	Very low (up to 20.00 per cent)	1	0.56
2	Low (20.01 to 40.00 per cent)	31	17.22
3	Medium (40.01 to 60.00 per cent)	62	34.44
4	High (60.01 to 80.00 per cent)	66	36.67
5	Very high (above 80.00 per cent)	20	11.11
	Total	180	100.00
Sr.No.	Sensitivity category	Frequency	Per cent
1	Very low (up to 20.00 per cent)	0	0.00
2	Low (20.01 to 40.00 per cent)	6	3.33
3	Medium (40.01 to 60.00 per cent)	6	3.33
4	High (60.01 to 80.00 per cent)	28	15.56
5	Very high (above 80.00 per cent)	140	77.78
	Total	180	100.00

as a symptom of TB in men. They have enough knowledge about the prevention of tuberculosis through cleaning feed and water troughs (90.56%), providing pasteurized milk (68.33%), and vaccination (52.22%). The mean percentage score for sensitivity was 89.89 (SD: 14.22). The majority of dairy farmers (92.22%) were sensitive towards hand washing with soap before & after handling animals and using face masks, hand gloves, and boots while handling animals. Similar findings were reported by Cakmur et al. (2015) study in Kars, Turkey, which stated that 91.4 per cent of the farmers washed their hands. About 92.1 per cent of them thought to use gloves while contacting animals. Regarding using masks, 84.1 per cent of the participants considered it necessary. Regarding wearing water-resistant boots during contact with animals, 89.4 per cent of the farmers felt it essential.

The majority of the dairy farmers had a positive attitude toward the control of tuberculosis, like vaccination (82.33 Mean Percent Score), awareness (81.44 MPS), and constant monitoring (79.89 MPS). These observations were more encouraging than the finding of Bihon et al. (2021); they reported that two-thirds of cattle owners (67.60%) had a positive attitude towards vaccination against TB. Most dairy farmers felt tuberculosis was hazardous to human health (84.44 MPS). Two-thirds of cattle owners (66.70%) regarded bovine tuberculosis as a significant public health threat reported by Bihon et al. (2021). Most dairy farmers (80.22 MPS) believed tuberculosis could be successfully controlled through managemental practices. Perceived health hazards effect of tuberculosis as a zoonotic disease on animals and human beings by the dairy farmers as well as economic losses caused by such a disease, the primary reasons for the formation of positivism toward control of tuberculosis might be the possible explanation for this result. The mean percentage score for attitude was 78.44 (SD: 9.30). The detailed analysis of knowledge, sensitivity, and attitude of dairy farmers towards control of tuberculosis is described in Table 1.

The data presented in Table 2 revealed that the majority of dairy farmers had a high (36.67%) to medium (34.44%) level of knowledge about tuberculosis. The government of India has run awareness programmes on tuberculosis for a long time, which might be the possible explanation for the high to medium level of knowledge about tuberculosis. Similar findings are reported by Thakkar, (2013). More than a third-fourth (77.78%) of dairy farmers had a very high sensitivity towards tuberculosis disease. A contrast finding is reported by Munisamy et al. (2017). More than half (58.33%) of dairy farmers had a favourable attitude towards

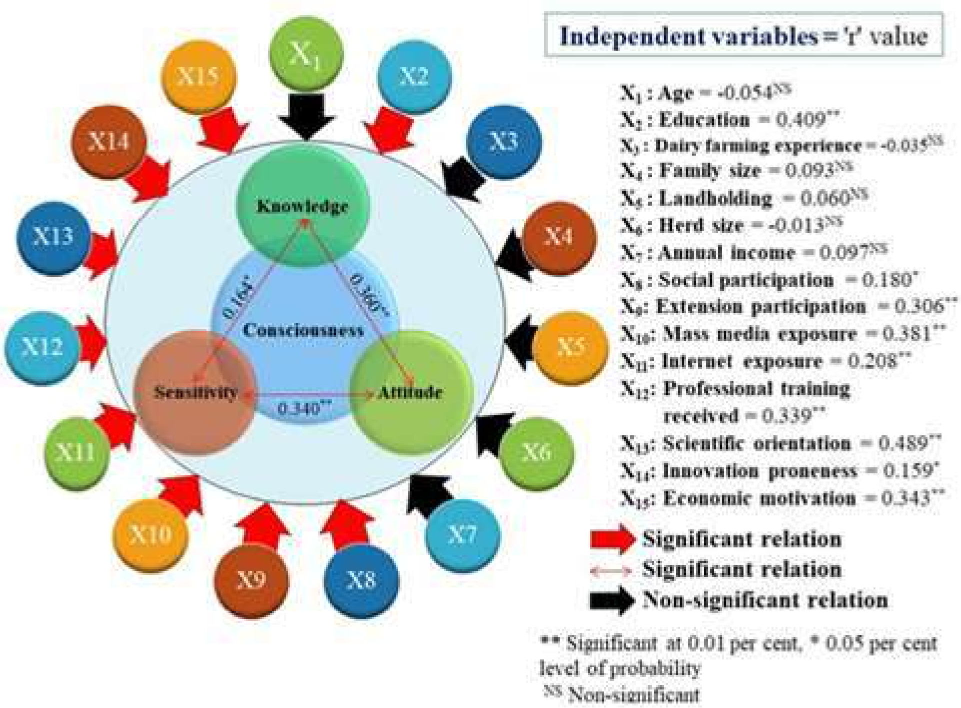

Fig. 1 An empirical model shows the cause of independent variables on consciousness of dairy farmers about bovine tuberculosis

control of tuberculosis disease, followed by 37.22 and 4.45 per cent of them had the most favorable and neutral attitude towards control of tuberculosis disease. This is because most respondents were literate and understood the seriousness of tuberculosis disease's effect on human and animal health. A similar finding reported by Yadav et al. (2022) that women in India have moderately good knowledge and a correct attitude towards tuberculosis.

The majority (62.78%) of dairy farmers had a high level of consciousness about tuberculosis, followed by 32.22 per cent very high level of consciousness (Table 3). A contrasting finding was reported by Bihon et al. (2021) in Ethiopia, who stated that only 18.6 per cent of cattle owners had a good KAP (Knowledge, Attitude, Practice) score level. The knowledge of dairy farmers about tuberculosis as a component of consciousness is still working to enhance the consciousness level of dairy farmers regarding tuberculosis as zoonosis.

The Pearson correlation analysis was conducted to identify the relationship between factors affecting the consciousness of dairy farmers about tuberculosis and independent variables. In Fig. 1, out of fifteen independent variables, nine had established a positive and significant relationship with the consciousness of dairy farmers about tuberculosis. The result shows that the consciousness of dairy farmers about tuberculosis was significantly increasing with an increase in education, participation in social-extension activities & training programmes, exposure to mass media & internet with scientific orientation, innovation proneness, and economic motivation. This result is in accordance with the observation of Mesfin et al. 2005; Bati et al. 2013; Bihon et al. 2021; Yadav et al. 2022 who stated that the education status of respondents was significantly associated with the KAP score of TB. The correlation between knowledge level with sensitivity level (0.164) and attitude level (0.360), and sensitivity with attitude (0.340) of dairy farmers regarding tuberculosis was found to be positive and significant. There was a non-significant relationship between age, experience in dairy farming, family size, landholding, herd size, and annual income with the consciousness of dairy farmers about tuberculosis as zoonosis. Cakmur et al. (2015) obtained a similar finding, which reported no statistically significant difference in knowledge-attitude-practice scores about zoonoses according to age.

Fig 2.

Path analysis - direct effect

The data in Fig. 2 revealed that the major variables contributing the maximum direct positive effect on the consciousness of tuberculosis were scientific orientation (0.356), mass media exposure (0.195), education, family size, professional training received, internet exposure, age, and landholding in descending order.

Path analysis - total indirect effect

Fourteen variables had a positive total indirect effect out of fifteen on the consciousness of dairy farmers about tuberculosis. Further, it can be observed that economic motivation had a

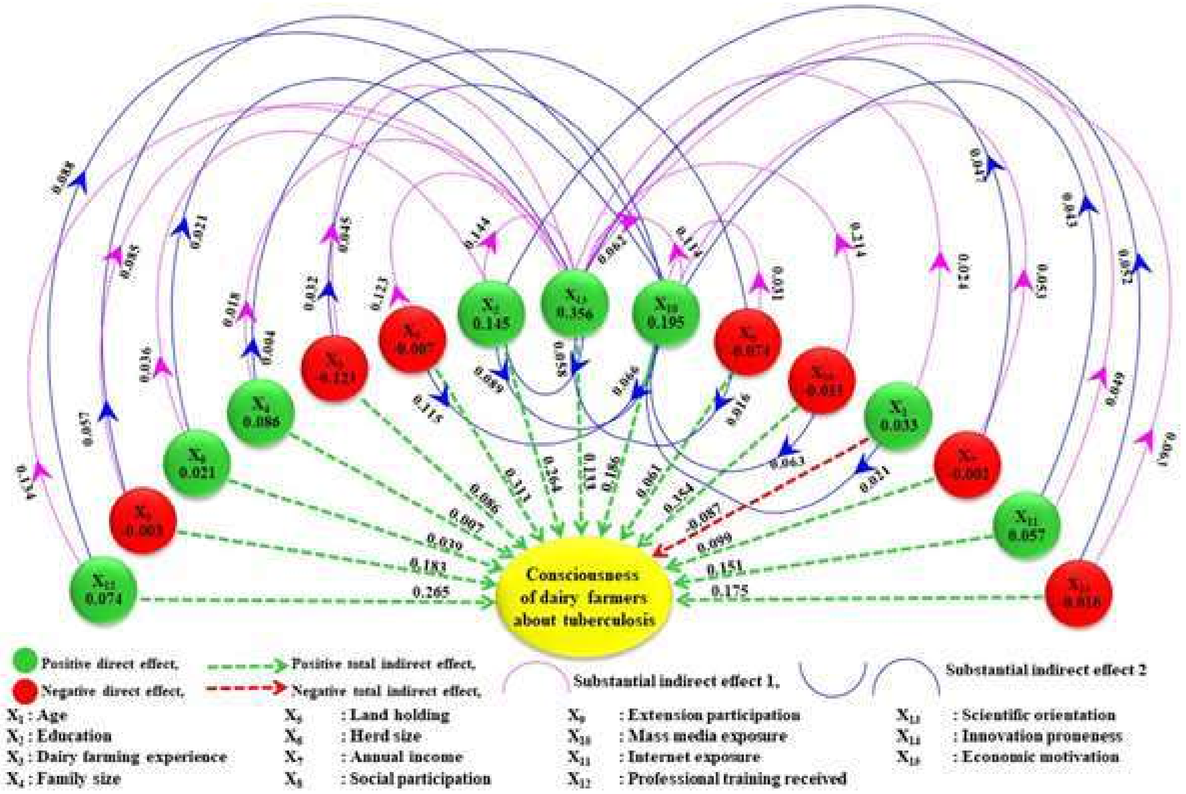

Fig. 2 Path diagram of direct and indirect effect of independent variables on consciousness of dairy farmers about bovine tuberculosis

maximum total indirect effect (0.354) through substantial indirect effect-1 [scientific orientation (0.214)] and substantial indirect effect-2 [mass media exposure (0.063)].

Path analysis - substantial indirect effect

Data further revealed that out of 30 substantial indirect effects, fourteen routed through scientific orientation, eleven through mass media exposure, four through education, and one through herd size.

To epitomize the result, scientific orientation and mass media exposure of dairy farmers was the critical variables in exerting a considerable direct and substantial effect on the determination of consciousness of tuberculosis. Economic motivation, extension participation, training, and education had exerted a higher indirect effect on dairy farmers' consciousness about tuberculosis, as seen in Fig. 2. A similar finding was reported by Jadav and Patel, 2022 for the dairy farmers' consciousness of rabies disease.

Conclusions

The majority of dairy farmers had high to very high level of consciousness about bovine tuberculosis. The majority of dairy farmers had a medium to high level of knowledge and a very high level of sensitivity with favorable to most favorable attitude towards the control of bovine tuberculosis. Veterinary extension wings should organize training programmes to improve the knowledge of bovine tuberculosis, especially on prevention & control aspects to enhance the consciousness of dairy farmers about bovine tuberculosis through education, scientific orientation, mass media exposure, economic motivation, & extension participation.

Acknowledgements

We immensely thank all respondents for their contribution towards the survey work.

Conflict of interest

The authors have not any conflict of interest to declare.

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SHORT COMMUNICATION

Prioritization of extension interventions for empowering resource poor dairy farm households in Haryana state

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Abstract: Dairy farming is practised mainly by resource poor farmers whose limited access to knowledge, skill, training and credit hamper their socio-economic and technological empowerment. Extension interventions would help these farmers to enhance their access to knowledge and technologies leading to better standard of living. Therefore, the present study was undertaken in three districts of Haryana state namely Karnal, Jind and Jhajjar representing three different Agro climatic zones in Haryana in 2022 to understand their existing level of knowledge, adoption and needs in dairy farming. The study constituted a sample size of 120 respondents by selecting 40 respondents in a cluster of villages with the predominance of resource poor dairy households in each of selected districts. The findings revealed that most of the respondents were middle aged (44.17%), male (81.67%), acquired secondary level of education (26.67%), land holding upto 1 acre (35.83%), belonged to medium level of income group and had joint family (58.33%). Extension interventions were prioritised as per the expert opinion by using Analytical Hierarchy Process methodology which indicated that training has acquired the highest rank with a value of 0.35 followed by demonstration with value of 0.34. Among policy interventions, subsidy has acquired the highest rank with a value of 0.37 and then incentive acquired 2nd rank with value of 0.25. The study suggests the importance of intensive training, demonstration and exposure visit to research institution and progressive farmers for gradual empowerment of the resources poor farmers.

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Keywords: Resource poor farmers, Interventions, Dairy farming, Analytical Hierarchy Process

India is primarily an agrarian country with vast biological and ecological diversity. About 56.6 per cent of the population is engaged in agriculture and allied sectors. The average size of operational holdings has decreased from 2.28 hectares in 1970-71 to 1.84 hectares in 1980-81, to 1.41 hectares in 1995-96 and to 1.08 hectares in 2015-16. About 82 per cent of farmers are small and marginal engaged in agriculture and allied sectors (FAO, 2021). The share of Agriculture and Allied Sectors in Gross Value Added (GVA) of the country in the year 2020-21 is 20.2 per cent and share of livestock in the GVA of the country is 4.11 per cent (NSO, 2021). India has about 535.78 million livestock population as per the 20th livestock census. India maintains first rank in milk production at global level since 1998 and the Indian dairy farming is dominated by resource poor farm households. A family whose resources like water, land, labour and capital don't allow a secure livelihood of family is known as resource poor farm family. Even farmers with more than 2 ha of land holding but whose land is not fertile are prone to flood or erosion and having low and unreliable rainfall (Chamber and Ghidyal, 1984) who are also in the bracket of resource poor. Resources poor farmers are not able to harness the technologies because there is lacuna of sources and resources (Ponnusamy et al. 2021).

Empowering resource poor farmers is very vital because it will augment their income and harness the resources so that milk yield will increase and then it will increase the economy of the country (Ponnusamy and Padaria, 2021). Ponnusamy and Ambasankar (2006) observed that productivity of milch animals and profitablity of farmers could considerably be increased through introduction of various technological interventions like compound feed, salt lick, ectoparasiticide, minerals and vitamins to the cows and buffalos. Patel and Ponnusamy (2019) developed and validated extension strategies for managing reproductive problems of dairy animals with 60 respondents from three villages *viz.,* Dilawara, Chundipur and Dhakwada of Karnal district. Among 14 selected extension strategies, development and demonstration of video on specific reproductive issues with Rank Based Quotient (RBQ) value of 81.19 and preparation of extension literature with RBQ value of 72.38 were found to be the two best strategies for faster dissemination of information on management of reproductive problems. This explains the effectiveness of extension strategies in improving the socio-economic conditions of resource poor dairy farm households. Therefore, a study was undertaken to determine the appropriate extension strategies for the empowerment of poor dairy farm households.

The present study was conducted in the Haryana state in three districts namely, Karnal, Jind, and Jhajjar which were randomly selected representing three different Agro climatic zones of the state as classified by Haryana Kisan Aayog. From the selected districts, cluster of villages with the predominance of resource poor dairy households were selected in consultation with the Krishi Vigyan Kendra of respective districts. From each selected district, 40 respondents were selected randomly. Thus, the study was conducted with 120 respondents. The dairy farmers having less than or equal to one ha of land and possession of herd size up to five dairy animals were selected wherein it was ensured that more than 50 per cent income should come from dairy farming at the time of investigation as respondents for the study.

The data were collected personally through well-structured and pre tested interview schedule. Suitable statistical tools and technique like Analytical Hierarchy Process (AHP), Frequency and Percentage and Cumulative Square Root Frequency were used in data analysis in order to draw meaningful conclusions.

Tabular analysis was used to understand the socio-economic conditions of resource poor dairy farm households. Procedure for Analytical Hierarchy Process: When application of AHP is done for any decision-making, the first requirement is the breaking up of the decision problem into decision element in a hierarchical fashion. The predecessor of AHP is pair-wise comparison in psychological measurement. AHP is based on pair-wise comparison, but associated with hierarchical formulation of multi-criteria. Thus, this has obvious advantage of providing 'objective decision', based on subjective and personal preference of an individual. The advantage of AHP includes its ability to make both qualitative and quantitative decision attributes commensurable, and its flexibility with regard to the setting of objectives (Kangas, 1992). Subjective preference, expert knowledge and objective information can all be included in the one and the same decision analysis (Kurttila *et al*, 2000).

In order to better understand the prioritisation of extension interventions, it is important to know the socio-economic background of the resource poor dairy farm households as both are closed related.

The socio-economic variables which were identified for assessing the status of respondents are listed in Table 1. Most of the respondents belonged to middle aged group (44.17%), middle to secondary educated and medium family size with joint family type. Majority of them (65%) did not have any social participation which deprives them in accessing useful information.

Almost one fourth of them (22.50%) had no land and 35. 83 per cent of respondents possessed upto one acre of land. Only 7.50 per cent had land with more than 2 acres. About 43.33 per cent of them owned two lactating animals followed by 19.17 per cent with single lactating animal. About 58.34 per cent of respondents produce less than 8 litres of milk per day and 61 per cent of them sold milk between 4 and 7 litres per day. Three-fifth (60%) of them could generate the annual income ranging between Rs. 57,600-98,000. This clearly explains the poor socio-economic conditions and the need arises to design appropriate interventions for improving their socio-economic conditions.

The prioritisation of extension interventions is depicted in Table 2. Analytical Hierarchy process (AHP) was used to prioritise the extension interventions. AHP utilised pair-wise comparison method to understand the overall priority of each intervention which can help to empower the resource poor households.

The relevant factors were identified under Extension, Policy, Technological and Input service interventions in relation to resource poor dairy farm households. The quantitative importance of each factor in the overall importance in specified intervention was determined as could be seen in the Table 2.

Factors *viz*. Training, Field trip, Demonstration and Dairy mela among Extension interventions were prioritized with lambda value 4.18 and consistency ratio of 0.06138 by using AHP methodology. Among the factors prioritized, training has acquired the highest rank with a value of 0.35 and then demonstration acquired 2nd rank with value 0.34. Field trip and dairy mela acquired 3rd and 4th rank which accounts to the value of 0.17 and 0.14 respectively. As per the expert opinion, among the four factors of extension interventions, training and demonstration with the highest values emerged as most useful strategies for empowering resource poor dairy farm households as compared to field trip and dairy mela.

Similarly, for policy intervention factors viz. Subsidy, Incentives, Credit facility and Insurance with lambda value 4.10 and consistency ratio of 0.037549 were prioritised. Among the prioritized factors, subsidy has acquired the highest rank with a value of 0.37 and then Incentive acquired 2nd rank with value 0.25. Insurance and credit facility acquired 3rd and 4th rank which accounts to the value of 0.20 and 0.17 respectively. Subsidy and incentive would always serve as motivational factors as both factors can be financially supporting the resource poor dairy farmer households for adoption of advance scientific technologies. Utilization of credit and insurance policy can play significant role once the farmers acquire substantial socio-economic status. There is a greater need to create awareness about schemes having subsidy component among resource poor dairy farm households.

Further for technological interventions factors viz. Artificial Insemination (A.I), mineral mixture, silage preparation and vaccination and deworming with lambda value 4.19 and consistency ratio of 0.071474 were determined. Among the factors prioritized, A.I. has acquired the highest rank with a value of 0.41 and then mineral mixture acquired 2nd rank with value 0.26. Vaccination and deworming and Silage preparation acquired 3rd and 4th rank which accounts for the value of 0.17 and 0.14 respectively. A.I and mineral mixture with the highest values come out as most potential strategies for empowering the income level of poor dairy farm households as compared to vaccination and deworming as well as silage preparation. It has been observed that the calves born through A.I technique is more towards true to type breed characters than the calves born through natural service.

Table 1: Socio-economic profile of resource poor dairy farm households

Variables	Categories	Frequency	Percentage
Age	Young (upto 35 years)	24	20.00
	Middle (36-50 years)	53	44.17
	Elder (>50 years)	43	35.83
Sex	Male	98	81.67
	Female	22	18.33
Education	Illiterate	12	10.00
	Primary	22	18.33
	Middle	29	24.17
	Secondary	32	26.67
	Intermediate	24	20.00
	Graduate and above	1	0.83
Family Size	Small (<5)	18	15.00
	Medium (5-7)	77	64.17
	High (>7)	25	20.83
Family type	Nuclear	50	41.67
	Joint	70	58.33
Experience in Dairy Farming	Upto 10 years	27	22.50
	11-20 years	53	44.17
	>20 years	40	33.33
Social Participation	No	78	65
	Yes	42	35
Land Holding	Landless	27	22.50
	Up to 1 acre	43	35.83
	1.1 to 2 acres	41	34.17
	>2 acres	9	7.50
Herd size (Lactating)	Single	23	19.17
	Two	52	43.33
	>2	45	37.50
Milk production (in litres/day)	Low (<8)	70	58.34
	Medium (8-10)	34	28.33
	High (>10)	16	13.33
Milk Consumption (litres/day)	Low (<3)	67	55.80
	Medium (3-4)	36	30.04
	High (>4)	17	14.16
Milk sale (litres/day)	Low (<4)	24	20.00
	Medium (4-7)	73	61.00
	High (>7)	19	19.00
Milk sale rate (Rupees)	Low (<40)	69	57.50
	Medium (40-59)	49	40.83
	High (>59)	2	1.67
Annual Income from Dairy Farming (in Rs)	Low (<57,600)	27	22.50
	Medium (57,600-98,000)	72	60.00
	High (>98,000)	21	17.50

For input service intervention factors *viz*. milking equipment, chaff cutter, medicine and veterinary drug and fodder seed/sapling with lambda value 4.06 and consistency ratio of 0.025412 were prioritised. Among the factors prioritized, milking equipment has acquired the highest rank with a value of 0.45 and then medicine and veterinarian drug acquired 2nd rank with value 0.25. Chaff cutter and Fodder seed/sapling got 3rd and 4th rank which accounts to the value of 0.18 and 0.11 respectively. Milking equipment and medicine and veterinarian drug with the highest values were found to be most useful and affordable strategies for empowering resource poor dairy farm households as compared to chaff cutter and fodder seed/sapling. However, incentive structures are to be worked out for motivating the resource poor farmers in order to enhance the adoption of improved dairy farming practices.

Conclusions

Resource poor dairy farm households have relatively lesser access to technologies, resources and finance. The study suggests that there is a dire need to realign the extension interventions such as training, demonstration, subsidy, incentives, A.I., mineral mixture, milking equipment and veterinary drugs for popularising improved dairy farming practices with the active involvement of resource poor farmers. As per the opinion of experts by using AHP methodology, training would empower the resource poor farmers followed by demonstration, field trip and dairy mela while, subsidy has acquired the highest rank followed by incentives among policy interventions. A.I. has acquired the highest rank followed by mineral mixture among technological interventions. The findings of this study would be helpful for formulating policies and interventions by policy makers, scientists, extension workers, private agencies and NGOs engaged in the development project related to resource poor dairy farmers. The realigned extension interventions have the potential to augment the milk productivity and thereby improving the livelihood of resource poor dairy farm households through increased revenue generation.

Table 2: Priority weights of categorized extension intervention factors in relation to empowering resource poor dairy respondents

Interventions	Factors	λ_{max} = Lamda	Consistency ratio (CR)	Priority of the factors within interventions
Extension Interventions	Training	4.18	0.06138	0.347451
	Field trip			0.177471
	Demonstration			0.334205
	Dairy mela			0.140874
Policy Interventions	Subsidy	4.10	0.03754	0.365262
	Incentives			0.255696
	Credit facility			0.172653
	Insurance			0.20639
Technological Interventions	A.I.	4.19	0.07147	0.415321
	Mineral mixture			0.268744
	Silage preparation			0.142544
	Vaccination and deworming			0.17339
Input Service Interventions	Milking equipment	4.06	0.02541	0.453645
	Chaff cutter			0.180744
	Medicine and veterinary drug			0.251025
	Fodder seed/sapling			0.114586

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SHORT COMMUNICATION

Effect of fat on quality characteristics of whey obtained from manufacture of Ricotta cheese

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Abstract: The Ricotta cheese whey (RCW) is obtained during the manufacturing of Ricotta cheese as byproduct. It is highly nutritious and can be utilized in the manufacture of whey-based beverages. In the present study, RCW was analyzed for its sensory and physico-chemical properties obtained during the manufacture of Ricotta cheese from mixture of Mozzarella cheese whey and milk (80:20) with different fat levels (0.1%, 1.5%, 3.0% and 4.5% in milk). The results indicated that an increase in the fat content of milk used in the manufacturing of Ricotta cheese has a significant effect on the composition of RCW. The fat content of RCW increased with the increase in the fat content of milk used in the manufacture of Ricotta. The protein content was reported to be highest in the RCW prepared from milk with no-fat. The L* value of the sample increased while a* and b* values of the sample decreased with increase in the fat content of RCW. Sensory results revealed that RCW with higher fat content showed the highest acceptability as compared with the other samples.

Keywords: Ricotta cheese, Ricotta cheese whey, Sensory properties, Physico-chemical properties

Ricotta is a soft Italian unripened whey cheese. It is popular in Italy and Ibero-American countries such as Argentina. Ricotta cheese is obtained by direct acidification of cheese whey or mixtures of cheese whey and milk using citric acid at 90°C (Martins et al. 2010; Ruble et al. 2019; Gautam et al. 2023). After coagulation and precipitation of protein, the curd was then separated from greenish-tinged liquid usually called ricotta cheese whey (RCW) or scotta, which is considered a byproduct of the cheese industry. RCW is highly nutritious and can be utilized in the preparation of whey-based beverages. It is usually discarded by the cheese industries, but discarding whey contributes to environmental pollution due to its high BOD and COD values of about 50 and 80 g/L, respectively, and leads to a big loss for the cheese industries (Sansonetti et al. 2009; Maragkoudakis et al. 2016; Cortellino and Rizzolo, 2018; Pires et al. 2021).

Nowadays, consumers want nutritionally rich food that is low in calories, thus, fat reduction is the best option to reduce the calories of any food as well as beverages. In the present study attempts have been made to evaluate the sensory and physico-chemical characteristics of RCW obtained from Ricotta cheeses prepared by addition of milk with different fat levels to the Mozzarella cheese whey. This study also evaluated the effect of fat on the composition and suitability of RCW for the preparation of whey-based beverages varying in fat content.

Mozzarella cheese whey (7% solids, 0.78% fat and 0.9% protein) was collected during Mozzarella cheese preparation from the Experimental Dairy Plant (EDP) of the College of Dairy Science and Technology, Ludhiana. Raw mixed milk (5.0% fat and 8.5% non-fat solids) was also obtained from EDP of the college to prepare a milk with different fat content viz. 0.1% (no-fat milk, NFM), 1.5% (low-fat milk, LFM), 3.0% (medium-fat milk, MFM) and 4.5% (full-fat milk, FFM). Citric acid was obtained from the local market.

Ricotta cheese was prepared as per the mechanized method described by Gautam et al. (2023) using basket centrifuge (Deepali United Manufacturing Pvt. Ltd., Mumbai, India). Ricotta cheese was prepared using mixture of whey and milk in the ratio 80:20. Sweet Mozzarella cheese whey was heated to 50°C to stop the growth of starter culture followed by addition of mixed milk varying in fat content (0.1%, 1.5%, 3.0% and 4.5%). The whey/milk mixture was heated to 90°C and citric acid (5%, w/v) was added to bring down the pH to 5.4 of the mixture. The flocculated

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protein rises to the surface and the curd along with whey was allowed to rest for 20 minutes to complete the coagulation process. Later, the curd was separated from whey using basket centrifuge (5000 rpm for 5 min). Four varieties of RCW produced (NFM-, LFM-, MFM and FFM-RCW) were thus collected and stored at 4°C until further analysis.

Total solids, fat, protein, lactose and ash content of RCW were determined as per the methods described for milk in IS: SP-18 (1981). pH of RCW was determined by using digital calibrated hand pH meter (pHep+, Hanna instruments) at 20°C. The titratable acidity of RCW was determined as per method for milk in IS: SP-18 (1981). Colour of RCW was determined by using a colorflex reflectance meter (Hunter lab, Reston, Virginia, USA). The instrument provides 3 parameters i.e., a* (greenness to redness), b* (blueness to yellowness) and L* (darkness to lightness) were recorded. All the measurements were carried out in triplicate.

Sensory evaluation of RCW samples was done using the 9-point hedonic scale (1 for disliked extremely and 9 for liked extremely) as per the method described in Indian standards (IS, 1971). Ten semi-trained panelists were served with 50 ml of sample each in glass beaker (100 ml), and panelists were asked to score for the attributes like flavour, sourness, colour and appearance and saltiness. Panel members, who were familiar with cheese whey and had a good knowledge on the sensory evaluation of dairy products and they were in the age group of 25 to 45 years.

Mean values and standard deviations (SD) of triplicate determinations were calculated using Microsoft Excel (Microsoft Office, 2010). All statistical analyses were performed using SPSS 16. A one-way analysis of variance (ANOVA) was used to determine differences among treatment means at the 95% confidence interval.

The physico-chemical analysis of RCW obtained after manufacturing Ricotta cheese with different fat content is given in Table 1. The total solids content of RCW samples ranged from 6.47 to 6.91% and did not differ significantly among all the treatments. Variation in the fat content (0.1-4.5%) of milk used for the preparation of Ricotta cheese resulted in significant difference in the fat content of the RCW varying from 0.07 to 0.11%. It was found to be highest in FFM-RCW while lowest in NFM-RCW. However, the protein content of LFM-RCW was found to be significantly higher ($P<0.05$) than other RCW samples, which might be due to the presence of more protein in the NFM and significant difference was not observed between LFM-, MFM- and FFM-RCW. The lactose and ash content did not differ significantly among all the treatments and found in the range of 5.51–6.01% and 0.46–0.51%, respectively, for all the samples. Cortellino and Rizzolo (2018) reported the chemical composition of RCW which comprised 5.39% total solids, 0.34% protein, 0.74% ash, and 3.76% lactose. Pisponen et al. (2013) showed similar results with respect to its compositional evaluation. They reported that the scotta has 5.43% dry matter, 0.26% fat, 0.5%

Table 1 Effect of fat on compositional and physico-chemical properties of Ricotta cheese whey (RCW)

Parameters	NFM-RCW	LFM-RCW	MFM-RCW	FFM-RCW
Total solids (%)	6.74 ± 0.42^a	6.60 ± 0.39^a	6.91 ± 0.52^a	6.47 ± 0.16^a
Fat (%)	0.07 ± 0.05^b	0.09 ± 0.17^{ab}	0.10 ± 0.09^a	0.11 ± 0.12^a
Protein (%)	0.59 ± 0.14^a	0.38 ± 0.11^b	0.37 ± 0.06^b	0.36 ± 0.09^b
Ash (%)	0.51 ± 0.21^a	0.48 ± 0.08^a	0.47 ± 0.13^a	0.46 ± 0.10^a
Lactose (%)	5.62 ± 0.23^a	5.67 ± 0.18^a	6.01 ± 0.23^a	5.51 ± 0.31^a
pH	5.51 ± 0.05^b	5.54 ± 0.02^b	5.42 ± 0.01^b	5.74 ± 0.09^a
Acidity (% lactic acid)	0.183 ± 0.01^a	0.186 ± 0.04^a	0.189 ± 0.07^a	0.162 ± 0.06^b
L*	28.98 ± 0.12^d	31.29 ± 0.07^c	32.44 ± 0.18^b	35.03 ± 0.01^a
a*	-3.50 ± 0.01^a	-3.39 ± 0.08^b	-3.23 ± 0.07^c	-3.02 ± 0.05^d
b*	-1.93 ± 0.12^a	-1.11 ± 0.04^b	-0.73 ± 0.04^c	-0.50 ± 0.03^d

Data are presented as Mean ± SD, n =3. Means with different lowercase superscripts (a, b, c, d) in each row are significantly different ($P < 0.05$) from each other.

Table 2 Effect of fat on sensory properties of Ricotta cheese whey (RCW)

Attributes	NFM-RCW	LFM-RCW	MFM-RCW	FFM-RCW
Flavour	6.06 ± 0.42^c	6.49 ± 0.39^b	6.42 ± 0.52^b	7.12 ± 0.16^a
Sourness	7.11 ± 0.39^a	6.88 ± 0.26^a	6.13 ± 0.55^b	5.95 ± 0.19^b
Colour and appearance	6.02 ± 0.21^b	6.84 ± 0.48^a	6.70 ± 0.33^a	6.88 ± 0.30^a
Saltiness	6.87 ± 0.31^a	6.05 ± 0.18^b	6.11 ± 0.23^b	5.96 ± 0.34^b

Data are presented as Mean ± SD, n =3. Means with different lowercase superscripts (a, b, c) in each row are significantly different ($P < 0.05$) from each other.

protein, and 4.14% lactose and ash content of unsalted and salted scotta was around 0.44% and 0.62%, respectively.

The pH values (at 20°C) of all RCW samples were in the range of 5.51 to 5.74. The pH value was found to be significantly higher ($P<0.05$) in FFM-RCW when compared to other treatments (Table 1). However, significant difference was not observed between NFM-, LFM- and MMF-RCW. High pH value in FFM-RCW might be due to the addition of FFM (contains 4.5% fat) which decreased the level of protein in the whey that is also a major contributor for pH in the milk. Maragkoudakis et al. (2016) reported that pH of scotta was in the range of 5.5 to 5.9. The titratable acidity values of RCW ranges from 0.162 to 0.189 % lactic acid, the highest value being observed for MFM-RCW and did not differ significantly from NFM- and LFM-RCW. The effect of colour profile on RCW is given in a Table 1. A L* value of RCW represents the brightness of the samples, which was in the range of 35.03–28.98. All the samples reported low brightness value because most of the casein has been removed from Scotta. The highest brightness value ($P<0.05$) was observed in FFM-RCW sample which might be due to the presence of a higher levels of fat when compared with other RCW samples. Results also showed that reducing the fat content in RCW has an inverse relationship with the L* value. The negative a* value represents greenness in the RCW samples due to the presence of riboflavin in whey, which ranges from -3.02 to -3.50. It was significantly higher ($P<0.05$) in LFM-RCW than the other treatments and lowest in FFM-RCW. The negative b* value represents the redness in the samples, which was in the range of -0.50 to -1.93. Results showed that there was a significant difference in b* value among RCW samples, which might be due to the varying fat and protein content in the samples. Reducing the fat content leads to lower carotene content and makes the sample less yellow in colour.

The sensory study has been done to check the acceptability score of the RCW as raw material which can be used for making beverages. The results of sensory evaluation of RCW samples are presented in the Table 2. The score for the flavour attribute was in the range of 6.06 to 7.12. The highest score observed in the FFM-RCW sample, which might be due to the presence of a higher levels of fat as compared with the other samples, as fat is a major contributor to the flavour of any product. As expected the lowest flavour score was observed for NFM-RCW sample. The sourness in the samples was mainly due to the addition of citric acid (5% w/v) during the preparation of Ricotta cheese. Sourness score for NFM-RCW was found to be highest among the treatments and differed significantly ($P<0.05$) from MFM- and FFM-RCW. Scores for colour and appearance did not differ significantly ($P>0.05$) among LFM-, MFM- and FFM-RCW samples while lower score was observed in the NFM-RCW. All the RCW samples were reported to have a salty taste, which might be due to the ash content in the samples. The score for saltiness of the NFM-RCW sample was reported to be the highest, which might be due to the combined effect of the higher amount of ash and protein content in the sample compared to other samples. However, significant difference ($P>0.05$) was not observed between LFM-, MFM- and FFM-RCW samples.

Conclusions

In the present investigation, effect of variation in the fat content of milk and whey mixture used in the manufacture of Ricotta cheese on physico-chemical and sensory properties of RCW was studied. FFM-RCW had higher fat, pH, L* value and lower protein, acidity, b* and a* values when compared to other RCW samples. Fat content of RCW positively influenced the flavour and colour scores and reduced the saltiness and sourness scores with increase in the fat content of RCW.

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