Vol. 77 No.3, 2024

ISSN 0019-5146 (Print)
ISSN 2454-2172 (Online)

INDIAN JOURNAL *of* DAIRY SCIENCE

an IDA *publication*

Regd. No. 15665/68
Covered by Clarivate Analytics Services: Emerging Sources Citation Index
https://mjl.clarivate.com/search-results

Contents

ISSN 0019-5146 (Print)
ISSN 2454-2172 (Online)

CENTRAL OFFICE : Indian Dairy Association, IDA House, Sector IV, R.K. Puram, New Delhi-110022. Phones: 011-26170781, 26165237, 26165355. Email: idahq@rediffmail.com /www.indiandairyassociation.org

ZONAL BRANCHES & CHAPTERS : **South Zone:** Dr. Satish Kulkarni, Chairman, IDA House, NDRI Campus, Adugodi, Bangalore-560 030. Ph.: 080-25710661 Fax: 080-25710161. **West Zone:** Dr.J.B. Prajapati, Chairman; A-501, Dynasty Business Park, Andheri-Kurla Road, Andheri (East), Mumbai 400059 Email: chairman@idawz.org / secretary@idawz.org Ph.: 91 22 49784009 **North Zone :** Shri S.S. Mann, Chairman; c/o IDA House, Sector IV, R.K. Puram, New Delhi - 110 022 Phones: 011-26170781, 26165355. **East Zone:** Shri Sudhir Kumar Singh, Chairman, c/o NDDB, Block-DK, Sector-II, Salt Lake City, Kolkata-700 091 Phones: 033-23591884-7. **Gujarat State Chapter:** Dr. Amit Moolchand Vyas, Chairman; c/o SMC College of Dairy Science, AAU Campus, Anand-388110 Gujarat. Email: idagscac@gmail.com **Kerala State Chapter:** Dr. S.N. Rajakumar, Chairman; c/o Prof. and Head, KVASU Dairy Plant, Mannuthy, E mail: idakeralachapter@gmail.com **Rajasthan State Chapter:** Shri Rahul Saxena, Chairman; Cabin no 1, Ground Floor, Manoram, #2, Ambeshwar Colony, New Sanganer Road, Near Shyam Nagar Metro Station, Jaipur-302019 E-mail: idarajchapter@yahoo.com **Punjab State Chapter:** Dr. Inderjit Singh, Chairman, H.No. 1620, Sector-80, SAS Nagar, Mohali-140 308 (Punjab) Email: secretaryidapb2023@gmail.com **Bihar State Chapter:** Shri D.K. Srivastava, Chairman; c/o Former Managing Director, Mithila Milk Union; House No. 16 Mangalam Enclave, Baily Road, Near Saguna SBI, Patna-814146 (Bihar). E-mail: idabihar2019@gmail.com **Haryana State Chapter:** Dr. S.K. Kanawjia, Chairman; c/o D.T. Division, NDRI, Karnal-132 001 (Haryana). Ph.: 09896782850 Email: skkanawjia@rediffmail.com. **Tamil Nadu State Chapter:** Shri Kanna K.S., Chairman; c/o Department of Dairy Science, Madras Veterinary College, Vepery, Chennai-600007. **Andhra Pradesh Local Chapter:** Prof. Ravi Kumar Sreebhashyam, Chairman; c/o College of Dairy Technology, Sri Venkateshwara Veterinary University, Thirupathi - 517502 Email: idaap2020@gmail.com **Eastern UP Local Chapter:** Dr. Arvind, Chairman; Assistant Professor, Department of Dairy Science & Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005 Ph.: 7007314450 Email: arvind1@bhu.ac.in **Western UP Local Chapter:** Dr. Ashok Kumar Tripathi, Chairman; c/o Flat no. 1003/8, Zen Spire, Ramprastha Greens, Vaishali, Ghaziabad-201010 (UP). Email: ttreddy@arvinddairy.com **Jharkhand Local Chapter:** Shri Pavan Kumar Marwah, Chairman; c/o Jharkhand Milk Federation, FTC Complex, Dhurwa Sector-2, Ranchi, Jharkhand-834004 Email: jharkhandida@gmail.com **Telangana Local Chapter:** Shri Rajeshwar Rao Chalimeda, Chairman; c/o Dodla Dairy Ltd Corporate Office, # 8-2-293/82/A, 270/Q, Road No 10-C, Jubilee Hills, Hyderabad – 500 033 Telangana.

Printed and published by Shri Hariom Gulati and edited by Dr. (Mrs.) Bimlesh Mann on behalf of the Indian Dairy Association and printed at National Printers, B-56, Naraina Industrial Area, Phase II, New Delhi and published at IDA House, Sector-IV, R.K. Puram, New Delhi-110022.

Indian J Dairy Sci 77(3): 195-202
https://doi.org/10.33785/IJDS.2024.v77i03.001

RESEARCH ARTICLE

Development of low-fat frozen yoghurt incorporated with strawberry and beetroot powder

Dhaval Patel· CN Dharaiya (✉), DH Patel and AM Patel

Received: 15 June 2023 / Accepted: 24 September 2023 / Published online: 23 June 2024

Abstract: Low-fat frozen yoghurt was prepared using a blend of strawberry and beetroot powder as one of the ingredients. Response Surface Methodology (RSM) was used to optimize the level of strawberry and beetroot powder, stabilizer-emulsifier mixture and sugar. The responses were sensory attributes such as flavor, body and texture, melting characteristics, color and appearance and total score as well as physical attributes like melting rate and overrun. Based on the output, RSM suggested the rate of addition of strawberry and beetroot powder, stabilizer-emulsifier mixture and sugar to be 4.06 per cent, 0.21 per cent and 15.45 per cent respectively. The experimental frozen yoghurt was prepared as per the suggestions from RSM and compared with control low-fat frozen yoghurt where strawberry and beetroot powder were replaced with strawberry flavor and matching color. The experimental frozen yoghurt was statistically similar in terms of compositional parameters except moisture and carbohydrate content while the experimental sample was superior to control sample in terms of sensory attributes. Aerobic plate count of both the samples were statistically similar while coliform as well as yeast and mold were absent in the product.

Key words: Frozen yoghurt; Strawberry; Beetroot; Response Surface Methodology; Sensory, Overrun

Dairy Technology Department, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India

Chetan N Dharaiya (✉)
Dairy Technology Department, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India
Email: chetandharaiya@gmail.com
Mobile #: +91 75730 13697

Introduction

Frozen yoghurt is a frozen fermented milk product manufactured by freezing of either stirred yoghurt prepared by fermentation of ice-cream mix or a mixture of stirred yoghurt and ice-cream mix or partially fermented ice-cream mix with addition of coloring and flavoring material. Frozen yoghurt contains the live strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*. Hence, it contains the nutritional benefits of yoghurt and sensory benefits of ice-cream and therefore, it is enjoyed by the people of age group (Tamime and Robinson, 2007).

Strawberry (*Fragaria ananassa*) is the most economically valuable berry fruit, owing to its high nutritional value and pleasant flavor. (Hummer and Hancock, 2009). Strawberries possess appreciable amounts of minerals such as potassium, calcium and magnesium as well as vitamins such as ascorbic acid and folic acid (Beattie et al. 2005). Strawberries are an excellent source of phytochemicals, mainly phenolic compounds and biologically active non-nutrients with considerable biological functions (Häkkinen & Törrönen, 2000). Strawberry phenolics are well recognised for their anti-inflammatory, anti-hypertensive and antioxidant characteristics as well as anti-allergy capabilities and the potential to block the actions of certain physiological enzymes, preventing oxidative stress-related disorders (Giampieri et al. 2014). Beetroot (*Beta vulgaris* L.) is high in a variety of biologically active phytochemicals such as betalains (betacyanins and betaxanthins), polyphenols, flavonoids, and nitrate (NO_3); as well as high in minerals like calcium, magnesium, potassium, sodium, phosphorus, zinc, manganese and iron (Mirmiran et al. 2020) as well as a minor quantity of vitamin C and vitamin E, which have been demonstrated to have good antioxidant capacity (Apak et al. 2004). Beetroot is a rich source of anthocyanins (1.44 to 8.45 mg/100 g) and carotenoids (1.9 to 2.3 mg/100 g) (Rebecca et al. 2014). According to studies, it also reduced low-density lipoprotein (LDL) by 50 percent and blood glucose by 40 percent after beetroot consumption (Ceclu and Nistor, 2020). Hence, beetroot is an excellent nutritional supplement.

Response Surface Methodology (RSM) has been widely used in recent years for the development of new products as well as improvement in existing products. RSM delineates the effect of the independent variables on responses of importance and is regarded as an effective method to optimize the new product formulations. It is a robust tool for data analysis that focuses on an adequate approximation relationship between input and output variables and determines the best operating circumstances for a system (Myers et al. 2004).

With a changing lifestyle and increasing awareness towards health and nutrition, consumers are moving towards low-fat diet to reduce the risk of obesity, coronary heart disease, atherosclerosis and hypertension (Dharaiya et al. 2021). High fat diet is also linked with psychiatric disorders (Jeong et al. 2019). Fat, being a costliest constituent in milk, increases the cost of final product and make the product unaffordable by low-income group people. However, reduction in fat content of frozen yoghurt influences sensory and rheological characteristics of the product. Incorporation of strawberry and beetroot will make up for the deterioration taken place in the quality of frozen yoghurt by reduction of fat along with improvement in the nutritional quality of the final product. Therefore, in current investigation, low-fat frozen yoghurt has been developed with incorporation of strawberry and beetroot powder.

Materials and Methods

Whole milk and skimmed milk were obtained from nearby commercial dairy plant. Skimmed milk powder of *Sagar* brand, marketed by Gujarat Cooperative Milk Marketing Federation (GCMMF) Ltd., Anand was used for standardization. Commercial grade cane sugar of *Madhur* brand was obtained from local market. Fresh beetroots were purchased from local vegetable market. Strawberry powder (Holy Natural brand, Earth Expo Company, Bhavnagar, India) was purchased online. Starter cultures *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* were obtained from Dairy Microbiology Division of the institute. Sodium alginate, guar gum, carrageenan and pectin were supplied by HiMedia, Mumbai. Glycerol Monostearate (GMS) was supplied by Loba Chemical, Mumbai. Strawberry flavor (International Flavors & Fragrances India Pvt. Ltd., Chennai) and erythrosine supra color (AB Enterprises, Mumbai, India) was used to prepare control frozen yoghurt.

Preparation of beetroot powder

Raw beetroot was brought from the local market and cleaned properly to remove foreign materials. Then trimmed properly and was sliced into thin pieces (3 mm) with the help of slicer. Sliced beetroot was blanched at 80°C, followed by drying at 55°C for 4 h by a cabinet drier. Dried beetroot was ground to a fine powder (65 mesh size sieve) and packed in airtight colored glass bottles for further use (Srivastava and Singh, 2016).

Preparation of stabilizer-emulsifier blend

A blend of stabilizers (i.e., sodium alginate, guar gum, carrageenan and pectin) and emulsifier (i.e., glycerol monostearate) was prepared and used in frozen yoghurt at a level suggested by RSM. The blend contained sodium alginate, guar gum, carrageenan, pectin and GMS in the ratio of 2:1:1:2:2. The ratio was decided on the basis of preliminary trials. A combination of different stabilizers has synergistic effect on the quality of frozen yoghurt which can ultimately reduce the use of stabilizers (Milani and Koocheki, 2011). Hence, a combination of stabilizers and emulsifiers has been used in current investigation.

Preparation of frozen yoghurt

Frozen yoghurt has been prepared using the method suggested by Agarwal and Prasad (2013) with minor modifications. The detailed method is illustrated hereafter:

Whole milk and skimmed milk are blended together and heated to 55°C. All the dry ingredients such as sugar, skimmed milk powder, a blend of strawberry powder and beetroot powder as well as stabilizer-emulsifier mixture were mixed together and added to whole milk and skimmed milk blend. The mixture was then homogenized at 65°C followed by heat treatment at 85°C/30 min. The heated mix was cooled to 42±2°C and inoculated with starter cultures *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* at the rate of 2 percent (w/w) of the quantity of mix followed by incubation at 42±2°C till the pH reached to 5.0. The yoghurt mix was then cooled to 4°C and stirred. The stirred mix was aged at 4°C for 6 h followed by freezing, packaging and hardening at -25°C for 24 h. The frozen yoghurt was stored at -18°C after hardening.

Analysis of low-fat frozen yoghurt incorporated with strawberry and beetroot powder

Whole milk and skimmed milk were analysed for fat, total solids and acidity as per the method described by FSSAI (2015). The prepared frozen yoghurt was analysed for fat, protein, ash, total solids and pH as per the method described by FSSAI (2015). Carbohydrates are calculated by difference. Viscosity of yoghurt mix, using Brookfield viscometer, as well as melting rate of frozen yoghurt was analysed by the method suggested by Muse and Hartel (2004). Overrun of frozen yoghurt was calculated as per the formulae used by Ilansuriyan and Shanmugam (2018). Aerobic plate count, coliform count and yeast and Mold count were analysed using the method given by FSSAI (2012).

Sensory evaluation of low-fat frozen yoghurt incorporated with strawberry and beetroot powder

The frozen yoghurt samples were stored at -13±2°C for 24 h before serving to the semi-trained judges (n=12). The judges were from the faculty of the institute who have basic idea about the product. Sensory analysis of the product was performed in isolated sensory booths illuminated with incandescent light maintained at 22±2°C. The well-labelled samples were presented in polystyrene cups in completely randomized order. The frozen yoghurt samples were evaluated using 100-point score card (Marshall et al. 2013).

Statistical analysis

A Central Composite Rotatable Design (CCRD) of the Response Surface Methodology (RSM) technique was adopted for the optimization of strawberry and beetroot powder, stabilizer-emulsifier blend and sugar. The minimum and maximum levels of the blend of strawberry powder and beetroot powder, stabilizer-emulsifier blend and sugar were selected as 2 and 6 percent, 0.2 and 0.5 percent as well as 12 and 16 percent respectively, on the basis of preliminary trials. The CCRD of three factors contained 20 combinations, including lower and upper limits, along with their responses for sensory parameters as well as melting rate and overrun are displayed in Table 1. The data generated for different responses were analysed using Design Expert® software (13.0.2 version) (Stat-Ease, Inc., 2021 E. Hennepin Avenue, Minnepolis, USA). A general polynomial equation given below was fitted for each response.

$$Y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_{12} + a_{22}x_{22} + a_{33}x_{32} + a_{12}x_1x_2 + a_{23}x_2x_3 + a_{13}x_1x_3 + \text{Error term}$$

where Y represents the predicted response; a_0 the constant coefficient; a_{11}, a_{22} and a_{33} denote quadratic coefficients; a_{12}, a_{23} and a_{13} denote interaction coefficients; x_1, x_2 and x_3 denote rate of addition of strawberry and beetroot powder, stabilizer-emulsifier blend and sugar respectively.

Adequacy of the model was evaluated using coefficient of determination (R^2) and statistical significance was examined by F value. The effect of independent variables and individual responses was described at $P<0.05$. t-test for two samples assuming equal variance was applied using Microsoft Excel for comparison of predicted values with the actual values of the responses. The variation between control and strawberry and beetroot incorporated low-fat frozen yoghurt samples was analysed using independent t-test.

Results and Discussion

The optimization of the rate of addition of the blend of strawberry and beetroot powder, stabilizer-emulsifier blend and sugar were carried out on the basis of sensory properties of the low-fat frozen yoghurt such as flavor, body & texture, melting characteristics, color & appearance and total score as well as quantity of frozen yoghurt melted in specific time and overrun. The successive regression analysis of the responses produced the quadratic models for each response. The variation in experimental data of fitted quadratic model was given by coefficient of determination (R^2) which ranged from 88 percent to 92 percent (Table 2). The model F-value of the fitted quadratic model for all responses was found to be significant. The sufficient accuracy for predicting all response variables of the frozen yoghurt prepared from any combinations of variables within the range was evaluated by non-significant lack of fit. These indicate that the obtained quadratic model fitted the data strongly. The signal to noise ratio called *Adequate Precision Value (APV)* for a well fitted model should be above four. This measure also fulfilled for the obtained mode with APVs ranging from 8.12 to 11.27. All these results firmly recommended that the model could be used to develop strawberry and beetroot incorporated low-fat frozen yoghurt.

Effect of variables on flavour

Flavor is an amalgamation of taste, odour and mouthfeel. It is a major factor in sensory evaluation and consumer acceptance for majority of the dairy products. The flavor score of the frozen yoghurt between 34.87 and 43.12. The minimum flavor score for the frozen yoghurt was obtained when strawberry and beetroot powder, stabilizer-emulsifier blend and sugar were added at the rate of 7.36%, 0.35% and 14% respectively while maximum flavor score was obtained when strawberry and beetroot powder, stabilizer-emulsifier blend and sugar were added at the rate of 4%, 0.35% and 14% respectively (Table 1). Strawberry and beetroot powder and sugar significantly ($P<0.05$) improved flavor of the final product at linear level. Similarly, the interaction of both of them also significantly ($P<0.05$) improved the flavor while all the three variables significantly deteriorated flavor at quadratic level because high intensity of beetroot flavor and very sweet taste were disliked by the judges. Flavor release was also reported to be slow by the experts when stabilizer-emulsifier mixture was added in higher amount. Incorporation of beetroot juice up to 4 percent level improved flavor followed by deterioration (Manoharan et al. 2013). Sudha and Madhvi (2015) incorporated beetroot pulp in ice-cream and observed improvement in flavor up to the addition of 6 percent beetroot pulp followed by deterioration at higher level. Similarly, Ateteallah et al. (2019) also reported improvement in flavor of ice-cream with addition of beetroot juice up to the level of 3 percent followed by deterioration. Moeenfard and Tehrani (2008) reported non-significant influence of rate of addition of stabilizers on flavor when added between 0.15 to 0.25 percent.

Effect of variables on body and texture

Body and texture is an important sensory characteristic for frozen yoghurt. The body and texture score of the low-fat frozen yoghurt containing strawberry and beetroot was ranged from 23 to 28.87. The frozen yoghurt displayed minimum body and texture score when it contained 7.36% strawberry and beetroot powder, 0.35% stabilizer-emulsifier mixture and 14% sugar while maximum body and texture score was observed when it contained 4% strawberry and beetroot powder, 0.35% stabilizer-emulsifier mixture and 14% sugar (Table 1). At linear level, all the three variables significantly ($P<0.05$) improved body and texture of the frozen yoghurt. Addition of strawberry and beetroot powder, stabilizer-emulsifier mixture and sugar increased total solids, which led to improvement water binding and increase in viscosity which assisted to get proper overrun resulting in smooth and firm body. At quadratic level, all the three variables reduced body and texture score which could be attributed to heavy and soggy body of the final product. Similar results were reported by different researchers when beetroot pulp (Sudha and Madhvi, 2015) and beetroot juice (Manoharan et al. 2013; Ateteallah et al. 2019) were added in ice-cream. Body and texture score also increased with increase in rate of addition of stabilizers (Moeenfard and Tehrani, 2008; Agarwal and Prasad, 2013).

Effect of variables on melting characteristics

The behaviour of frozen product upon melting has an utmost importance. The sensory score for melting quality ranged between 3 and 4.67 The minimum score for melting quality was obtained when the product contained 0.64% strawberry and beetroot powder, 0.35% stabilizer-emulsifier mixture and 14% sugar while maximum melting characteristics score was observed when it contained 4% strawberry and beetroot powder, 0.35% stabilizer-emulsifier mixture and 14% sugar (Table 1). Stabilizer-emulsifier mixture and sugar significantly ($P<0.05$) improved melting quality of the product at linear level which could be attributed to uniform melting at optimum rate. The addition of all the three variables at higher level resulted in very slow melting as well as presence of visible curd particles on melting, leading to lower score. Dabija et al. (2019) showed improvement in water holding capacity and therefore, reduction in syneresis in yoghurt. Melting characteristics of ice-cream improved by the addition of beetroot juice up to 3 percent followed by deterioration (Ateteallah et al. 2019). Addition of beetroot pulp in oat milk-based ice-cream improved melting characteristics of ice-cream (Butt et al. 2023). Similar results were reported by Manoharan et al (2013) in ice-cream.

Effect of variables on color and appearance

Color and appearance is the first sensory parameter that is observed while carrying out sensory evaluation. The color and appearance score of the frozen yoghurt was ranged between 2.50 and 4.62. The product obtained minimum score for color and appearance when strawberry and beetroot powder, stabilizer-emulsifier mixture and sugar were added at the rate of 0.64%, 0.35% and 14% respectively while maximum score was obtained when the variables were added at the rate of 4%, 0.35% and 14% respectively. The rate of addition of strawberry and beetroot powder significantly ($P<0.05$) improved color and appearance of the frozen yoghurt at linear level as the judges liked the red color given by strawberry and beetroot powder but significantly ($P<0.05$) deteriorated color and appearance at quadratic level due to high intensity of the color which was disliked by the judges. The color and appearance score of ice-creams incorporated with beetroot pulp increased up to the level of 6 percent followed by reduction at higher level (Manoharan et al. 2013; Sudha and Madhvi, 2015).

Effect of variables on total score

Table 1: Experimental design matrix showing factors and their responses for the development of strawberry and beetroot incorporated low-fat frozen yoghurt

Std Run	A: SBP** (% w/w)	B: S+E@ (% w/w)	C: Sugar (% w/w)	Response 1: Flavor	Response 2: Body & Texture	Response 3: Melting character-ristics	Response 4: C&A#	Response 5: Total score*	Response 6: Melting rate, %	Response 7: Overrun, %
1	4.00	0.35	14.00	42.87	28.12	4.37	4.42	94.78	47.16	90.62
2	4.00	0.35	14.00	42.62	28.52	4.52	4.62	95.28	46.98	89.84
3	6.00	0.20	12.00	35.00	24.87	3.52	3.50	81.89	63.04	77.26
4	4.00	0.35	14.00	42.67	28.62	4.67	4.42	95.38	47.25	91.25
5	4.00	0.60	14.00	36.25	28.00	4.40	4.08	87.73	43.48	82.26
6	7.36	0.35	14.00	34.87	23.00	4.12	3.12	80.11	48.14	84.76
7	4.00	0.35	17.36	39.75	26.12	4.52	4.32	89.71	59.61	97.88
8	4.00	0.35	14.00	42.82	28.87	4.50	4.38	95.57	48.23	90.16
9	2.00	0.20	16.00	42.12	26.87	3.82	3.87	91.68	72.64	72.45
10	6.00	0.50	12.00	35.25	25.37	3.87	4.32	83.81	46.01	86.14
11	6.00	0.20	16.00	36.25	25.12	4.18	4.28	84.83	70.14	85.05
12	2.00	0.50	12.00	38.25	26.52	4.43	3.50	87.70	48.92	84.95
13	4.00	0.10	14.00	35.50	24.37	3.37	3.62	81.86	68.34	72.88
14	4.00	0.35	14.00	43.12	28.50	4.31	4.62	95.55	47.65	88.98
15	0.64	0.35	14.00	35.50	25.25	3.00	2.50	81.25	55.14	83.12
16	2.00	0.50	16.00	42.37	26.50	4.22	3.87	91.96	54.69	97.16
17	4.00	0.35	10.64	35.87	23.52	4.06	3.57	82.02	48.25	87.02
18	2.00	0.20	12.00	35.12	24.00	3.00	3.42	80.54	68.96	78.38
19	6.00	0.50	16.00	36.25	25.12	4.00	4.06	84.43	57.31	80.18
20	4.00	0.35	14.00	43.00	28.37	4.38	4.32	95.07	48.05	91.52

*Score for bacteria (15) was added in the Total score; @ Stabilizer-emulsifier blend; #Color and appearance; **Strawberry and beetroot powder

Total score is sum of the scores of all the sensory attributes. The total score ranged from 80.11 to 95.57. The frozen yoghurt containing 7.36% strawberry and beetroot powder, 0.35% stabilizer-emulsifier mixture and 14% sugar showed minimum total score while one containing 4% carrot powder, 0.35% stabilizer-emulsifier mixture and 14% sugar showed maximum total score. All the three variables significantly (P<0.05) increased total score at linear level while all the three variables significantly (P<0.05) reduced total score at quadratic level. Several researchers observed similar trend in ice-cream (Manoharan et al. 2013; Sudha and Madhvi, 2015; Ateteallah et al. 2019, Butt et al. 2023).

Effect of variables on melting rate

Frozen product should have optimum melting time. Rapid or slow melting causes inconvenience to the consumer. Melting time is inversely related to melting rate. The melting rate of the frozen yoghurt ranged between 43.48 per cent and 72.64 percent. The frozen yoghurt containing 4 percent strawberry and beetroot powder, 0.6 percent stabilizer-emulsifier mixture and 14 percent sugar showed minimum melting while the one containing 2 percent strawberry and beetroot powder, 0.2 percent stabilizer-emulsifier mixture and 16 percent sugar showed maximum melting. At linear level, melting rate was significantly (P<0.05) reduced with increase in stabilizer-emulsifier quantity which could be attributed to increase in amount of bound water with addition of stabilizer and increased overrun while was significantly (P<0.05) increased with increase in sugar quantity leading to higher freezing point depression. At quadratic level, strawberry and beetroot powder as well as sugar resulted in significantly (P<0.05) higher melting rate due to higher freezing point depression while stabilizer-emulsifier blend reduced melting significantly by increasing the amount of bound water. Incorporation of beetroot pulp in strawberry flavored frozen yoghurt reduced melting rate (Chhikara et al. 2019). The deviation from the current

Table 2: Regression coefficients and ANOVA fitted quadratic model for the responses of strawberry and beetroot incorporated low-fat frozen yoghurt

Partial coefficients	Flavor	Body & texture	Melting characteristics	Color & appearance	Total score	Melting rate, %	Overrun, %
Intercept	42.77	28.24	4.29	4.45	94.16	47.29	91.89
A-SBP@	1.33*	0.52*	0.14*	0.18*	1.46*	-0.71	-0.42
B-S+E#	0.35	0.64*	0.33*	0.10	1.43*	-2.21*	1.78*
C-Sugar	1.60*	0.58*	0.18*	0.12	2.50*	1.43*	0.75*
AB	0.39	0.20	0.20*	0.06	0.49	1.14	0.32
AC	0.98*	-0.35	0.02	-0.03	-1.48	0.86	0.29
BC	0.39	0.42	0.19*	-0.14	-0.90	0.65	0.22
A^2	-1.64*	-1.39*	-0.33*	-0.50*	-1.89*	1.86*	0.74
B^2	-0.40	-1.66*	-0.63*	-0.13	-1.26*	-3.15*	-1.56*
C^2	-1.71*	-1.15*	-0.03	-0.10	-1.93*	2.59*	0.91*
			Model fit statistics				
Lack of fit	< 0.0001	0.0286	0.0394	0.0026	0.0001	< 0.0001	< 0.0001
Model F value	8.82	9.63	9.85	8.21	11.76	14.26	9.02
R^2	0.90	0.92	0.88	0.89	0.91	0.92	0.89
APV	8.12	9.43	10.24	9.74	11.27	10.56	9.12

@ Strawberry and beetroot powder; #Stabilizer-emulsifier blend; *significant effect at 5% level

Table 3: Regression equation for predicting sensory score, melted quantity and overrun of strawberry and beetroot incorporated low-fat frozen yoghurt

Property	Equation
Flavor	$42.77+1.33A+0.35B+1.60C+0.39AB+0.98AC+0.39BC-1.64A^2-0.40B^2-1.71C^2$
Body & texture	$28.24+0.52A+0.64B+0.58C+0.20AB-0.35AC+0.42BC-1.39A^2-1.66B^2-1.15C^2$
Melting characteristics	$4.29+0.14A+0.33B+0.18C+0.20AB+0.02AC+0.19BC-0.33A^2-0.63B^2-0.03C^2$
Color & appearance	$4.45+0.18A+0.10B+0.12C+0.06AB-0.03AC-0.14BC-0.50A^2-0.13B^2-0.10C^2$
Total score	$94.16+1.46A+1.43B+2.50C+0.49AB-1.48AC-0.90BC-1.89A^2-1.26B^2-1.93C^2$
Melting rate	$47.29-0.71A-2.21B+1.43C+1.14AB+0.86AC+0.65BC+1.86A^2-3.15B^2+2.59C^2$
Overrun	$91.89-0.42A+1.78B+0.75C+0.32AB+0.29AC+0.22BC+0.74A^2-1.56B^2+0.91C^2$

investigation may be due to addition of strawberry flavor instead of strawberry powder.

Effect of variables on overrun

Overrun, directly related to amount of air incorporated in ice cream, is an important characteristic as it influences product quality and profit of the producer as well as is also involved in meeting legal standards. Too high overrun results in fluffy ice cream while too little overrun produces soggy and heavy body (Patel et al. 2015). Overrun (%) of the frozen yoghurt ranged between 72.45 percent and 97.88 percent. The frozen yoghurt samples containing 2% strawberry and beetroot powder, 0.2% stabilizer-emulsifier blend and 16% sugar showed minimum overrun while the one containing 4% strawberry and beetroot powder, 0.35% stabilizer-emulsifier blend and 17.36% sugar showed maximum overrun. At linear level, the rate of addition of stabilizer-emulsifier mixture and sugar significantly ($P<0.05$) increased overrun due to increased water binding and thus viscosity while at quadratic level stabilizer-emulsifier mixture had significantly ($P<0.05$) negative impact on overrun due to drastic increase in viscosity leading to poor air incorporation (Syed and Shah, 2016). Sugar had significantly ($P<0.05$) positive impact even at quadratic level. Moeenfard and Tehrani (2008) reported increase in overrun up to the level of 0.25 per cent. Though the combination of stabilizers and emulsifiers was different as well as the process of preparation of frozen yoghurt was also different. Increase in carrageenan content from 0.05 to 0.15 percent reduced overrun by almost 5.0 percent while increasing corn starch from 2.0 to 3.0 percent reduced overrun rate by 6.0 percent (Skryplonek et al. 2018).

Optimization of variables for preparation of low-fat frozen yoghurt

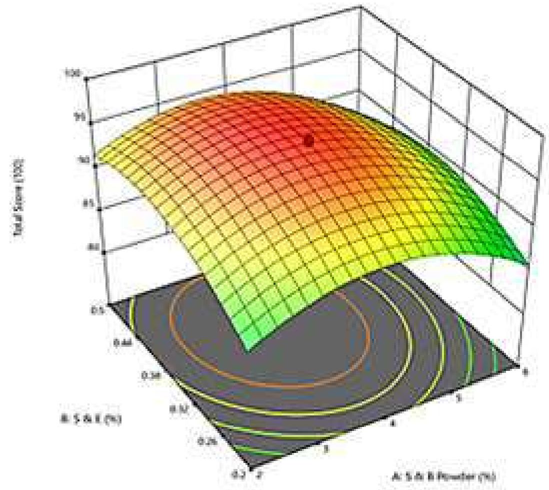
The optimization of different variables such as strawberry and beetroot powder, stabilizer-emulsifier mixture and sugar was carried out using numerical optimization technique. The criteria used for optimization are summarized in Table 4. Among the variables, strawberry and beetroot powder was maximized while stabilizer-emulsifier mixture and sugar were kept in range. Among the responses, the sensory parameters were maximized while melted quantity and overrun were set to the target of 50% and 90% respectively for the optimization process. RSM suggested the rate of addition of strawberry and beetroot powder, stabilizer-

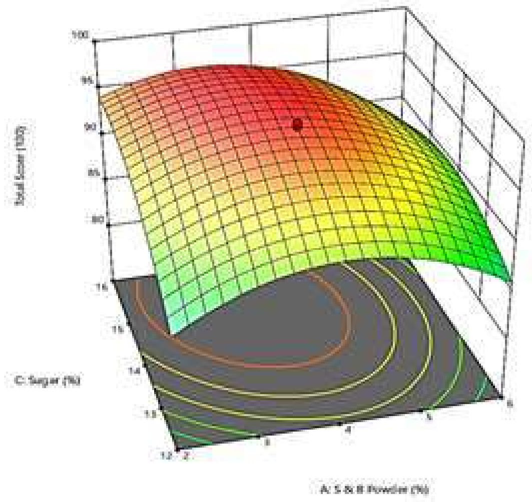
Table 5: Comparison of predicted values and observed values for strawberry and beetroot incorporated low-fat frozen yoghurt

Attribute	Predicted value	Observed value	t-value
Flavor	43.14	43.05	NS
Body & texture	28.02	27.81	NS
Melting characteristics	4.41	4.34	NS
Color & appearance	4.48	4.44	NS
Total score	94.23	94.14	NS
Melting rate, %	50.05	50.15	NS
Overrun, %	90.07	90.20	NS

Table 6: Comparison of strawberry and beetroot incorporated low-fat frozen yoghurt with control frozen yoghurt

Parameter	Control frozen yoghurt	Carrot incorporated low-fat frozen yoghurt	t-value
Chemical composition			
Moisture, %	68.18±0.11	64.12±0.15	0.15
Fat, %	2.47±0.07	2.41±0.09	NS
Protein, %	5.18±0.10	5.08±0.15	NS
Ash, %	1.09±0.05	1.19±0.08	NS
Carbohydrates, %	23.10±0.18	27.18±0.23	0.22
Physical characteirstics			
Melting rate, %	50.62±0.42	50.15±0.26	NS
Overrun, %	91.45±1.15	90.20±1.18	NS
Sensory characteristics			
Flavor	40.85±0.51	43.05±0.78	0.32
Body & texture	25.54±0.69	27.81±0.64	0.29
Melting characteristics	4.23±0.16	4.34±0.32	0.15
Color & appearance	4.49±0.14	4.44±0.26	NS
Total Score*	90.11±0.64	94.14±0.54	1.09
Microbial analysis			
APC ($\log_{10}$cfu/g)	9.04 ± 1.24	9.15 ± 1.29	NS
Coliform	Absent in 1 g		
Y&M	Absent in 1 g		





emulsifier mixture and sugar to be 4.06 percent, 0.21 percent and 15.45 percent respectively with desirability of 0.96. Strawberry and beetroot-based low-fat frozen yoghurt was prepared by adding strawberry and beetroot powder, stabilizer-emulsifier mixture and sugar as suggested by RSM. The predicted values for flavor, body and texture, melting quality, color and appearance, total score, melted quantity and overrun for the frozen yoghurt were 43.14, 28.02, 4.41, 4.48, 94.23, 50.05% and 90.07% respectively. It is evident from the table that the observed values were not significantly ($P>0.05$) different from predicted values with respect to all attributes. Hence, it was confirmed that the selected level of addition of strawberry and beetroot powder, stabilizer-emulsifier mixture and sugar is most suitable for the preparation of strawberry and beetroot-based low-fat frozen yoghurt with optimum sensory and physio-chemical attributes.

Analysis of strawberry and beetroot incorporated low-fat frozen yoghurt

strawberry and beetroot-based low-fat frozen yoghurt was analysed and compared with control frozen yogurt for its compositional parameters, physical characteristics as well as sensory attributes and analysed statistically using t-test. Moisture content of experimental frozen yoghurt was significantly ($P<0.05$) lower while carbohydrates content was significantly ($P<0.05$) higher due to addition of strawberry and beetroot powder. Sensory attributes such as flavor, body and texture, melting quality and total score of experimental frozen yoghurt were significantly ($P<0.05$) higher than those of control frozen yoghurt. Aerobic plate count of the experimental and control frozen yoghurt were statistically at par. Both the yoghurt samples were free from coliform as well as yeast and Mold (Table 6).

Conclusion

Strawberry and beetroot-based low-fat frozen yoghurt was prepared using response surface methodology and the rate of addition of strawberry and beetroot powder, stabilizer-emulsifier mixture and sugar were optimized to obtain sensorially acceptable product and similar physical characteristics to those of control. At linear level, strawberry and beetroot powder improved flavor, body and texture, color and appearance as well as total score of the frozen yoghurt while stabilizer-emulsifier mixture improved body and texture, melting characteristics, total score and overrun. It also reduced melted quantity of frozen yoghurt. Sugar improved flavor, body and texture, melting characteristics, total score, melted quantity as well as overrun. At quadratic level, all the variables had negative impact on sensory attributes while strawberry and beetroot powder increased melted quality, stabilizer-emulsifier blend reduced melted quality and overrun while sugar increased both of them. On the basis of the results, RSM suggested to prepare frozen yoghurt using 4.06 percent strawberry and beetroot powder, 0.21 percent stabilizer-emulsifier

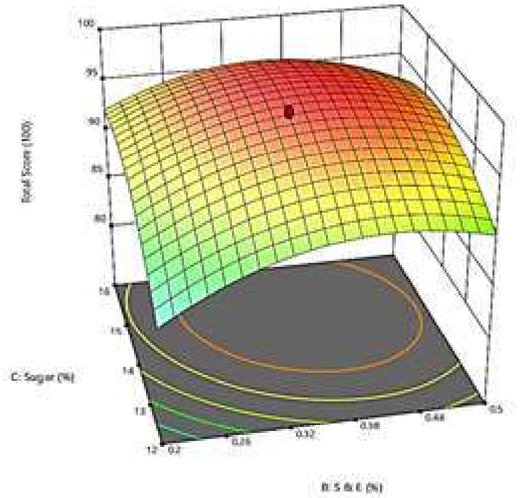


Fig. 1: Effect of different variables on total score of strawberry and beetroot incorporated low-fat frozen yoghurt

mixture and 15.45 percent sugar. The standardized frozen yoghurt was highly acceptable. Hence, an acceptable quality carrot-based low-fat frozen yoghurt can be developed by using response surface methodology.

References

Agarwal S, Prasad R (2013) Effect of stabilizer on sensory characteristics and microbial analysis of low-fat frozen yoghurt incorporated with carrot pulp. Int J Agric Food Sci Technol 4: 797-806

Apak R, Güçlü K, Özyürek M, Karademir SE (2004) Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem 52:7970-7981

Ateteallah H, Abd-Elkarim N, Hassan NA (2019) Effect of adding beetroot juice and carrot pulps on rheological, chemical, nutritional and organoleptic properties of ice cream. J Food Dairy Sci 10:175-179

Beattie J, Crozier A, Duthie GG (2005) Potential health benefits of berries. Curr Nutr Food Sci 1:71-86

Butt AY, Haq A, Aamir SH, Ashraf S, Ali R (2023) Development of functional ice cream by incorporation of oat milk and beetroot. J Agric Sci Food Res 14:1-6

Ceclu L, Nistor OV (2020) Red beetroot: Composition and health effects—A review. J Nutr Med Diet Care 6:1-9

Chhikara N, Kushwaha K, Sharma P, Gat Y, Panghal A (2019) Bioactive compounds of beetroot and utilization in food processing industry: A critical review. Food Chem 272:192-200

Dabija A, Codină GG, Ropciuc S, Stroe SG (2019) Studies regarding the production of a novel yogurt using some local plant raw materials. Journal of Food Processing and Preservation 43:1-9

Dharaiya CN, Jana A, Patel AM, Patel DH (2021) Comparison of natural Mozzarella cheese with acid casein-based Mozzarella cheese analogue. Indian J Dairy Sci 74:301-308

FSSAI (2012) Manual of Methods of Analysis of Foods: Microbiological Testing. Food Safety and Standards Authority of India, New Delhi

FSSAI (2015) Manual of Methods of Analysis of Foods: Milk and Milk Products. Food Safety and Standards Authority of India, New Delhi

Giampieri F, Alvarez-Suarez JM, Battino M (2014) Strawberry and human health: Effects beyond antioxidant activity. J Agric Food Chem 62:3867-3876

Häkkinen SH, Törrönen AR (2000) Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique. Food Res Int 33:517-524

Hummer KE, Hancock J (2009) Strawberry genomics: botanical history, cultivation, traditional breeding, and new technologies. In Genetics and genomics of Rosaceae. Springer, New York, NY. pp. 413-435

Ilansuriyan P, Shanmugam M (2018) Rheological, physicochemical and sensory properties of no fat to high fat ice creams samples prepared using stabilizer/emulsifier blends created with liquid and powder polysorbate-80. Int Food Res J 25:2579-2584

Jeong MY, Jang HM, Kim DH (2019) High-fat diet causes psychiatric disorders in mice by increasing Proteobacteria population. Neurosci Lett 698:51-57

Manoharan AP, Ramasamy D, Jayachandran S (2013) Organoleptic analysis of herbal ice cream with artificial sweeteners and natural color beetroot juice. Int J Food Agric Vet Sci 3:177-184

Marshall RT, Goff HD, Hartel RW (2013) Analyzing frozen desserts. In HD Goff & RW Hartel (Eds.). Ice cream (7th edn., pp. 403-435). New York

Milani E, Koocheki A (2011) The effects of date syrup and guar gum on physical, rheological and sensory properties of low fat frozen yoghurt dessert. Int J Dairy Technol, 64:121-129

Mirmiran P, Houshialsadat Z, Gaeini Z, Bahadoran Z, Azizi F (2020) Functional properties of beetroot (Beta vulgaris) in management of cardio-metabolic diseases. Nutr Metab 17:1-15

Moeenfard M, Tehrani MM (2008) Effect of Some Stabilizers on the Physicochemical and Sensory Properties of Ice Cream Type Frozen Yogurt. American-Eurasian J Agric Environ Sci 4: 584-589

Muse MR, Hartel RW (2004) Ice cream structural elements that affect melting rate and hardness. J Dairy Sci 87:1-10

Myers RH, Montgomery DC, Vining GG, Borror CM, Kowalski SM (2004) Response surface methodology: A retrospective and literature survey. J Qual Technol 36: 53-77

Patel IJ, Dharaiya CN, Pinto SV (2015) Development of technology for manufacture of ragi ice cream. J Food Sci Technol 52: 4015-4028

Rebecca LJ, Sharmila S, Das MP, Seshiah C (2014) Extraction and purification of carotenoids from vegetables. J Chem Pharm Res 6:594-598

Skryplonek K, Henriques M, Gomes D, Viegas J, Fonseca C, Pereira C, Dmytrów I, Mituniewicz-Małek A (2018) Characteristics of lactose-free frozen yogurt with κ-carrageenan and corn starch as stabilizers. J. Dairy Sci 102:7838–7848

Srivastava S, Singh K (2016) Physical, Sensory and Nutritional Evaluation of Biscuits Prepared By Using Beetroot (Beta vulgaris) Powder. Int J Innov Res Adv Stud 3:281-283

Sudha S, Madhvi D (2015) Evaluation of organoleptic properties of beetroot green pulp incorporated vanilla ice cream. Int J Food Nutr Sci 4:105-106

Syed QA, Shah MSU (2016) Impact of stabilizers on ice cream quality characteristics. MOJ Food Process Technol 3:246-252

Tamime AY, Robinson RK (2007) Background, standards and marketing of frozen yogurt: Yoghurt: Science and Technology. pp. 392-393. Boca Raton, CRC Press.

Indian J Dairy Sci 77(3): 203-208
https://doi.org/10.33785/IJDS.2024.v77i03.002

RESEARCH ARTICLE

Modelling and Optimizing Buttermilk Concentration and Coagulation Temperature for *Paneer* Production

Gaurav Chaudhary [ae], Ameeta Salaria[b], Yogeshkumar V. Vekariya[c], Shalini Arora[de](✉) and A.K. Singh[e]

Received: 27 Julyl 2023 / Accepted: 06 October 2023 / Published online: 23 June 2024

Abstract: This study explores the utilization of buttermilk, a significant byproduct of butter manufacture, in the production of *paneer*, a heat and acid-coagulated dairy product. Response Surface Methodology (RSM) was employed to investigate the impact of buttermilk addition (7.5-25.00%) and coagulation temperature (70-85°C) on various compositional parameters of *paneer*. The *paneer* yield ranged from 16% to 21%, while total solids, fat, protein, and lactose content varied from 40.5% to 50.5%, 21.0% to 32.5%, 10.1% to 18.8%, and 1.9% to 2.8%, respectively, at different buttermilk levels. Through optimization, it was found that replacing 13.99% of milk with buttermilk and coagulating at 85°C resulted in the production of *paneer* with enhanced nutritional quality. This study demonstrates an innovative and sustainable approach to improving the value of buttermilk and obtaining high-quality *paneer* with desirable nutritional attributes.

Keywords: Buttermilk; Coagulation temperature; Milk product; *Paneer*; Modelling

Introduction

India holds the distinction of being the world's leading milk producer, with a substantial output of 209.96 million tons (MT) in 2020-21, surpassing the previous year's production of 198.44 MT (FAOSTAT, 2021). The traditional sector plays a pivotal role, converting approximately 50-55% of processed milk into a diverse range of dairy products, contributing 20.17 percent to the sector (Arora, 2019). Amongst the cherished processed dairy products in India is *paneer,* a classic cottage cheese delicacy derived from buffalo milk, often used as a key ingredient in traditional Indian desserts (USDA, 2015; Arora et al. 2019; Arora et al. 2022; Gurditta et al. 2019). The Indian market witnesses a significant consumption of approximately 3 million tonnes of locally produced sweets, further showcasing the popularity of *paneer* in Indian cuisine (Gurditta, et al. 2014).

During the butter-making process, an essential byproduct known as buttermilk is produced. Buttermilk constitutes the liquid phase resulting from churning cream into butter. On a global scale, around 6.5%–7.0% of milk production is utilized for butter production, leading to the substantial generation of buttermilk as a byproduct (3.2 million tonnes annually). Additionally, the process of making butter and curd produces sour buttermilk, also known as *lassi* (Kumar et al. 2015).

Buttermilk boasts emulsifying and flavor-enhancing properties, making it a crucial dairy ingredient in various culinary applications. Both sweet and cultured buttermilk share a chemical composition akin to skim milk, although natural buttermilk exhibits compositional variations depending on the type of milk used for curd preparation and the amount of water added during churning. On average, it contains approximately 4% total solids, 3% to 4% lactose, 1.2% lactic acid, 1.3% protein, and 0.8% fat. Notably, buttermilk is a rich source of calcium, providing about 28% of the body's daily calcium requirement in one cup of low-fat buttermilk (Sodini et al. 2006). Consumption of around 500 ml of buttermilk can fulfill the body's entire daily calcium needs. Furthermore, well-packaged buttermilk typically possesses a pH of 4.5 and a smooth, thick consistency. Its consumption contributes to increased protein intake (approx.3.73% in sweet cream buttermilk), making it a popular domestic beverage in India, prized for its high nutritive value. Its mild acidity also makes it an excellent thirst quencher. buttermilk contains up to seven times higher concentrations (about 0.89 mg/g) of phospholipids than whole milk (Barukèiæ et al. 2019).

[a]Department of Food Science and Technology, Chaudhary Charan Singh University, Meerut (UP), (India)

[b]Faculty of Dairy Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology, RS Pura, Jammu, J&K, (India)

[c]Dairy Engineering Department, SMC College of Dairy Science, Kamdhenu University, Gandhinagar, Gujarat (India)

[d]Department of Dairy Technology, CoDST, Lala Lajpat Rai University of Veterinary and Animal Sciences, (Hisar), Haryana (India)

[e]Dairy Technology Division, National Dairy Research Institute (NDRI), Karnal, Haryana (India)
(✉)
Email: shaliniarora.luvas@gmail.com

In dairy product development, Response Surface Methodology (RSM) stands as a powerful statistical tool to optimize various process variables through experimental design (Henika, 1982). Researchers have effectively employed RSM to optimize process variables in diverse dairy products such as pearl millet-based dairy desserts, desiccated *chhana-murki* and low-calorie high-fiber *chhana* balls (Singh et al. 2019; Jha et al. 2013; Arora et al. 2021). However, the application of RSM to determine optimal *paneer* production parameters, specifically related to buttermilk addition and coagulation temperature, remains unexplored. The main objective of the numerical optimization in this study was to produce a *paneer* product with a high level of acceptance or quality. To achieve this goal, various constraints and parameters were taken into consideration. This study aims to investigate the impact of these variables on the compositional properties of paneer using Response Surface Methodology (RSM).

Materials and Methods

Raw materials

Freshly pooled mixed milk (6-7% fat content and 8.5-9% SNF), was procured from the Experimental Dairy Plant, Dairy Technology Division, ICAR-National Dairy Research Institute, Karnal, India in a steam-sterilized aluminium can. The citric acid (SQ grade, Qualigens Fine chemicals, Mumbai) was used to adjust milk pH for *paneer* preparation. Buttermilk produced during the process of butter making was used, with fat content varying from 0.1-0.5% and total solids ranged from 2.6-3.2%.

Experimental Design

RSM was used to study the optimum condition for producing buttermilk-added *paneer*. The experimental design and statistical analysis were executed using Stat-Ease software (Design-Expert 13 Trial, Stat-Ease, Inc., MN, USA). A five-level-two factor central composite rotatable design (CCRD) was used to assess the mutual effect of two process variables (%) buttermilk and coagulation temperature (CT) of milk, coded as A, and B respectively. The minimum and maximum values for A, and B was 7.5-25% and 70-85°C, respectively. Table 1 represents the codes and values of the process variables for *paneer* making experiment. The complete design consisted of 13 combinations. The actual value of two process variables and the experiment results of the studied responses' such as proximate composition and yield is shown in Table 2.

Preparation of paneer

The 13 different *paneer* samples were prepared using different combinations of buttermilk level and coagulated at different coagulation temperature to ascertain the optimized solution.

The preparation of *paneer* was carried out following the method of Sachdeva and Singh (1988). Desired quantity of buttermilk (as per RSM) was added in the standardized mixed milk (4.5% fat and 8.5% SNF) and it was heated to 90°C without holding and coagulated at the desired temperature as per RSM design. The milk solids were pressed by weight corresponding with a weight of 4 kg placed over planks measuring 35×28×10 cm sitting over the filled hoops for 25-30 minutes. Cooling was done with chilled water (4-5°C) for 2 hours.

Analytical procedures

The moisture, fat, protein and ash content of *paneer* samples was estimated using moisture analyzer, Gerber method, Kjeldahl assembly, and Muffle furnace, respectively as per methods described by IS: SP18 (Part XI). Lactose content was estimated by Lane Eynon method as described by Ranganna (1986). The yield of the *paneer* obtained by mixing buttermilk with milk was calculated. This was done by weighing the total milk amount before *paneer* making and then weighing the product obtained. Then yield percentage was calculated using formula given below:

$$\text{Yield (\%)}\ \frac{100 \times W_1}{W_2}$$

Where, W_1 = Weight of the product (*paneer*) obtained

W_2 = Weight of milk taken for *paneer* preparation including buttermilk

Statistical analysis

Analysis of variance (ANOVA) was used to evaluate the effect of independent variables on responses. All trials were carried out in triplicate and the statistical significance was evaluated and p d"0.01 and p d"0.05 was taken as significant.

Result and Discussion

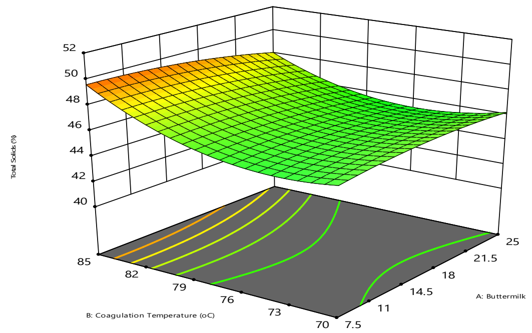
Total Solids content

The findings, presented in Table 2, Table 3, and Fig. 1a, revealed that the total solid content of the paneer samples exhibited a range from 40.55% to 50.58%.

The *paneer* prepared using 16.66% buttermilk at a coagulation temperature of 66.89°C displayed the lowest total solid content. This can be attributed to the combination of a lower coagulation temperature and a relatively higher buttermilk concentration, which resulted in a substantial entrapment of water within the casein structure. Consequently, the entrapped water led to a reduction in the overall solid content of the *paneer*.

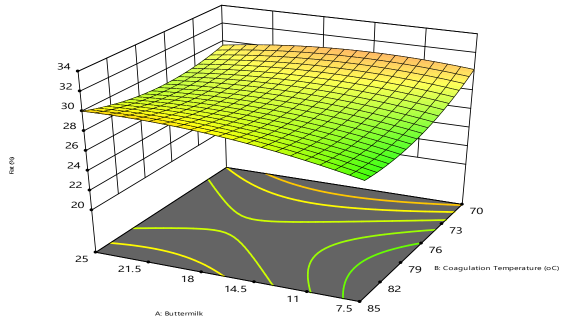
Conversely, the highest total solids (50.58%) content was observed in the *paneer* sample produced with 16.25% buttermilk

(a)



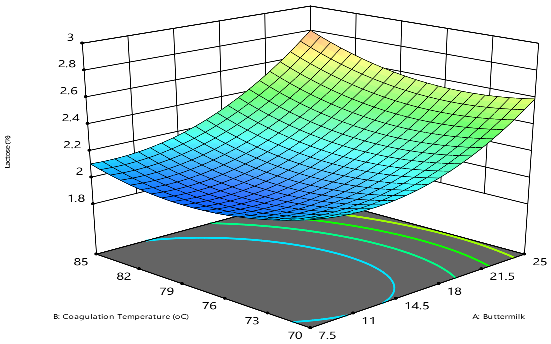
1a Response surface plots showing the effect of buttermilk and coagulation temperature on Total solids content

(b)



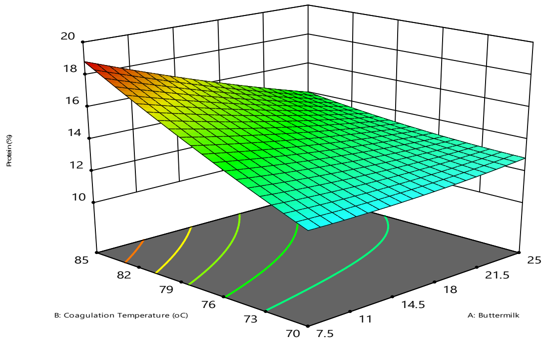
1b Response surface plots showing the effect of buttermilk and coagulation temperature on Fat content

(c)



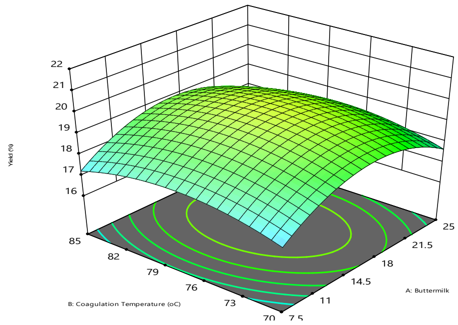
1c Response surface plots showing the effect of buttermilk and coagulation temperature on Lactose content

(d)



1d Response surface plots showing the effect of buttermilk and coagulation temperature on Protein content

(e)



1e Response surface plots showing the effect of buttermilk and coagulation temperature on %Yield

Fig 1. Response surface plots showing the effect of buttermilk and coagulation temperature

at a higher coagulation temperature of 88.11°C. The elevated coagulation temperature facilitated maximum casein coagulation, resulting in a significant enhancement of the total solid concentration in the *paneer*.

The results provide valuable insights for optimizing the *paneer* production process to achieve desired solid concentrations. The results are also following earlier reports of Deshmukh et al. (2009); Kantha and Kanawjia (2009) and Bhatt (2013).

Fat concentration

The study investigated the fat percentage in *paneer* produced using different combinations of buttermilk concentration and coagulation temperatures. The results, presented in Table 2, Table 3, and Fig. 1b, revealed that the fat content of the *paneer* samples varied in the range of 21.00% to 32.50%.

The *paneer* sample prepared with 7.5% buttermilk and a coagulation temperature of 70°C exhibited the highest fat percentage (32.50%). This can be attributed to the relatively lower buttermilk concentration and the consequent reduction in fat losses during the whey-draining process. The lower buttermilk concentration allowed for a higher retention of fat within the *paneer* structure.

On the other hand, the paneer sample made with 16.25% buttermilk and a coagulation temperature of 66.89°C had the lowest fat percentage (21.00%). The higher buttermilk concentration and lower coagulation temperature in this case led to increased fat loss in the whey, which was drained off during the *paneer*-making process. The resulting *paneer* had higher moisture content, contributing to the reduced fat level and a very soft texture.

These findings are in line with earlier reports by Chandan (2007), Deshmukh et al. (2009), Kantha and Kanawjia (2009), and Bhatt (2013), further supporting the consistency and validity of the study's results.

Protein concentration

The study focused on analyzing the protein concentration in *paneer* produced using different combinations of buttermilk concentrations and coagulation temperatures. The results,

Table 1: Experimental variables for *paneer* making using milk admixed with buttermilk (coded and actual values)

Independent Variable	Coded Values	Coded Level				
		-1.7[a]	-1	0[b]	+1	+1.7[a]
Buttermilk (%)	A	3.88	7.5	16.25	25.0	28.62
Coagulation Temperature (°C)	B	66.89	70	77.50	85	88.11

a = ± á, b = centre point

Table 2: Chemical composition and yield of *paneer* made with different levels of buttermilk and coagulation temperature

Run	A: Buttermilk (%)	B: Coagulation Temperature °C	Total Solids (%)	Fat (%)	Protein (%)	Lactose (%)	Yield (%)
1.	16.25	77.50	47.40	30.50	13.67	2.10	18.00
2.	7.50	70.00	46.02	32.50	10.11	2.38	17.40
3.	16.25	77.50	47.82	31.50	13.16	2.06	21.34
4.	16.25	77.50	47.81	31.50	13.16	2.14	21.30
5.	16.25	77.50	47.40	30.50	13.74	2.18	18.00
6.	28.62	77.50	44.32	28.25	12.19	2.86	17.20
7.	3.88	77.50	45.45	25.00	17.46	1.91	16.00
8.	25.00	85.00	49.03	29.50	15.63	2.80	17.80
9.	25.00	70.00	46.64	30.50	12.29	2.71	17.60
10.	16.25	77.50	47.40	30.50	13.81	2.09	18.00
11.	7.50	85.00	50.32	28.25	18.77	2.21	17.60
12.	16.25	88.11	50.58	31.00	16.12	2.38	18.00
13.	16.25	66.89	40.55	21.00	16.66	1.95	19.80

Table 3: Coefficients of quadratic models for the response variables as a function of process variables

	Total Solids	p-values	Fat	p-values	Protein	p-values	Lactose	p-values	Yield	p-values
Intercept	46.20		29.00		14.11		2.08		19.69	
A	-0.28	0.76	0.48	0.72	-1.05	0.15	0.28	0.00	0.26	0.58
B	1.40	0.15	-0.57	0.67	1.93	0.02	0.04	0.50	0.05	0.92
A^2	-0.39	0.69	-0.81	0.58	0.19	0.79	0.20	0.01	-1.47	0.02
B^2	1.66	0.12	1.25	0.40	0.23	0.75	0.13	0.06	-0.77	0.16
AB	-0.48	0.71	0.81	0.67	-1.33	0.19	0.07	0.43	0.00	1.00
R^2	0.67		0.7		0.66		0.86		0.66	

Highlited significant value $p<0.05$

Table 4: Goals set for constraints to get an optimum combination of the process variables

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Buttermilk	is in range	7.5	25	1	1	3
B:Coagulation Temperature	is in range	70	85	1	1	3
Total Solids	maximize	40.55	50.58	1	1	3
Fat	is in range	21	32.5	1	1	3
Protein	maximize	10.11	18.77	1	1	3
Lactose	is in range	1.91	2.86	1	1	3
Yield	maximize	16	21.34	1	1	3

presented in Table 2, Table 3, and Fig. 1c, demonstrated that the protein content of the *paneer* samples varied between 10.11% and 18.77%.

The *paneer* sample prepared with 7.5% buttermilk and a coagulation temperature of 70°C exhibited the lowest protein percentage (10.11%). This can be attributed to the coagulation of casein particles, the major milk protein responsible for the formation of *paneer*. The coagulam formed at 70°C are relatively softer due to the gentle coagulation process. The softer curds retain more moisture, and as a result, the protein content in the curds might be relatively lower because of the higher water content.

Conversely, the *paneer* sample made with 7.5% buttermilk and a coagulation temperature of 85°C displayed the highest protein percentage (18.77%). The higher temperature leads to more whey expulsion, concentrating the proteins in the curds. As a result, the protein content in the curds formed at 85°C is likely to be higher compared to curds formed at 70°C. Further due to denaturation, whey protein also get entrapped with the casein particles leading to higher protein content in the resultant product.

These findings align with earlier research by Chandan (2007), Deshmukh et al. (2009), Kantha and Kanawjia (2009), and Bhatt (2013), providing further support for the consistency and validity of the study's results.

Lactose concentration

The study investigated the lactose concentration in *paneer* produced using different combinations of buttermilk concentrations and coagulation temperatures. The results, presented in Table 2, Table 3, and Fig. 1d, revealed that the lactose content of the *paneer* samples varied between 1.912% and 2.86%.

The highest lactose percentage (2.86%) was found in the *paneer* sample made from 18.62% buttermilk and a coagulation temperature of 77.5°C. This can be attributed to the fact that buttermilk is a good source of lactose, and the higher concentration of buttermilk in this case led to an increase in the lactose content of the resulting *paneer*.

Conversely, the *paneer* sample prepared with 3.88% buttermilk and a coagulation temperature of 77.5°C displayed the lowest lactose percentage (1.91%). Most of the lactose is lost in the whey during the *paneer*-making process. The lower buttermilk concentration in this sample resulted in a reduced lactose content in the final product.

These findings align with previous studies conducted by Chandan (2007), Kantha and Kanawjia (2009), and Bhatt (2013), providing further support for the consistency and validity of the study's results.

Yield of the *paneer*

The study examined the yield of *paneer* produced using different combinations of buttermilk concentrations and coagulation temperatures. The results, presented in Table 2, Table 3, and Fig. 1e, revealed that the yield of the *paneer* samples varied between 16.00% and 21.34%.

The highest yield of *paneer* (21.34%) was obtained from the sample prepared with 16.25% buttermilk at a coagulation temperature of 77.5°C. This higher yield can be attributed to the better retention of moisture and other constituents during the *paneer*-making process. The optimal combination of buttermilk concentration and coagulation temperature allowed for the maximum incorporation of water and other components, resulting in a higher yield of *paneer*.

Conversely, the *paneer* sample made with 3.88% buttermilk and a coagulation temperature of 77.5°C displayed the lowest yield percentage (16.00%). The lower buttermilk concentration in this case led to reduced moisture retention during the *paneer*-making process, resulting in a lower yield of the final product.

These findings are consistent with previous reports by Chandan (2007), Kantha and Kanawjia (2009), and Bhatt (2013), further reinforcing the validity and reliability of the study's results.

Optimization

Table 4 provides a summary of these constraints that were applied during the optimization process to attain the highest value for each parameter.

By maximizing the objectives for each parameter, the researchers were able to determine the optimal combination of buttermilk concentration and coagulation temperature that would result in a *paneer* product with the best over all quality. According to the numerical optimization results, the ratio of 13.99% buttermilk to 85°C coagulation temperature was identified as the ideal combination, producing a paneer of optimum quality. This was determined based on a desirability value of 0.79. This specific combination resulted in paneer with the following composition: 49.03% total solids, 29.50% fat, 17.1% protein, and 2.10% lactose, a higher *paneer* yield of up to 18.7%, indicating that the selected settings of the manufacturing procedure were highly favorable for achieving good results.

Conclusion

In conclusion, the results of the present experiments suggest that the optimal combination of buttermilk concentration and coagulation temperature for producing nutritionally high-quality *paneer* is achieved. This specific combination resulted in *paneer* with the following composition: 49.03% total solids, 29.50% fat, 17.1% protein, and 2.10% lactose. Additionally, the use of 13.99% buttermilk and 85°C coagulation temperature led to a higher *paneer* yield of up to 18.7%.

The findings indicate that *paneer* prepared with this combination has a desirable nutrient profile, with significant levels of total solids, fat, and protein. Moreover, the higher yield obtained from this combination is advantageous from both a nutritional and economic standpoint.

References

Arora S (2019) Food safety issues and strategies to improve trade ofIndian livestock products. Ind Dairyman 10:60-67

Arora S, Gurditta H, Patel AA, Singh U, Singh RRB, Rekha (2021) Modelling and optimization of process parameters for productionof desiccated Chhana-murki (Indian cottage cheese-based dessert). J Food Sci Technol 56:3043–3054 https://doi.org/10.1007/s13197-020-04920-6

Arora S, Patel AA, Gurditta H, Yadav U, Mahajan S (2019) Estimation of production cost for hard-variant of Chhana-murki (Indian cottage cheese based cheese-based dessert). Haryana Vet 58: 174-180

Arora S, Patel AA, Sindhu R, Yadav U, Singh TP, Chaudhary G (2022) Studies on the organoleptic perception of different flavours and consumer acceptance of desiccated Chhana-murki. Indian Dairy Sci 75:125-132

Barukèiæ I, Lisak Jakopoviæ, K, Božaniæ, R (2019) Valorisation of whey and buttermilk for production of functional beverages - An overview of current possibilities. Food Technol Biotechnol 57:448-460

Chandan RC (2007) Cheese varieties made by direct acidification of hot milk. In Hui YH (eds) Handbook of food products manufacturing Vol.-I. Wiley-Inter Science, John Eiley and Sons Inc. Pub., p 645–650

Deshmukh DS, Zanjad PN, Pawar VD, Machewad GM (2009) Studies on the use of acidified and cultured whey as coagulant in the manufacture of *paneer*. Int J Dairy Technol 6:174–181

FAOSTAT (2021) Food and Agriculture Organization of the United Nations Statistics Division. Food and Agriculture Organization of the United Nations

Gurditta H, Patel AA, Arora S (2014) Optimisation of sweetener and bulking agent levels for the preparation of functional *chhana-murki*. Int J Dairy Technol 67:1-8

Gurditta H, Patel AA, Arora S (2019) Selection of dietary fibres for sucrose replacement in functional *Chhana-murki* (Indian Cottage cheese-based dessert) and their effect on sensory, physical and instrumental texture parameters. Int J Dairy Technol 72: 633-642. https://doi.org/10.1111/1471-0307.12624

Henika RG (1982) Use of response surface methodology in sensory evaluation. Food Technol 36:96–101

Jha A, Tripathi AD, Alam T, Yadav R (2013) Process optimization for manufacture of pearl millet-based dairy dessert by using response surface methodology. J Food Sci Technol 50:367–373

Kantha KL, Kanawjia SK (2009) Response surface analysis of sensory attributes and yield of low fat paneer enriched with soy fiber. Indian J Dairy Sci 60:230–238

Kumar R, Kaur M, Garsa AK, Shrivastava B, Reddy VP, Tyagi A (2015) Natural and cultured buttermilk. Chapter: Editors: AK Puniya, Natural and Cultured ButtermilkPublisher: CRC Press/ Taylor and Francis, 203-225

Ranganna S (1986) Handbook of analysis and quality control for fruit and vegetable products, 2nd Edition, McGraw-Hill Publishing Co. Ltd., New Delhi

Sachdeva S, Singh S (1988) Optimisation of processing parameters in the manufacture of paneer. J Food Sci Technol 25:142–145

Singh TP, Chauhan G, Mendiratta SK, Agrawal RK, Arora S (2019) Optimization of ingredients for preparation of low calorie fiber enriched *chhana* balls-Sandesh like product. J Food Sci Technol 5:3043-3054

Sodini I, Morin P, Olabi A, Jiménez-Flores R (2006) Compositional and functional properties of buttermilk: a comparison between sweet, sour, and whey buttermilk. J Dairy Sci 89:525– 536

USDA (2015) Foreign Agricultural Service Dairy and Products Annual GAIN Report No IN5131 October

Indian J Dairy Sci 77(3): 209-215
https://doi.org/10.33785/IJDS.2024.v77i03.003

RESEARCH ARTICLE

Evaluation of antimicrobial effect of lemon grass essential oil on biofilm forming pathogens in broth medium and on stainless steel chip

Jamunkar Shivani Rajuji[1], Santosh Kumar Mishra(✉)[1], Pranav Kumar Singh[2] and Vikas Sangwan[1]

Received: 28 August 2023 / Accepted: 05 October 2023 / Published online: 23 June 2024

Abstract: The objectives of this study was to check effect of lemon grass essential oil on biofilm forming selected pathogens in broth medium and on stainless steel chip. The antimicrobial effect of lemon grass essential oil (LMO) by well agar assay showed all four pathogenic strains were susceptible to LMO. 0.9% MIC was observed in strain *B. cereus* ATCC 10876 and it was 0.8% for both *S. aureus* ATCC 700698 and *Proteus mirabilis* ATCC 12453 strains. *E. coli* ATCC 10536 was found to be the species bearing the least MIC (0.6%). Time kill analysis with all the four planktonic pathogens in broth medium by using 0.8% lemongrass essential oil (selected based on MIC) was checked at their stationary growth phase. *E. coli* was found most sensitive to LMO treatment and gave complete reduction within 20 min contact time, while *S. aureus* gave 71% inhibition, *Proteus mirabilis* gave 53% inhibition after 80 min of treatment with LMO. *Bacillus cereus* was the most resistant among all against LMO and gave only 27% inhibition after 80 min of treatment. However all the pathogens inhibited completely after 24 h treatment with 0.8% LMO. On stainless steel chip against biofilm of *S. aureus* and *E. coli,* LMO (0.8%) treatment gave complete reduction within 24 h. This study demonstrated that LMO could be a reliable foodborne pathogen biofilm disrupter.

[1] Department of Dairy Microbiology, College of Dairy Science & Technology, Ludhiana-141004, Punjab, India

[2] Department of Dairy Technology, College of Dairy Science & Technology, Ludhiana-141004, Punjab, India

Santosh Kumar Mishra(✉)
Department of Dairy Microbiology,
College of Dairy Science and Technology,
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India 141 004
Phone: +91-9464995049
E Mail: skmishra84@gmail.com

Keywords: Biofilm, Pathogens, Stainless steel chip, Lemon grass essential oil, Antimicrobial effect

Introduction

A food industry, particularly those in the dairy sector, always strives to satisfy customers and avoid microbial contamination, which can adversely affect product quality and consumer health (Mnif et al. 2020; Mishra et al. 2011). Internationally, both developed and developing countries see foodborne illnesses as a rising public health issue. The dairy industry's equipment are highly susceptible to microorganisms, especially in milk storage tanks and milk processing lines, and is the leading cause of milk contamination (Vishwakarma, 2020). The dairy industry often struggles with biofilm problems due to the presence of foodborne pathogens or spoilage microorganisms. Due to this major spoilage, dairy industry connected to both economic and public health consequences. Biofilms of pathogenic bacteria may also raise the risk of foodborne illness in milking equipment and milk storage tanks (Panebianco et al. 2022; Manju & Grover 2023). Due to their difficulty in eliminating, bacteria within biofilms act as a source of recurrent contamination of plants, products, and personnel after they have been established (Makwana et al. 2018)

To date, a wide range of commercially available disinfectants have been broadly used for lowering microbial contamination in order to produce safer and longer-lasting products. In order to eradicate biofilm, several factors need to be considered, such as concentration, pH, temperature, exposure time, type of surface, and relative humidity.

Traditionally, plants essential oils have been sought out as safe and natural alternatives to synthetic antiseptics and antibacterial drugs (Burt, 2004). Research has shown that essential oils and their component have antimicrobial properties against some bacteria and fungi. Several studies have implicated lemongrass in antidepressants, antioxidants, antiseptics, astringents, bactericidal, fungicidal, nervine, and sedative activities. LMO is having GRAS (Generally Recognised As Safe) status (Faheem et al. 2022). Reports state that LMO (probably referring to a specific chemical) has been discovered to have higher FRAC (ferric reducing antioxidant capacity) but lower scavenging activity. In comparison to compounds like ascorbic acid and butylated hydroxyl toluene, LMO is hence more efficient at chelating iron (II) ions (Viuda-Martos et al 2010). Lemongrass essential oil (LMO) is rich in terpenes like citral (85%) and geraniol (1.5%) (Ortega-Ramirez et al. 2020).

The objectives of this study was to check effect of lemon grass essential oil on biofilm forming selected pathogens in broth medium and on stainless steel chip.

Materials and Methods

Pathogenic cultures

Four pathogenic cultures for screening of biofilm formation were obtained from the Department of Dairy Microbiology, College of Dairy Science and Technology, Ludhiana, Punjab (Table 1). Stock cultures were preserved at -80ºC. Before any assay, strains were revived by transferring stock cultures into Brain Heart Infusion (BHI, Hi media) broth and further placed at 37ºC for 24 hours. Purity of every culture was ascertained by doing Gram staining and catalase test. The storage of cultures was done below 5ºC between transfers.

Procurement of Lemongrass essential oil (LMO)

LMO (extracted from *Cymbopogon citratus*) was obtained from Satuguru Trading firm located in Tarntaran, Punjab, India. The manufacturer confirms that the lemongrass oil was isolated through distillation from lemongrass leaves cultivated in Punjab. Furthermore, the oil's purity has been verified to be 100% using a chiral method.

Preparation of LMO concentrations

Various concentrations, specifically 5%, 10%, 15%, 20%, 25%, and 30%, (v/v) of lemongrass oil were prepared in sterile 0.6% tween-80 (v/v) in aseptic environment.

Evaluation of antimicrobial effect of LMO by disc diffusion assay

Using disc diffusion method of Adukwu et al. (2016), the antimicrobial effect of LMO was tested on bacterial cultures at different concentrations (5 %, 10 %, 15%, 20%, 25% and 30%). BHI agar having 0.6% of tween 80 was prepared and 15 mL agar was added to specific petri plates and allowed to solidify. A total of 100 µL of pathogenic suspension was spread on to agar aseptically. At the same time, 6 mm diameter sterile discs were soaked with 20 µL of various concentrations of LMO (5 %, 10 %, 15%, 20%, 25%, 30%) and then placed on the agar plates. For negative control only disc with tween 80 was placed. Inhibition Zone in mm was checked after the incubation period was over.

Evaluation of antimicrobial effect of LMO by well agar assay

Using agar well diffusion method, the inhibitory effect of LMO was tested on bacterial cultures at different concentrations (5 %, 10 %, 15%, 20%, 25% and 30%) as described by Adukwu et al. (2016). BHI broth, having 0.6% of tween 80 was prepared and 15 mL of agar was added to specific petri plates and allowed to solidify. A total of 100 µL of pathogenic suspension was spread on to agar aseptically. Wells of 6 mm diameter were made in solidified agar. A total of 50 µL of each LMO concentration was pipetted out and filled into wells. For negative control only tween 80 was added to the well. All the petri dishes were incubated at 37! for 24-36 hrs. Inhibition zone in mm was checked after the incubation period was over.

Evaluation of minimum inhibitory concentration (MIC) of LMO

The determination of the MIC for LMO on the pathogenic strain was conducted through the broth dilution method, following the protocol outlined by Hammer et al. (1999). To prepare the test strain cultures, the pathogenic strain was inoculated into sterilized test tubes containing 5 mL of nutrient broth. Subsequently, the tubes were placed in an incubator and allowed to incubate overnight at a temperature of 37±1ºC. The MIC was defined as the minimum amount of any compound capable of inhibiting the growth of pathogen. A total of 10 mL of tryptic soy broth (TSB, Hi media) supplemented with 0.6% (v/v) tween-80 was spiked with varying concentrations of LMO, spanning from 1% to 0.015% (v/v) in different test tubes. As a positive growth control, TSB with 0.6% (v/v) tween-80 and without LMO was employed. Uniformly, 25 µL of pathogenic suspension was added to individual tubes. Subsequently, the tubes were placed in an incubator at a temperature of 37±1ºC for incubation periods of both 24 and 48 hours. After the designated incubation periods, the tubes were assessed for turbidity. Additionally, to evaluate bacterial growth, the tubes that exhibited no increase in turbidity during each time interval of 24 to 36 h were streaked onto nutrient agar. This step aimed to confirm the absence of pathogenic growth. To ensure accuracy, each trial was repeated three times.

Time kill analysis of planktonic pathogens by LMO in broth medium at stationary phase of their growth

Method of Mitic-Culafic et al. (2005) was subjected to further modifications. 100 µL of each pathogenic culture was inoculated into 10 mL BHI (pH 6.80) tubes in duplicate and incubated at 37! for 16 hrs. The pathogenic cultures were centrifuged at 3000 rpm for 6 minutes and pellet were washed and diluted using sterile phosphate buffer to the final OD of 0.5 at 600 nm wavelength to obtain cell number about 8.7×10^8 colony forming unit (CFU) per mL. The tubes containing test strains were inoculated with 0.8 % concentration of lemongrass oil (above the calculated MIC for each pathogen) along with tubes without any lemon grass oil as control. All the test tubes were incubated at 37! for a period 20, 40, 60, 80 min and 24 h. After every designated incubation period the dilutions were made for each tube and were plated on BHI agar. Plates were incubated at 37! for 24-48 hrs. Percent inhibition was calculated at each time interval in comparison with control.

Development of biofilm on stainless steel

The method initially proposed by Moltz and Martin (2005) was subjected to further modifications. A volume of 0.5 mL from cultures of selected pathogens (*E. coli* ATCC 10536 and *S. aureus* ATCC 700698) was inoculated into 500 mL of BHI broth at a pH of 6.80. This process was carried out in duplicate and the mixtures were then incubated at a temperature of 37°C for a period of 24 hours. These cultures were supplemented with 304 stainless steel chips measuring 2.54 x 2.54 cm^2 each. Before utilization, all stainless steel (SS) chips were immersed in a 70% ethanol solution for a period of 10 minutes, following which they were rinsed three times using sterile deionized water. Subsequently, the chips underwent autoclaving at a temperature of 121°C for 15 minutes and were then dried under a biosafety hood for 30 minutes. The incubation of the broth was executed on a shaker to replicate the bacterial growth on the SS chips. After the incubation, the chips were subjected to washing using a sterile phosphate buffer in order to eliminate any detached cells present on the surface of the SS chips.

Evaluation of antimicrobial effect of LMO on selected pathogenic biofilm developed on stainless steel

Stainless steel chips with biofilm were dipped in 0.8 % lemon grass solution and placed in a shaker incubator at 37! for 24 hrs for further incubation. After 24 hrs incubation stainless steel chip was taken out. The surface of the stainless steel chip was scraped using sterile swab. The swab was then used to make dilutions, and the diluted samples were plated on BHI agar and were incubated at 37! for 24 hrs.

Statistical analysis

Under the supervision of a statistician, data gathered from numerous experiments during the screening and comparative analysis process were analyzed for two-way analysis of variance (ANOVA) and t-test using SAS 9.3 version. Microsoft excel was used to calculate the mean, standard error of data, when needed.

Results and Discussion

Assessing purity of commercial pathogenic strains

During this study four pathogenic cultures for screening of biofilm formation were obtained from the Department of Dairy Microbiology, College of Dairy Science and Technology, Ludhiana, Punjab. All the strains were found to be pure under microscope, shown characteristic appearance on their selective growth medium and were catalase positive (Table 1 & Figure 1).

Evaluation of antimicrobial activity of lemongrass essential oil by Disc diffusion assay

The antimicrobial activity of lemongrass essential oil gradually increased with increased in concentration (Figure 2). Both Gram positive and Gram negative pathogens showed sensitivity towards LMO. Highest inhibition zone was observed at concentration 30%.

Evaluation of antimicrobial activity of LMO by well agar assay

As given in Figure 3 and Table 2, for *B. cereus* ATCC 10876, *S. aureus* ATCC 700698, *E. coli* ATCC 10536 and *Proteus mirabilis* ATCC 12453 highest inhibition zone was observed at 30% LMO concentration. The findings of the current investigation unequivocally demonstrate the considerable antibacterial potential of lemongrass oil against the examined microorganisms. The outcomes derived from agar diffusion assay align with the prevailing notion that Gram-positive organisms exhibit more susceptibility to the LMO compared to Gram-negative bacteria. Comparable conclusions were also drawn by Cimanga et al. (2002).

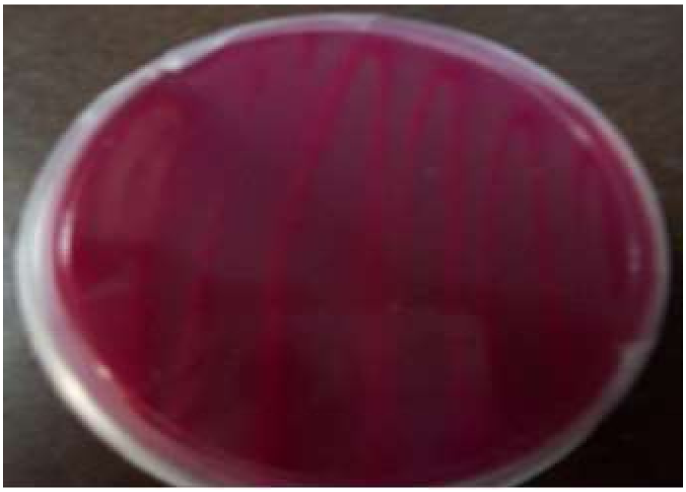


Fig.1 *E. coli* on Violet red bile Agar

Table 1: Purity testing of pathogenic strains

Sr. No.	Name of Lactic acid bacteria	Gram's staining	Catalase test
1	*Staphylococcus aureus* ATCC 700698	Gram +ve	Catalase +ve
2	*Escherichia coli* ATCC 10536	Gram -ve	Catalase +ve
3	*Proteus mirabilis* ATCC 12453	Gram -ve	Catalase +ve
4	*Bacillus cereus* ATCC 10876	Gram +ve	Catalase +ve

Fig. 2 Disc diffusion assay using LMO against pathogens

Staphylococcus aureus ATCC 700698 *Escherichia coli* ATCC 10536

Bacillus cereus ATCC 10876 *Proteus mirabilis* ATCC 12453

Table 2: Diameter of zone of inhibition of selective pathogen

Pathogen	Zone of inhibition (mm)					
	5%	10%	15%	20%	25%	30%
Escherichia coli ATCC 10536	10±0.2	13±0.4	17±0.6	18±0.2	19±0.4	20±0.3
Staphylococcus aureus ATCC 700698	14±0.5	15±0.3	18±0.4	18±0.4	18±0.5	19±0.4
Proteus mirabilis ATCC 12453	10±0.3	13±0.4	17±0.2	18±0.4	18±0.6	19±0.3
Bacillus cereus ATCC 10876	13±0.4	14±0.3	19±0.5	20±0.6	21±0.2	21±0.6

* - Average of triplicate trials

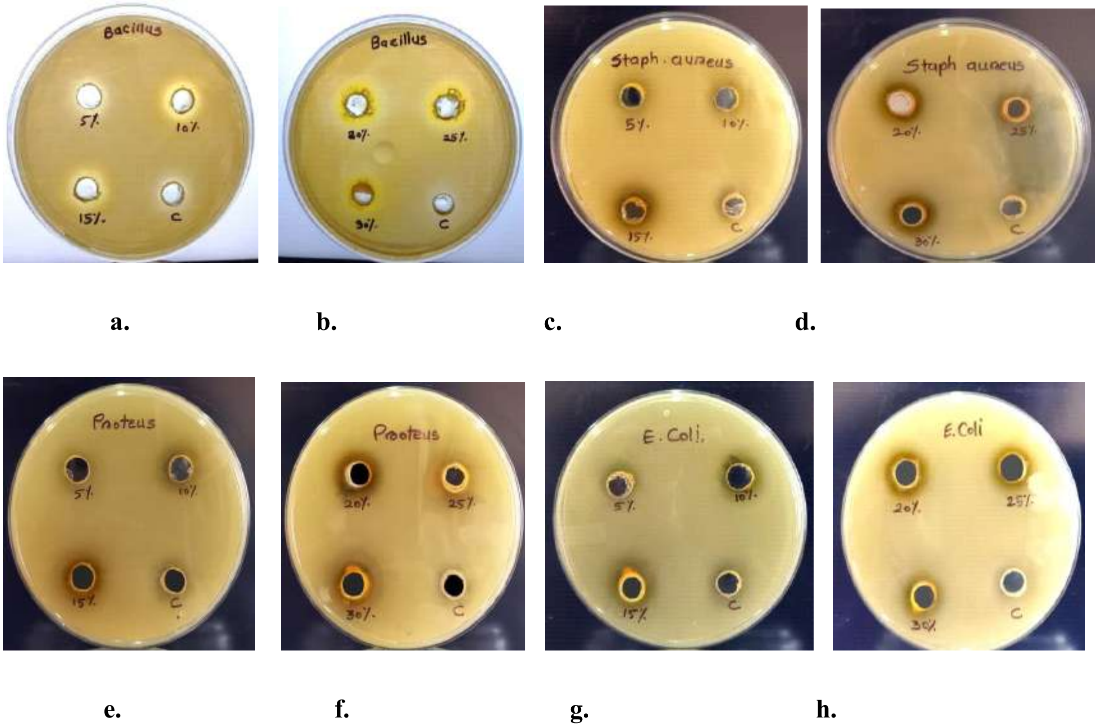
Table 3: Minimum Inhibitory Concentration (in %) of LMO against the pathogens

Pathogen	Minimum Inhibitory Concentration (MIC in % LMO)
Escherichia coli ATCC 10536	0.6
Staphylococcus aureus ATCC 700698	0.8
Proteus mirabilis ATCC 12453	0.8
Bacillus cereus ATCC 10876	0.9

Determination of Minimum Inhibitory Concentration of LMO by broth dilution method

Minimal inhibitory concentration (MIC) was performed by serial micro dilutions based method MIC values of each strain are given in Table 3. MIC of LMO tested for all 4 pathogenic bacteria ranged from 0.6 to 0.9% (V/V). Among the species majority of the strains were having MIC in the range of 0.8%. MIC of 0.9% was observed for strain *B. cereus* ATCC 10876 and 0.8% MIC was detected for *S. aureus* ATCC 700698 and *Proteus mirabilis* ATCC 12453,

Fig. 3 Well agar assay using different concentration (5, 10, 15, 20, 25, 30% and C-negative control) of LMO. Where, a and b - *Bacillus cereus* ATCC 10876, c and d- *Staphylococcus aureus* ATCC 700698, e and f- *Proteus mirabilis* ATCC 12453, g and h- *Escherichia coli* ATCC 10536



respectively. *E. coli* ATCC 10536 was found to be the species bearing the least MIC of 0.6%. Lemongrass oil exhibited notable inhibition of the test organisms at notably low concentrations in the broth dilution method when contrasted with the agar diffusion method. This observation coincides with the findings of Tortorano et al. (1998). Discrepancies in results between these two methods are attributed to various factors inherent to each assay, as discussed in prior research (Hili et al. 1997). These encompass variations in microbial growth, the extent of microorganism exposure to LMO, LMO solubility, and the type and quality of emulsifier employed, among other factors.

The MIC values determined in this study surpass those documented in previous research. This variation can be attributed to a multitude of factors, including disparities in plant cultivation conditions, specific plant material used, the technique employed for essential oil extraction, and the choice of solvent (Burt, 2004; Alma et al. 2007; Guan et al. 2007; Polatoglu et al. 2010). Furthermore, variations strains of *S. aureus* also contribute to this diversity. Fluit et al. (2001) reported a broad spectrum of antimicrobial susceptibility among 3,051 *S. aureus* isolates, exhibiting varying MIC levels for gentamicin (ranging from 0.12 to >8 mg/L).

Time kill analysis of planktonic cells of pathogens by using LMO (0.8%) in broth medium at their stationary phase of growth

Time kill analysis with all the four planktonic pathogens in broth medium by using 0.8% LMO (selected based on MIC) was checked in this experiment at their stationary growth phase.

As shown in Figure 4, the logarithmic value of all pathogens in LMO treated broth started to reduce at 20 minutes onwards. Increasing the contact time with LMO from 0 to 1440 min (24 h) increased cell death. For LMO treated *S. aureus* ATCC 700698 in broth medium growth inhibition was observed as 6, 26, 55, 71, and 100% after 20, 40, 60, 80 min and 24 h, respectively. *E. coli* ATCC 10536 was found to be most sensitive and got inhibited within 20 min at 0.8% concentration of lemongrass essential oil. For LMO treated *B. cereus* ATCC 10876 in broth medium growth inhibition was observed as 13, 15, 24, 27, 100% after 20, 40, 60, 80 min and 24 h, respectively. Similarly, for LMO treated *Proteus mirabilis* ATCC 12453 in broth medium growth inhibition was observed as 7, 39, 45, 53 and 100% after 20, 40, 60, 80 min and 24 h, respectively.

Time kill analysis of pathogenic biofilm formed on stainless steel chip by using LMO (0.8%)

Time kill analysis with one Gram's positive (*S. aureus* ATCC 700698) and one Gram's negative (*E. coli* ATCC 10536) pathogenic biofilms formed on stainless steel chip after overnight growth at 37°C by using 0.8% LMO (selected based on MIC) was checked in this experiment. As shown in Figure 5, after 24 h treatment of LMO, *E. coli* ATCC 10536 reduced by 8.10 log showing 100% inhibition and for control, it cells number increased by 0.17 log value. Similarly, for *S. aureus* ATCC 700698 reduced by 8.08 log showing 100% inhibition and for control, it cells number increased by 0.52 log value.

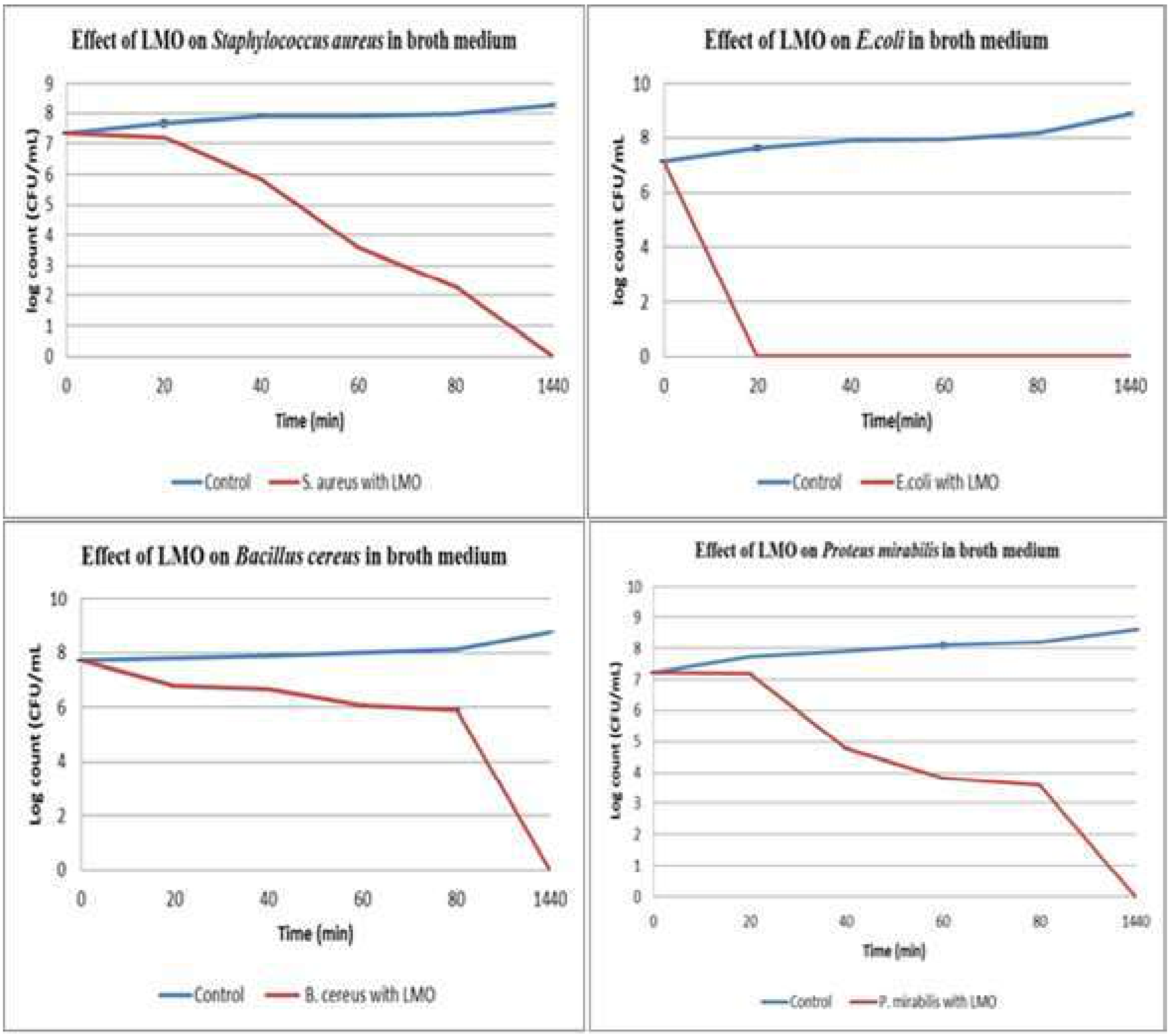
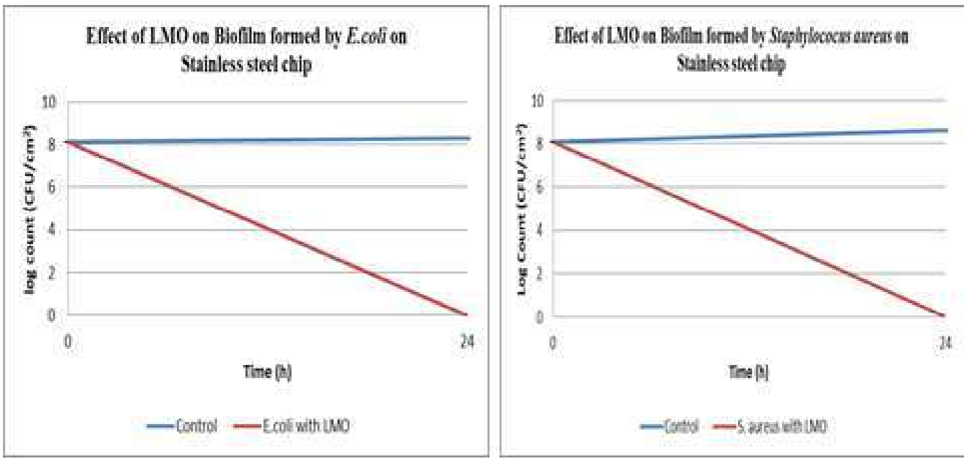


Fig. 4 Time kill analysis of planktonic cells of *S. aureus* ATCC 700698, *E. coli* ATCC 10536, *B. cereus* ATCC 10876 and *P. mirabilis* ATCC 12453 by using 0.8% LMO treatment in broth medium at their stationary phase of growth

Fig. 5 Antimicrobial effect of LMO (0.8%) on *E. coli* ATCC 10536 and *S. aureus* ATCC 700698 biofilm grown on stainless steel chip



This study stands among the limited research endeavors exploring the anti-biofilm characteristics of lemongrass essential oil concerning biofilms formed by *S. aureus* and *E. coli* on stainless steel chips. Prior research (Bearden et al. 2008) focused on examining commercial formulations containing essential oils in relation to MRSA.

Conclusions

In conclusion, as far as our understanding extends, this study represents the inaugural comprehensive examination of the anti-biofilm attributes of LMO in the context of biofilms formed by *S. aureus* and *E. coli* on stainless steel chips. The findings of this study underscore the potential of LMO as a dependable disruptor of foodborne pathogen biofilms. This treatment presents a noteworthy alternative to conventional sanitizers and holds promise for addressing biofilm concerns in food processing facilities, while also economically safeguarding food products against cross-contamination.

Acknowledgments

The authors express gratitude to Guru Angad Dev Veterinary and Animal Sciences University for providing financial support for this research.

Ethical Considerations: This article does not involve any studies involving human or animal subjects.

Declaration of Conflicts of Interest: The authors confirm that there are no conflicts of interest to disclose.

References

Adukwu, EC, Bowles M, Edwards-Jones V, Bone H (2016) Antimicrobial activity, cytotoxicity and chemical analysis of lemongrass essential oil (*Cymbopogon flexuosus*) and pure citral. Appl Microbio Biotech 100(22):9619-9627

Alma MH, Ertaş M, Nitz S, Kollmannsberger, H (2007) Chemical composition and content of essential oil from the bud of cultivated Turkish clove (Syzygium aromaticum L.). Bio Res 2(2): 265-269

Bearden DT, Allen GP, Christensen JM (2008) Comparative in vitro activities of topical wound care products against community-associated methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 62(4):769-772

Bhatt JD (2013) Standardization of method for preparation of reduced fat paneer using whey protein concentrates and selected emulsifiers. M. Tech Thesis in Dairy Technology submitted to Anand Agricultural University, Anand (Gujarat)

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods-a review. Inter J Food Microbio 94(3):223-253

Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Vlietinck A J (2002) Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 79(2):213-220

Faheem F, Liu ZW, Rabail R, Haq IU, Gul M, Bryła M, Aadil RM (2022) Uncovering the industrial potentials of lemongrass essential oil as a food preservative: A review. Antioxidants 11(4):720

Guan W, Li S, Yan R, Tang S, Quan C (2007) Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. Food Chem 101(4):1558-1564

Hammer KA, Carson CF, Riley TV (1999) Antimicrobial activity of essential oils and other plant extracts. J Appl Microbio 86(6):985-990

Hili P, Evans CS, Veness RG (1997) Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. Lett Appl Microbio 24(4):269-275

Makwana M, Grover CR, Kumar N (2018) Biocides resistance profiles of biofilm forming bacteria of dairy niche and their control. Inter J Current Microbiol Appl Sci 7(02):1194-1205

Manju G, Grover CR (2023) Assessment of bioactive components of essential oils for antimicrobial activity in the dairy food matrix. Ind J Dairy Sci 76(3):252-258

Mishra SK, Malik RK, Kaur G, Manju G, Pandey N, Singroha G (2011) Potential bioprotective effect of reuterin produced by *L. reuteri* BPL-36 alone and in combination with nisin against food borne pathogens. Ind J Dairy Sci 64(5):406-411

Mitic-Culafic D, VukoviC-Gacic BS, Knezevic-Vukcevic JB, Stankovic S, Simic DM (2005) Comparative study on the antibacterial activity of volatiles from sage (Salvia officinalis L.). Archives Biol Sci 57(3): 173-178.

Mnif S, Jardak M, Yaich A, Aifa S (2020) Enzyme-based strategy to eradicate monospecies *Macrococcus caseolyticus* biofilm contamination in dairy industries. Int Dairy J 100:104560

Moltz AG, Martin SE (2005) Formation of biofilms by *Listeria monocytogenes* under various growth conditions. J Food Protect 68(1):92-97

Ortega-Ramirez LA, Gutiérrez-Pacheco MM, Vargas-Arispuro I, González-Aguilar GA, Martínez-Téllez MA, Ayala-Zavala JF (2020) Inhibition of glucosyltransferase activity and glucan production as an antibiofilm mechanism of lemongrass essential oil against *Escherichia coli* O157: H7. Antibiotics 9(3):102

Polatoglu K, Demirci F, Demirci B, Gören N, Baser KHC (2010) Antibacterial activity and the variation of *Tanacetum parthenium* (L.) *Schultz Bip.* essential oils from Turkey. J Oleo Sci 59(4):177-184

Panebianco F, Rubiola S, Di Ciccio PA (2022) The use of ozone as an eco-friendly strategy against microbial biofilm in dairy manufacturing plants: a review. Microorganisms 10(1): 162

Tortorano AM, Viviani M.A, Barchiesi F, Arzeni D, Rigoni AL, Cogliati M, Scalise G (1998) Comparison of three methods for testing azole susceptibilities of *Candida albicans* strains isolated sequentially from oral cavities of AIDS patients. J Clinic Microbio 36(6):1578-1583

Viuda-Martos M, El Gendy AENG, Sendra E, Fernandez-Lopez J, Abd El Razik KA, Omer E A, Perez-Alvarez, JA (2010) Chemical composition and antioxidant and anti-Listeria activities of essential oils obtained from some Egyptian plants. J Agri Food Chem, 58(16):9063-9070

Vishwakarma V (2020) Impact of environmental biofilms: Industrial components and its remediation. J Basic Microbio 60(3):198-206

Indian J Dairy Sci 77(3): 216-223
https://doi.org/10.33785/IJDS.2024.v77i03.004

RESEARCH ARTICLE

Characterization and identification of some probiotic properties of lactic acid bacteria isolated from curdled cow and goat milk

Gunjan Patil[1] (✉) and Farida Minocheherhomji[2]

Received: 31 May 2023 / Accepted: 24 September 2023 / Published online: 23 June 2024

Abstract: Since ancient times, curd is the most common fermented milk product consumed in India. Curd comprises brilliant gut healers that improves immune system. Raw milk of cow and goat is rich in various nutrients, minerals and vitamins. Additionally, it also contains mixture of probiotic lactic acid bacteria (LAB). The widespread use of synthetic antimicrobial agents, such as antibiotics, antifungals, and antivirals, can lead to the development of resistance in infectious microbes. This phenomenon is commonly referred to as antimicrobial resistance (AMR). For this reason, identification of natural antimicrobials like LAB appears to be essential for prevention and treatment. The purpose of this article is to isolate LAB from curd made from cow and goat milk and identify eminent probiotic LAB isolates which acquire safety aspects. The raw milk samples of cow and goat were allowed to curdle naturally and used for isolation of LAB. The gram positive and catalase negative LAB were isolated from curd samples. A total of 22 isolates were presumed as LAB form the curdled milk samples collected aseptically. Primarily these isolates were screened for cultural, microscopic and biochemical characteristics. Selected LAB strains were exposed to in vitro gastrointestinal conditions like low pH, bile salt, fluctuating NaCl and phenol conditions. Also, these isolated strains were evaluated for survival in stimulated gastric juice and stimulated intestinal juice. Further, safety property of these isolates was checked by their hemolytic, deoxyribonuclease (DNase) activity and susceptibility to antibiotics. On the basis of the evaluated results, LAB strains showing notable probiotic properties were further subjected to molecular identification. In conclusion *Lactiplantibacillus plajomi, Enterococcus italicus* and *Lactobacillus pentosus* are identified as LAB possessing impressing probiotic properties.

Keywords: LAB, probiotic, isolation, stimulated gastrointestinal conditions, safety property, molecular identification.

[1]Department of Microbiology, B. P. Baria Science Institute, Navsari, Gujarat, India 396445.
Email: gunjanpatil30@gmail.com
[2]Department of Microbiology, B. P. Baria Science Institute, Navsari, Gujarat, India 396445.
Email: minocheherhomji@yahoo.co.in

Gunjan Patil (✉)
[1]Department of Microbiology, B. P. Baria Science Institute, Navsari, Gujarat, India 396445.
Email: gunjanpatil30@gmail.com

Introduction

In India Cow and goat milk is consumed since olden times due to its quality of having rich nutrients, it is also considered to be a healing agent and is used widely for medicinal benefits. Cow and goat's milk almost rank similar in mineral content but both have different aids, combination of raw cow and goat milk may fulfill all the downsides lacking (Zhang, Lai et al. 2022) and (Mittu and Girdhar 2015). Lactic acid bacteria (LAB) are noble source of Probiotics i.e., live microorganism which on consumption in adequate quantity can confer apparent health benefits. They are well known for their medicinal properties. LAB was found significantly higher in raw milk as compared to pasteurized milk and higher macronutrients could be considered as one of the factors for the survival of LAB. LAB dominant in small and large intestine can inhibit the growth of pathogenic microbes by producing organic acids, bacteriocins and hydrogen peroxides. LAB stabilizes intestinal microflora after a long use of antibiotics LAB improves absorption of nutrients in gastrointestinal tract and decreases lactose intolerance. LAB in milk use enzymes to produce ATP from lactose, meanwhile by-product obtained during ATP production is Lactic acid that curdles milk and forms curd (Liptáková, Matejčeková et al. 2017) (König, Fröhlich et al. 2017) and (Zhang, Lai et al. 2022).

LABs are gram-positive bacteria (cocci, rods or coco bacilli) which does not form spores, non- pathogenic and typically are non-respiring but aerotolerant, fastidious, acid tolerant and catalase negative. LAB ferment carbohydrates and metabolically yields lactic acid as the key product during fermentation and other organic acids by products (Ibrahim, Naufalin et al. 2021).

Widespread use of synthetic antimicrobial agents causes resistance of infectious microbes to these compounds. For this reason, identification of natural antimicrobials like LAB appears to be essential for prevention and treatment. New methods have been developed for preventing various multidrug This article documents the isolation of probiotic Lactic acid bacteria from cow and goat milk and its physiological, molecular identification and characterization.

Materials and methods

Collection and enrichment of samples

Fresh goat and cow milk samples were collected from different local milk farms of Surat district (latitude 21.170240 and longitude 72.831062), in sterilized autoclavable plastic bottles.

Within 3 hours of collection Milk samples were brought to the laboratory and stored at -2 to -4°C before the experiments. Goat milk was mixed with the cow milk at a ratio of 100:0 (T1); 50:50 (T2) and 0:100 (T3) and heated up to 40-45°C for 10 minutes to promote growth of bacteria as reported by (Temerbayeva et al. 2018)

All above test samples were incubated at 37°C overnight for curdling of milk by Lactic acid bacteria present naturally in raw milk samples. No starter culture is added, Prior to isolation of Lactic acid bacteria all samples were subjected to Enrichment step. Where, 0.1ml of homogenized sample was suspended to sterile de Mann Rogosa and Shapre broth (MRS) media (Hi-Media, India) and incubated at 37° C for 48 hours.

Isolation of LAB

The enriched MRS broth with samples were further streaked on sterile MRS medium and incubated in microaerophilic conditions at 37° C for 24 hours. The colonies with calcified zone were selected and further sub cultured onto MRS agar plates. Selected LAB isolates were maintained at -20°C in glycerol stock for further analysis.

Physiological and Biochemical identification of LAB

The isolates were subjected to preliminary screening to confirm whether the isolated colonies belong to the group of lactic acid bacteria. Lactic acid bacteria are known to give creamy white distinct round colonies on MRS media. According to (Sadia et al. 2021 and Somashekaraiah et al. 2019) LAB are gram positive, non-motile, non-sporulating, catalase negative isolates that were further subjected to biochemical analysis; Such as Simmon citrate, Indole, Methyl red, Voges Proskauer, Oxidase test, Urea hydrolysis and Carbohydrate fermentation test as recommended in Bergey's Manual of Determinative Bacteriology.

Evaluation of Probiotic properties

Tolerance to Low pH

The tolerance of isolated LAB strain to low pH is very important criteria for screening of probiotic organism. Staying time of food in stomach is approximately 3 h at 3 pH. 24 h old culture of selected LAB was inoculated in Phosphate Buffer Saline (PBS) of pH 2, 3 and 4 respectively using 0.1N Hydrochloric acid.

0.1 ml of suspension from respective PBS inoculated was transferred to sterile MRS broth after the time interval of 0, 1, 2 and 3 h of inoculation. These MRS broths were incubated at 37° C for 24 hours. Growth of LAB was monitored at OD_{620} as suggested by (Shaikh et al. 2013 and Estifanos et al. 2014).

Tolerance to Bile salt

The mean concentration of intestine is supposed to be 0.3% and staying time of food is suggested to be 4h. Fresh cultures of selected LAB strain were inoculated in PBS containing 0.2%, 0.3%, 0.4% Bile salts. Inoculation of 0.1 ml from this inoculated PBS was carried out after interval of 0, 1, 2, 3 and 4 h into sterile MRS broth. Further inoculated broth tubes were incubated at 37° C for 24 hours. Growth was observed by measuring absorbance at OD_{620} (Shaikh et al. 2013; Estifanos 2014).

Tolerance to NaCl

The isolates were incubated overnight in MRS broth for 24 h and harvested by centrifugation (7000 rpm,4°C,10 min). Sterile MRS broth of different NaCl concentration range (0-6%) were inoculated with 0.1 ml of selected LAB strains and incubated at 37° C for 24 hours. After incubation growth was monitored at OD_{620}. (Shaikh et al. 2013; Prabhurajeshwar et al. 2019)

Tolerance to Phenol

Determination of ability of LAB to resist phenol was carried out by inoculation of 0.1 ml of overnight grown LAB isolates in MRS broth supplemented with 0.4% and 0.6% v/v phenol (Shaikh et al. 2013; Somashekaraiah et al. 2019). Growth was observed by measuring absorbance at OD_{620} after incubation at 37° C for 24 hours.

Response to Stimulated Gastric Juice (SGJ)

Tolerance of LAB strain to stimulated gastric conditions was estimated by using the stimulated gastric solution that consists of Pepsin 3g/L at pH 2.5 using 0.1 N HCL (Ortakci et al. 2012) in sterile saline water (0.85%NaCl). Sterile SGJ was inoculated with overnight grown LAB isolates suspensions adjusted to 0.5 McFarland Standard and incubated at 37° C for 3h. MRS broth were inoculated with 0.1ml suspension from inoculated SGJ at 0 and 3h in an orbital shaker at 200 rpm to stimulate Peristalsis and incubated at 37° C for 24 hours. After incubation growth was monitored at OD_{620}. (Somashekaraiah et al. 2019 and Prabhurajeshwar et al. 2019).

Response to Stimulated Intestinal Juice (SIJ)

SIJ was prepared by mixing pancreatin 1g/L and Bile salt 0.03g/L at pH 8 using 0.1 N NaOH (Musikasang et al. 2009; Asan et al. 2018) in sterile saline water (0.85%NaCl). A pancreatin solution was inoculated with overnight grown LAB isolates suspensions adjusted to 0.5 McFarland Standard and incubated at 37° C for 3h. After 0 and 3 h 0.1 ml SIJ solution maintained at 200 rpm on shaker was inoculated in MRS broth. Further incubation and growth were monitored same as SGJ (Somashekaraiah et al. 2019).

Evaluation of safe probiotic strains

Haemolytic Activity

For assessment of Haemolytic activity of LAB isolates 5% (w/v) Sheep blood agar plate (Somashekaraiah et al. 2019) was streaked with overnight grown cultures of selected LAB and incubated at 37°C for 2- 3 days. After incubation plates were then evaluated for the haemolytic reaction i.e. á haemolysis- green zone or â haemolysis- clear zone and ã haemolysis no clear zone around the colonies on blood agar plates. (Yadav et al. 2016)

DNase Activity

To check the production of DNase enzyme LAB isolates were streaked on DNase agar medium (HiMedia, India). Plates were then incubated at 37° C for 24-48 h. After incubation plates were observed for DNase activity. (Monique et al. 2020) reported that colonies surrounding with clear or a pinkish zone was considered as positive.

Antibiotic Susceptibility

LAB isolates were assessed for their resistance to antibiotics by Disc Diffusion method by using antibiotic discs of Streptomycin, Tetracycline, Erythromycin, Penicillin, Kanamycin, Chloramphenicol, Amikacin, Ampicillin and Clindamycin. This method was originally standardized as per ISO 10932/IDF 233 standards (Erginkaya et al. 2018). Actively grown culture was swabbed on the MRS agar plates. Antibiotic discs (HiMedia, India) were placed on the inoculated agar surface with three replicates and then incubated at 37° C for 24-48 h. Diameter of zone of inhibition around colonies were measured using Vernier caliper (Luana et al. 2014).

Molecular Identification of LAB

Identification of LAB isolates were carried out by 16s RNA gene sequencing using the primers 27F:52 - AGAGTTTGATYMTGGCTCAG and 1492R:52 - TACCTTGTTAYGACTT. This technique is fast and valid for molecular identification. The isolation of genomic DNA of LAB was done by using genomic purification kit (HiMedia, India). The quality of isolated DNA from overnight grown culture was evaluated on 1% Agarose gel, Gene fragments were amplified by PCR (polymerase chain reaction) and PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was performed with 16s BDT v3.1 cycle sequencing kit on ABI 3730xl Genetic Analyzer. The resultant sequences were examined for similarity in the database of NCBI GeneBank using nBLAST (www.ncbi.nlm.nih.gov/blast). The obtained sequences were submitted to the GeneBank for accession numbers. The Evolutionary analyses were conducted in Mega6. (Rine et al. 2019).

Results and Discussion

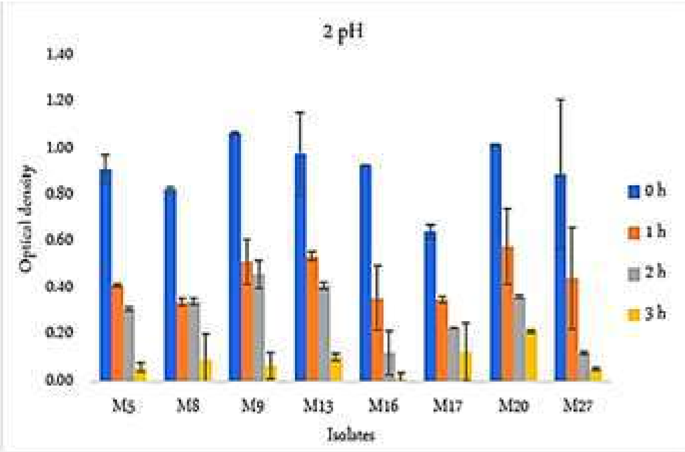
Isolation and Identification

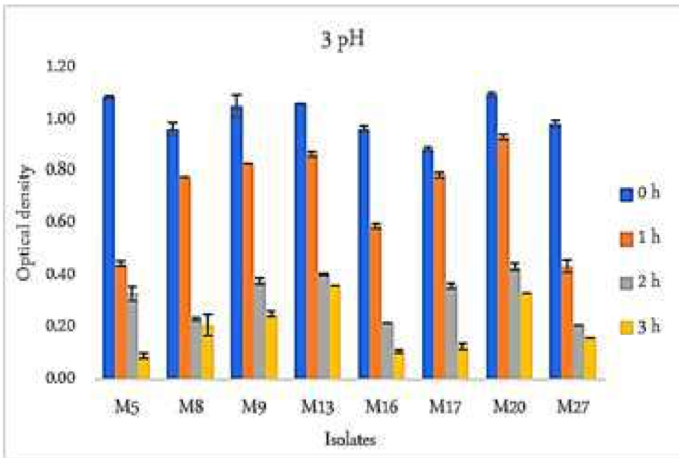
A total of 71 bacterial cultures were initially isolated from naturally curdled raw milk samples collected. These isolates were further proceeded to physiological and biochemical tests. Out of total isolates 22 of them were presumed as Latic acid bacteria on the basis of their morphological and colony characteristics. Only

Table 1: Morphological characteristics of LAB

Isolates	Gram character	Motility	Endospore staining	Capsule staining	Colony characteristics		
					Size	Shape	Color
M5	+, rods	-	-	-	Small	Round	Cream
M8	+, rods	-	-	-	Small	Round	White
M9	+, rods	-	-	-	Pin point	Round	Cream
M13	+, rods	-	-	-	Medium	Round	White
M16	+,cocco-bacilli	-	-	-	Large	Irregular	Dew drop
M17	+, rods	-	-	-	Small	Round	Cream
M20	-, rods	-	-	-	Large	Round	White
M27	+, rods	-	-	-	Small	Round	Cream

$^{-}$Positive and $^{+}$Negative





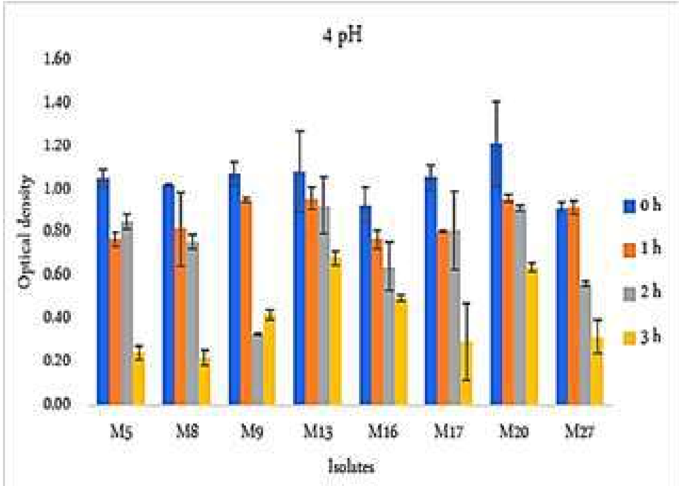


Fig 1. Growth of Isolates M5, M8, M9, M13, M16, M17, M20 and M27 at 2 pH, 3 pH and 4 pH at 0h, 1h, 2h and 3h were observed by measuring absorbance at OD_{620}. Values are given as mean = SD(n=3).

Gram positive, rod or cocci, catalase negative, non-spore forming and non- motile isolates were further identified by biochemical and carbohydrate fermentation tests. Information of only 8 isolates which fulfilled all the selection criteria and comprising of good probiotic properties are illustrated here in Table 1 and Table 2.

Evaluation of Probiotic properties

Tolerance to Low pH and Bile salts

Survival of LAB isolates to low pH and bile salt helps in studying the persistence of probiotic bacteria under gastric juice and their colonization in intestine. Ability of selected isolates to withstand low pH for 0 to 3h at 37°C has been represented in fig 1. Here, at 0h almost all isolates showed better growth but as time rises proliferation of M9, M13 and M20 showed improved viability. And survival of LAB cultures in bile salts for 4h at 37˚C mentioned in fig 2. Overall, among all the isolates tested, M9, M13, and M20 exhibited higher survival rates compared to the others. (Parisa S., et al. 2014)

Tolerance to NaCl

Almost all isolates showed significant growth but notable sustainability was observed in M13 and M20 for 24h. Thus, it can be observed that all isolates displayed resistance at various NaCl concentrations. The results have been shown in fig 3.

Tolerance to Phenol

Isolates showing resistance to above gastric conditions were further subjected to phenol resistance at concentration 0.4% and 0.6%. Results are displayed in fig 4. Isolates M9, M13 and M20 showed good tolerance and less sensitive to phenol concentration 0.4% and 0.6%. Whereas, M5, M8, M16, M17, M20 and M27 were more sensitive.

Tolerance to Stimulated Gastric and Intestinal juices

Survival in stimulated gastrointestinal conditions is very important criteria for selection of potential probiotic isolates. Lactic acid bacteria showing highest resistance to above gastric conditions were further subjected to evaluation for in vitro resistance to simulated gastrointestinal environment. As per results presented in fig 5. at 0h growth of all isolates is observed but after 3h of incubation only M9, M13, M20 and M27 showed higher tolerance to stimulated gastric juice and intestinal juice compared to M5, M8, M16 and M17.

Evaluation of safe probiotic strains

Haemolytic Activity

For the selection of safe probiotic strain safety properties of selected isolates is a principal measure. It is obligatory to evaluate invitro analysis of haemolytic activity on blood agar plate. The results exhibited that all selected LAB strains were ã haemolytic. Strain M5, M8 and M17 showed non hemolysis i.e. no clear zone around the colonies on blood agar plate.

DNase Activity

The DNase plates inoculated with LAB isolates showed no zone around the colonies which confirms and proves that none of the LAB strains has ability to produce DNase enzyme. Hence, they may be non-pathogenic probiotic isolates; however, for safe use in humans and animals, in vitro analysis should be conducted.

Antibiotic Susceptibility

Table 2: Biochemical and Carbohydrate fermentation test of LAB

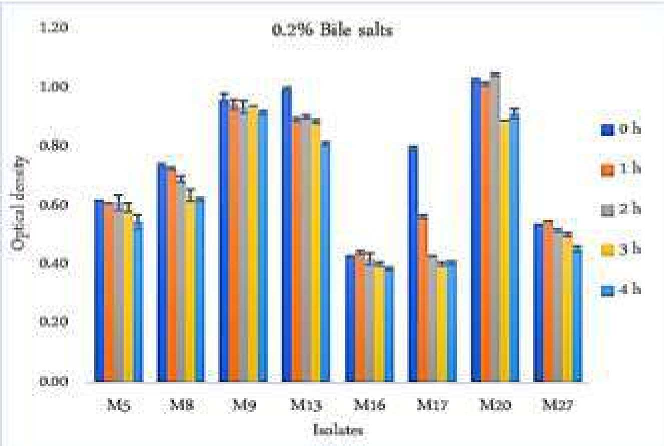
Parameters								
Biochemical tests	Isolates							
Catalase test	M5	M8	M9	M13	M16	M17	M20	M27
Oxidase test	-	-	-	-	-	-	-	-
Citrate utilization Test	-	-	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-	-	-
Voges prausker Test	-	-	-	-	-	-	-	-
Urea hydrolysis Test	-	-	-	-	-	-	-	-
Peptone utilization test	-	-	-	-	-	-	-	-
Gelatin hydrolysis Test	-	-	-	-	-	-	-	-
Indole test	-	-	-	-	-	-	-	-
Carbohydrate fermentation test								
Glucose	-	+	+	±	±	-	-	-
Fructose	+	+	+	+	+	+	±	±
Sucrose	+	±	+	+	±	±	±	-
Maltose	-	+	+	+	+	+	+	+
Mannitol	+	+	-	+	+	+	-	+
Lactose	+	-	-	+	-	-	+	±
Xylose	+	+	-	+	+	+	±	±

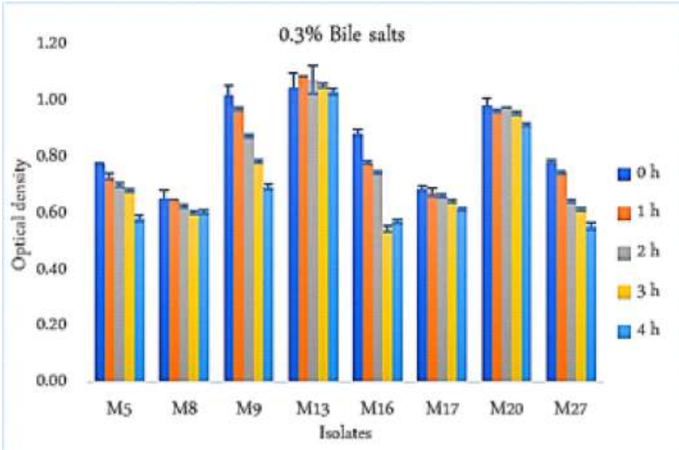
$^{-}$Positive, $^{+}$Negative and $^{\pm}$ Variable

Table 3: Antibiotic Susceptibility test

Antibiotic	Concentration (µg/disc)	M5	M8	M9	M13	M16	M17	M20	M27
Chloramphenicol	30	S	S	S	S	S	MS	S	S
Amikacin	30	R	S	S	MS	R	S	MS	R
Amphicillin	10	S	S	S	R	S	S	S	MS
Erytromycin	15	R	S	MS	R	S	S	S	MS
Streptomycin	25	S	S	S	S	S	MS	MS	S
Kannamycin	30	R	R	R	R	R	R	R	R
Penicillin	10	S	S	S	S	S	S	S	S
Clindamycin	20	S	S	S	S	S	R	S	S
Co Trimoxazole	25	S	S	MS	R	MS	S	S	S
Tetracycline	30	MS	S	S	S	S	S	S	S

S- susceptible, R- resistant and MS- moderately susceptible





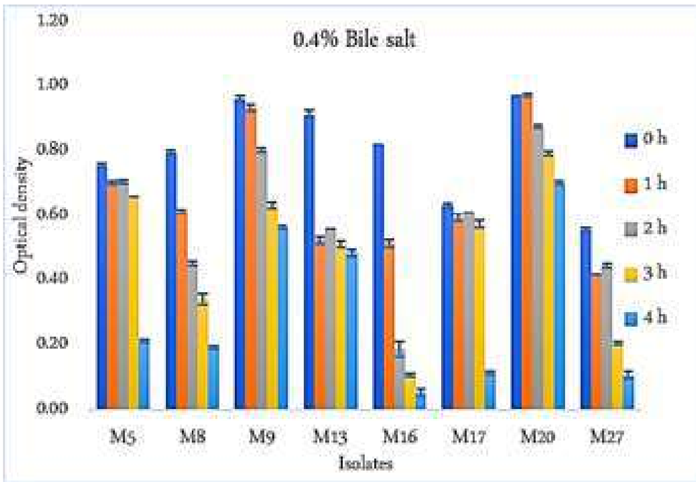


Fig 2. Growth of Isolates M5, M8, M9, M13, M16, M17, M20 and M27 at 0.2%, 0.3% and 0.4% bile salt concentration at 0h, 1h, 2h, 3h and 4h were observed by measuring absorbance at OD_{620}. Values are given as mean = SD(n=3).

One of the eminent probiotic property of a LAB strain is that it should be lacking antibiotic resistance. Any LAB must not carry any transportable antibiotic resistance gene that can be easily shifted to pathogenic organisms. Contrary to this, it is necessary for probiotic organisms to subsist in intestine during antibiotic treatments. Thus, inherent antibiotic resistant of LAB is considered natural and beneficial to host. As suggested by Morten et al. (2003) Two groups of antibiotics are recommended for selection of strain for instance antibiotics inhibiting cell wall synthesis (ampicillin and vancomycin) and antibiotics inhibiting protein synthesis (chloramphenicol, clindamycin, erythromycin, streptomycin, kanamycin and tetracycline). As per the results and diameter of zone measured were compared with the zone size interpretative chart (Hi-Media). Most of the isolates were sensitive to chloramphenicol, ampicillin, erythromycin, streptomycin, penicillin, clindamycin, co-trimoxazole and tetracycline and all isolates showed resistance to this Kanamycin as reported in (Table 3). The results attained are displayed in terms of susceptibility (S), moderate susceptibility (MS) and resistance (R). According to Elkins et al. (2004) most of the strains are resistant to Kanamycin and it has been reported earlier too, but it could be accreditable to the absence of cytochrome-mediated electro transport, which facilitates drug uptake. There is no trouble for low resistance towards kanamycin since the strains exhibited high susceptibility to clinically relevant antibiotics, so could be totally free of transferable antibiotic resistance gene.

Molecular Identification of LAB

Isolates showing renowned probiotic abilities were further identified by 16S r RNA gene sequencing and phylogenetic analysis as reported in (Table 4). On the basis of the results of Blast analysis it was found that 16S rRNA gene sequence of organism coded CG1 showed 98% similarity to *Lactiplantibacillus plajomi*, strain coded CG5 showed 100% similarity to *Enterococcus italicus*, strain coded CG13 showed 100% similarity to *Lactobacillus pentosus* sequence submitted to Gene Bank.

Table 4: Identification of the lactic acid bacterial isolates using 16S rRNA gene sequences

Isolates	Accession number	The nearest matched species from GenBank	Similarity %
CG9	OM 169332	*Lactiplantibacillus plajomi*	98
CG13	OM 670160	*Enterococuss italicus*	100
CG20	ON 495683	*Lactobacillus pentosus*	100

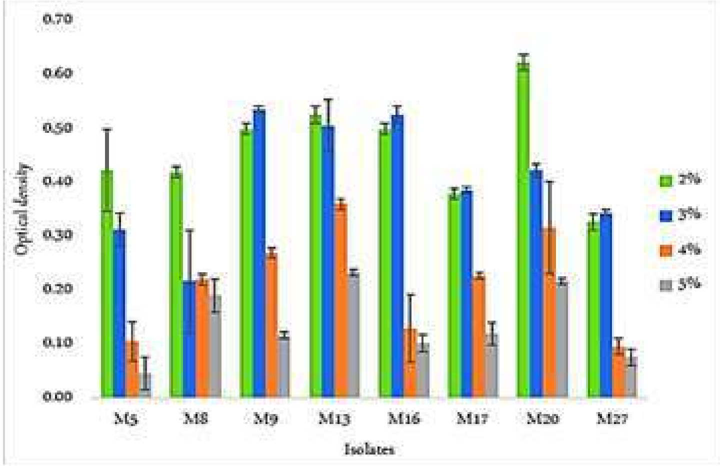


Fig 3. Growth of Isolates M5, M8, M9, M13, M16, M17, M20 and M27 at 2%, 3%, 4% and 5% salt concentration were observed by measuring absorbance at OD_{620}. Values are given as mean = SD(n=3).

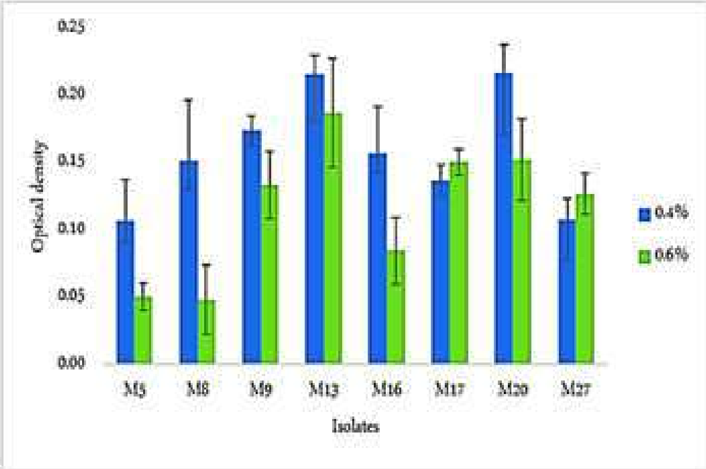


Fig 4. Growth of Isolates M5, M8, M9, M13, M16, M17, M20 and M27 at 0.4% and 0.6% phenol concentration were observed by measuring absorbance at OD_{620}. Values are given as mean = SD(n=3).

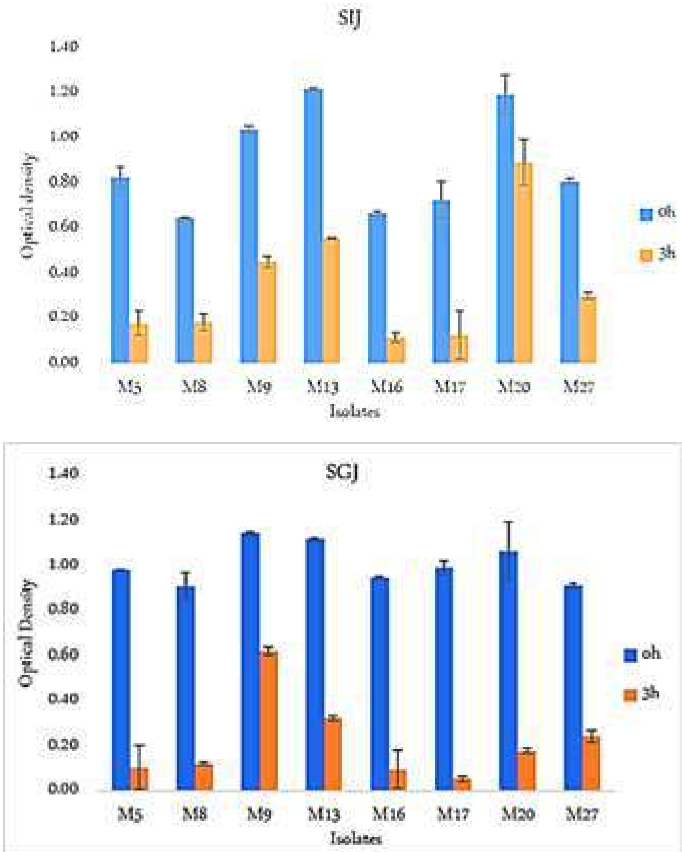


Fig 5. Growth of Isolates M5, M8, M9, M13, M16, M17, M20 and M27 in SGJ and SIJ at 0h and 3 h were observed by measuring absorbance at OD_{620}. Values are given as mean = SD(n=3).

Conclusion

This study focused on screening of probiotic lactic acid bacteria from naturally curdled milk of cow and goat. These probiotic strains were isolated and identified according to physiological and biochemical characteristics. All the LAB isolates could survive in GI tract but these three LAB isolated displayed striking tolerance to severe gastrointestinal conditions like resistance to low pH, bile salt, varying NaCl and phenol concentrations and survival in stimulated gastrointestinal conditions. Followed by susceptibility to several clinically effective antibiotics and evaluated as safe probiotic strains. Therefore, from these results we divulge that *in vitro* potential probiotic isolates *Lactiplantibacillus plajomi*, *Enterococcus italicus* and *Lactobacillus pentosus* are successfully isolated from naturally curdled cow and goat milk. However, further investigation can be performed to use these isolates reliably. Like many other safety parameters, *in vivo* assessments in animal models can be conducted. Further exploration of health benefits and potential applications can also be pursued.

References

Temerbayeva M, Rebezov M, Okuskhanova E, Zinina O, Gorelik O, Vagapova O, Beginer T, Gritsenko S, Serikova A, Yessimbekov Z (2018) Development of Yoghurt from Combination of Goat and Cow Milk. Annual Res Rev Biol 23:1-7

Asan M, Gunyakti A (2018) *Lactobacillus fermentum* strains from human breast milk with probiotic properties and cholesterol-lowering effects, Food Sci Biotechnol 28:501-509

Erginkaya ZE, Turhan EU, Tatl D (2018) Determination of antibiotic resistance of lactic acid bacteria isolated from traditional Turkish fermented dairy products. Iran J Vet Res 19:53-56

Elkins CA, Mullis LB (2004) Bile-mediated aminoglycoside sensitivity in Lactobacillus species likely results from increased membrane permeability attributable to cholic acid. Appl Environ Microbiol 70:7200-7209

Estifanos H (2014) Isolation and identification of probiotic lactic acid bacteria from curd and in vitro evaluation of its growth inhibition activities against pathogenic bacteria. African J Microbiol Res 8:1419-1425

König H, Unden G, Fröhlich J (Eds.) (2009) *Biology of Microorganisms on Grapes, in Must and in Wine* (pp. 3-29). Heidelberg: Springer.

Lamiaa AM, Abeer A (2015) Potent antagonistic activity of Egyptian *Lactobacillus plantarum* against multiresistant and virulent food-associated pathogens. Frontiers Microbiol 6:347

Liptáková D, Matejèeková Z, Valík L (2017) Lactic acid bacteria and fermentation of cereals and pseudocereals. Fermentation Processes 10: 65459

Luana P, Luís N (2014) Antagonistic lactic acid bacteria isolated from goat milk and identification of a novel nisin variant *Lactococcus lactis.* BMC Microbiol 14:1-9

Marina T, Maksim R, Eleonora O, Oksana Z, Olga G, Oksana V, Tatiana B, Svetlana G, Ainur S, Zhanibek Y (2018) Development of yoghurt from combination of goat and cow milk. Annual Res Rev Biol 23:1-7

Mittu, B., & Girdhar, Y. (2015). Role of lactic acid bacteria isolated from goat milk in cancer prevention. Autoimmun Infec Dis 1(2):2470-102

Musikasang H, Tani A, H-kittikun A, Maneerat S(2009) Probiotic potential of lactic acid bacteria isolated from chicken gastrointestinal digestive tract. World J Microbiol Biotechnol 25:1337-1345

Monique C, Luis N, Svetoslav T (2020) Safety profiles of beneficial lactic acid bacteria isolated from dairy systems, Braz J Microbiol 51:787-795

Morten D, Anette W (2003) Susceptibility of *Lactobacillus* spp. to antimicrobial agents. Int J Food Microbiol 82:1-11

Ortakci F, Broadbent R (2012) Survival of microencapsulated probiotic *Lactobacillus paracasei* LBC-1e during manufacture of Mozzarella cheese and simulated gastric digestion. J Dairy Sci 95:6274-6281

Parisa S, Chin Chin S, Ramasamy K, Juan L, Noorjahan A, Mohammad J, Yin H (2014) Probiotic potential of lactobacillus strains with antimicrobial activity against some human pathogenic strains

Prabhurajeshwar C, Chandrakanth K (2019) Evaluation of antimicrobial properties and their substances against pathogenic bacteria in-vitro by probiotic Lactobacilli strains isolated from commercial yoghurt. Clinical Nutr Experimental 23:97-115

Rine R, Pravas R, Shovon S, Rubayet A, Iqbal J (2019) Isolation, characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. BMC Microbiol 19:253

Sadia A, Hoque MA, Sarker AK, Satter MA, Bhuiyan MNI (2021) Characterization and profiling of bacteriocin-like substances produced by lactic acid bacteria from cheese samples. Access Microbiol. 3:234

Somashekaraiah R, Shruthi B, Deepthi BV, Sreenivasa MY (2019) Probiotic properties of LAB Isolated from Neera: A naturally fermenting coconut palm Nectar, Frontiers in Microbiology, 10:1382.

Shaikh M, Shah G (2013) Determination of probiotic properties of lactic acid bacteria from curd. Global J Biol Agric Health Sci 2:119-122

Yadav Anil P, Pratyoosh S (2016) Probiotic Properties of *Lactobacillus plantarum* RYPR1 from an indigenous fermented beverage raabadi. Front Microbiol 7:1683

Yuanyuan F, Lin Qiao, Rui L, Hongming Y, Changbin G (2017) Potential probiotic properties of lactic acid bacteria isolated from the intestinal mucosa of healthy piglets .Annals Microbiol 67: 239-253

Zhang W, Lai S, Zhou Z, Yang J, Liu H, Zhong Z, Peng G (2022) Screening and evaluation of lactic acid bacteria with probiotic potential from local Holstein raw milk. Frontiers in Microbiol 13: 918774

Indian J Dairy Sci 77(3): 224-230
https://doi.org/10.33785/IJDS.2024.v77i03.005

RESEARCH ARTICLE

Development of a technique to detect the presence of cow milk in goat milk

Vandhana P S and Divya M P(✉)

Received: 05 June 2023 / Accepted: 07 September 2023 / Published online: 23 June 2024

Abstract: Adulteration of Goat milk in terms of mixing with Cow milk has emerged to be a serious issue in the recent years. The higher price and nutritional value coupled with limited availability of the former serves as the driving factor for the mixing of relatively cheaper, easily available cow milk to goat milk. The current available analytical techniques for the detection of presence of cow milk in goat milk are highly sophisticated, complex and time consuming which cannot be adopted at the basic level by private goat milk producers. So, the development of a simple rapid laboratory technique for the same which can be applied at the farm and society level is a need of the hour. This study was conducted using pure goat milk, pure cow milk and cow milk mixed with goat milk at different proportions of 25%, 50 % and 75%. The physico chemical and compositional properties of samples were analysed and a significant variation between the samples was observed in terms of fat and chloride content. The ethanol stability was checked for the samples and found out that goat milk has lower ethanol stability compared to bovine milk and it improved by addition of cow milk. A blue colour which varies proportionately in intensity from light blue for pure goat milk to dark purple by increased addition of cow milk was observed by addition of Bromocresol purple and was verified using Hunterlab colorimeter. The addition of Seliwanoff' s reagent to the above said samples led to the development of coagulum with significant differences in appearance such as a completely dispersed coagulum in case of goat milk and a clearly settled coagulum on top portion for cow milk. The rate of settlement of coagulum to the top increases with increase in content of cow milk in goat milk. The above stated differences may be considered as the basis for detection of presence of cow milk in goat milk. Further the Scanning Electron Microscopic study of coagulum was carried out and fatty acid profile of samples was analysed using GC-MS, which finally confirmed our results. This led to the development of a rapid test for detection of inter- species adulteration of goat milk with cow milk.

Key words: Adulteration, Goat milk, Coagulum, Bromocresol purple, Seliwanoff's reagent

P S Vandhana
Verghese Kurien Institute of Dairy And Food Technology, Mannuthy, Thrissur Under Kerala Veterinary and Animal Sciences University, Kerala.
E Mail: Vandhu1693@Gmail.Com

M P Divya(✉)
Department of Dairy Chemistry, Verghese Kurien Institute of Dairy and Food Technology, Kerala Veterinary and Animal Sciences University, Kerala
E mail: divya@kvasu.ac.in

Introduction

Food quality is a term that indicates the overall properties and attributes of a food article that are most acceptable to a consumer. It serves to be an integral and inevitable part of any food industry. The present era of globalization with improved standard of living marks the fact that the consumers are highly health conscious and they always pick out foods that are assured of high quality.Adulteration is a very serious persistent problem that is seen in the food industry which is highly detrimental to both the consumers as well as producers. If a particular product contains ingredients other than that specified in the food label, it can be regarded as a mode of adulteration.According to Food Safety and Standards Authority of India (FSSAI), adulteration of any food is defined as the addition or subtraction of any substance to or from food, so that the natural composition and quality of food substance is affected. Milk, a rapidly perishable product, is the easiest source of adulteration due to its compositional characteristics. The admixture of milk of one species with another without any specification also falls under this category. This can be at times so lethal because of the allergenicity of human beings to milks of specific species.

The worthiness of goat milk leads to its greater demand in the present market having a direct positive correlation with its higher price value. Despite all these facts the thought-provoking factor is the limited availability of goat milk over bovine milk. This coupled with the higher price of former paves the way for unscrupulous adulteration (Kang et al 2022). This type of adulteration may lead to problems relating to health, religious, ethical or cultural objections and legal requirements. It is therefore desirable to confirm that goat milk offered for sale is free from admixtures of cow milk.

The private goat farmers constantly raise their suspicion of the practice of adding bovine milk in bulk goat milk by some producers. There is an alarming need for the development of a rapid laboratory test that would be able to detect the adulteration, as the currently available techniques are highly sophisticated, expensive and time consuming (Jing Yan Li et al 2023) The objective of this study to develop a rapid method for the detection of inter- species adulteration of goat milk with cow milk, which is of paramount importance and greatly beneficial for the layman.

Materials and methods

The initial step of procurement of raw materials was done by collecting pooled fresh goat milk and cow milk samples from University Goat & Livestock Farm and University Dairy Plant, Kerala Veterinary and Animal Sciences University, Kerala. As a preliminary trial the samples taken for analyses were pure goat milk, pure cow milk and cow milk mixed with goat milk at proportions of 5 %, 10 % and 25 %. Due toless sensitivity of the aforesaid proportions, further experiments were carried out with higher concentrations of 25%, 50 % and 75 % (a total of 5 samples).

Analysis of compositional and physico- chemical parameters

Various compositional and physico- chemical parameters of the samples were analysed with the primary aim of finding out a factor that exhibits a statistically significant difference between the samples. These parameters were analysed using the standard procedures prescribed by AOAC and BIS specifications.

Compositional parameters such as Fat (Gerber method), Protein (Kjeldahl method), Lactose (Lane- Eynon method), Chloride content (Mohr method) and Ash content (Gravimetric method) were analysed using standard method specified by BIS (IS: SP:18 [Part XI], 1981). Similarly physico- chemical aspects such as acidity and pH (IS: SP:18,1981), Electrical conductivity (Conductivitymeter, Systronics 306), Freezing point (IS1479 [Part 4: 2009), Refractive Index (AOAC 17thedn, 2000), Surface tension (AOAC 17thedn, 2000) and Specific gravity (IS 10083: 1982) were also analysed as part of the study.

Comparison of Ethanol stability

The differences in stability of the samples towards 70 % ethanol were analysed. A total of five samples (pure goat milk, pure cow milk, 25%, 50 % and 75 % mix of cow milk with goat milk) maintained at a temperature of 35- 40 °C were taken for the analysis. Five millilitreeach of thoroughly mixed milk samples and 70 %ethanol were taken in a test tube and shaken well. These were observed for any visible coagulation. The test samples were kept in a boiling water bath for about 3 minutes. The differences in coagulation as well as the separation of layers together with the nature of coagulum particles formed at the sides of the test tube were observed.

Reaction with Bromocresol purple reagent

Five millilitres of thoroughly mixed milk samples(pure goat milk, pure cow milk, 25 %t, 50 %and 75 %mix of cow milk with goat milk) maintained at a temperature of 35- 40 °C were taken in test tubes. Five millilitresof distilled water followed by 1 millilitre Bromocresol purple reagent was added and mixed well. The difference in the intensity of blue colour developed was noted and measured quantitatively by Hunterlab colorimeter.

Hunter Colorimeter

Colour of the samples was measured by reflectance spectroscopy technique using reflectance meter (color flex, Hunter lab Miniscan XE plus Spectrocolrimeter, Virginia, USA) with geometry of diffuse/8p (sphere- 8mm view) and an illuminant of D65/10p . Before the test, the instrument was calibrated with standard black glass and white tile as specified by the manufacturer. The light source was dual beam xenon flash lamp. Data was received from the software in terms of L^* [Lightness, ranges 0 (black) to 100 (white)], a^* [Redness- positive, Grey- zero, Greenness- negative] and b^*[Yellowness- positive, Grey- zero, Blueness- negative] values of the International Colour System.

Reaction with Bromocresol purple and Seliwanoff's reagent

Seliwanoff's reagent was added to the samples mixed with Bromocresol purple solution. To the obtained blue colour solution 1millilitre of freshly prepared Seliwanoff's reagent (0.5 % resorcinol in 3N HCl) was added and properly mixed. These test tubes were kept in a boiling water bath for about 5 minutes. The appearance of the coagulum formed was observed for the distinctive features. The coagulum formed was later subjected to centrifugation in a laboratory centrifuge at 4000 rpm for 5 minutes to find out the variations in the amount of sediment obtained for each sample.

Other parameters like microscopic view of milk coagulum by Scanning Electron Microscopy (SEM) and fatty acid profile of milk samples using Gas Chromatography- Mass Spectrometry (GC-MS) were also studied.

Statistical Analysis

The results obtained from the analysis were verified using One way Analysis of Variance (ANOVA) with Duncan test at 5 percent level of significance and correlation coefficients statistically using SPSS (Statistical Packages for Software Solutions) software, Version 21.0 designed by IBM Company, USA and data were expressed as Mean ± Standard Error.

Results and Discussion

Comparison of compositional and physico- chemical parameters

The results for compositional and physico- chemical parameters of the samples are shown in Table 1 and Table 2 respectively.One way ANOVA analysis of the parameters by Duncan tests at 5 percent level of significance indicated that there was a statistically non-significant difference between the samples in these parameters with an exception of fat, chloride content and refractive index that differs significantly. Fat is one of the most variable components and has shown a statistically significant difference between each sample which may account for the change in breed of the animals. A decrease in fat content was observed when the quantity of cow milk mixed with goat milk increased. The detection limit when considering the case of variation in fat content can be taken as 25%. Pure goat milk exhibited significant difference in chloride content with 50 %mix, 75 %t mix and pure cow milk but non-significant difference with 25 %mix of cow milk. This implies that if cow milk is being mixed with pure goat milk, the minimum detection limit in terms of change in chloride content is found out to be 50%. The electrical conductivity of samples varied almost linearly with the level of chloride content with the highest for goat milk. Both the parameters decreased proportionately with an increase in the proportion of cow milk being mixed with goat milk. The next variable factor is refractive index in the case of which pure goat milk varied significantly with 50%, 75 % mix and pure cow milk.

Relation between chloride content and electrical conductivity

A positive correlation was observed between the chloride content (percent) and electrical conductivity (mS) of samples. There was a proportionate increase in conductivity with increase in chloride content with goat milk possessing the highest value.

The above stated parameters cannot be relied upon since these may vary according to breed, season, stage of lactation, udder infection, temperature etc.

Difference in Ethanol stability

An immediate coagulation was observed in case of pure goat milk with no coagulation at all for pure cow milk. As the quantity of cow milk mixed with goat milk increases the rate of coagulation was found to decrease. The minimum level of detection limit was found to be 50 percent. Decreasing order of rate of coagulation is: Pure goat milk> 25 %mix> 50 %mix> 75 %t mix> Pure cow milk (Figure 1).The rate of coagulation was higher for goat milk with 70 % ethanol when compared to cow milk which shows that the former had lower ethanol stability than the latter. When the quantity of cow milk admixture with goat milk increases the rate of coagulation decreased proportionally i.e., an increase in ethanol stability was observed. The higher rate of coagulation of goat milk was attributed to the lower content or absence of αs1 casein in it (Feligini et al. 2009). The αs1 casein fraction in milk has the ability to trap calcium ions and withdraws them from the proteolysis of k casein, thereby retards the rate of curd formation. Hence greater amount of free calcium remains unbound by virtue of the lesser quantity of casein present in goat milk.

Table 2: Change in physico- chemical parameters for pure goat milk, pure cow milk and admixture samples

Parameter	Goat milk	25% mix	50% mix	75% mix	Cow milk
Fat (%)	5.111 ± 0.355^{a}	4.811 ± 0.289^{c}	4.411 ± 0.169^{d}	4.011 ± 0.136^{e}	3.667 ± 0.194^{b}
Lactose (%)	5.022 ± 0.151^{ns}	4.975 ± 0.171^{ns}	4.922 ± 0.201^{ns}	4.809 ± 0.125^{ns}	4.813 ± 0.267^{ns}
Chloride (%)	0.183 ± 0.013^{a}	0.170 ± 0.013^{bc}	0.161 ± 0.014^{cd}	0.149 ± 0.010^{d}	0.141 ± 0.015^{ab}
Protein (%)	3.496	3.494	3.485	3.470	3.482
Ash (%)	0.840	0.796	0.772	0.763	0.778

Figures are Mean average values and Mean± Standard error, $^{a-e}$ figures in row bearing different superscripts differ significantly, ^{ns}- non significant

Table 2: Change in physico- chemical parameters for pure goat milk, pure cow milk and admixture samples

Parameter	Goat milk	25% mix	50% mix	75% mix	Cow milk
Acidity(%LA)	0.186 ± 0.022^{ns}	0.171 ± 0.016^{ns}	0.171 ± 0.016^{ns}	0.171 ± 0.016^{ns}	0.158 ± 0.008^{ns}
pH	6.528 ± 0.031^{ns}	6.547 ± 0.065^{ns}	6.547 ± 0.065^{ns}	6.547 ± 0.065^{ns}	6.608 ± 0.094^{ns}
Electrical conductivity (mS)	6.628 ± 0.301^{ns}	6.524 ± 0.309^{ns}	6.394 ± 0.307^{ns}	6.307 ± 0.332^{ns}	6.172 ± 0.303^{ns}
Freezing point (℃)	-0.557 ± 0.017^{ns}	-0.550 ± 0.010^{ns}	-0.540 ± 0.002^{ns}	-0.537 ± 0.003^{ns}	-0.530 ± 0.007^{ns}
Refractive index	1.353 ± 0.002^{a}	1.351 ± 0.001^{b}	1.351 ± 0.001^{b}	1.350 ± 0.001^{b}	1.350 ± 0.001^{ab}
Surface tension (N/m)	0.059 ± 0.007^{ns}	0.060 ± 0.010^{ns}	0.060 ± 0.010^{ns}	0.060 ± 0.004^{ns}	0.063 ± 0.004^{ns}
Specific gravity	1.034 ± 0.002^{ns}	1.033 ± 0.002^{ns}	1.032 ± 0.001^{ns}	1.031 ± 0.001^{ns}	1.031 ± 0.002^{ns}

Figures are Mean± Standard error, $^{a-e}$ figures in row bearing different superscripts differ significantly, ^{ns}- non significant

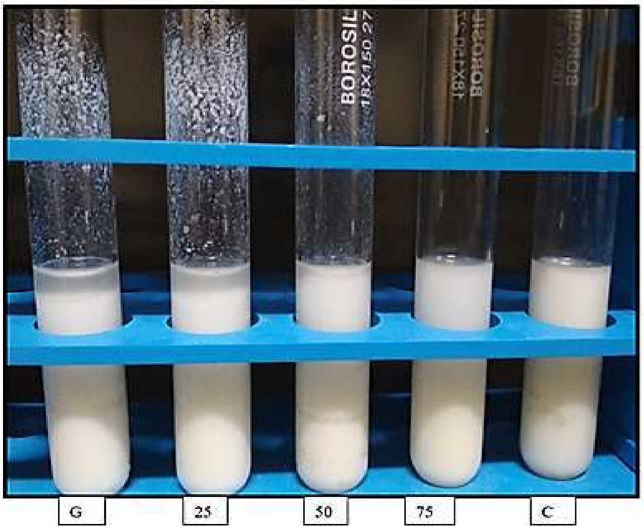


Fig. 1 Decreasing order of coagulation after addition of 70% ethanol

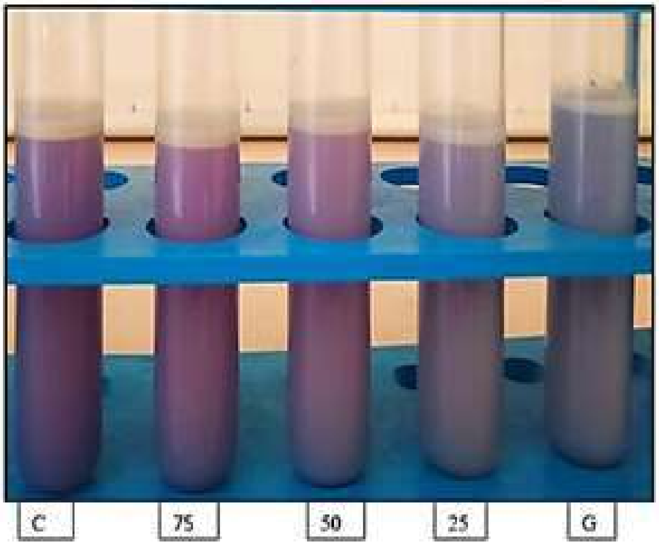


Fig. 2 Decreasing order of colour intensity after addition of Bromocresol purple reagent

The amount of casein, surface hydrophobicity, collision distance of proteins, size and integrity of micelles are some of the important factors affecting the coagulation properties of milk samples. The greater size of casein micelles causes shorter collision distance between the proteins that forms the basis for the reduced coagulation time of goat milk compared to cow milk (Bonomi et al. 1988).Moreover, surface hydrophobicity of goat milk is greater since it consists of β casein, the most hydrophobic of all the caseins as the major casein component (Mellema et al.1999).

Stocco (2018) found out that the fat content in milk has an effect on the coagulation characteristics. Better coagulation properties of goat milk may be attributed to its higher fat content as milk flocculation occurs due to the collision and aggregation of fat droplets. The fat globules that get entrapped in the casein network and thereby forming a surface coating, behaves to an extent like casein micelles and accelerates the kinetics of coagulation (Sweetsurand Muir1983).Studies conducted by Horne and Parker (1982) stated that the low ethanol stability of goat milk may be caused because of its lower micellar charge. The negative charge of protein gets neutralized by the more available free calcium (Ca^{2+}) in case of goat milk.

Difference in reaction with Bromocresol purple reagent

Goat milk and cow milk exhibited remarkable differences in their reaction with Bromocresol purple (BCP) reagent. Both the milks developed a purplish-blue colour when mixed with Bromocresol purple reagent, but the intensity of colour varied. A lighter colour was observed in case of pure goat milk and a relatively darker one for pure cow milk. The intensity of blue colour increases when the quantity of cow milk mixed with goat milk increases. The decreasing order of the samples according to the intensity of colour is: Pure cow milk > 75 %mix > 50 %mix > 25 % mix > Pure goat milk (Figure 2).

Bromocresol purple (BCP) or otherwise called 5', 5"- dibromo-o-cresolsulfophthalein is a dye of the triphenylmethane family. It is a commonly used pH indicator which is coloured yellow below pH 5.2 and purple above pH 6.8. Its structure changes with the pH with different colour for each structure. The chemical has a sulfonate structure in near neutral or alkaline pH that gives the solution a purple colour. It converts to a sultone (cyclic sulfonic ester) that colours the solution yellow as the pH decreases.The increase in pH to the alkaline range may be caused due to the addition of distilled water to the samples. This may enhance the identification of colour due to greater scattering of light. The indicator transfers a proton from it to water and thereby shifts the equilibrium to the side of the conjugate base and hence the development of purple colour (Pradeep and Dave 2013).

The lighter intensity of colour in case of pure goat milk may be due to the increased fat content as it may hinder the absorption of light by Bromo cresol purple. The intensity of colour increased with the quantity of cow milk being admixture with goat milk. Since cow milk has a lower fat content relative to goat milk, the former developed a comparatively intense purple colour. The increased colour intensity with increase in proportion of cow milk may be because of the decrease in fat content.Moreover, goat milk has a lower pH value compared to cow milk which may also contribute to the relatively less intense colour.

The colorimetric readings (L^*, a*, b*) confirmed the obtained results. From the results it is clear that since the intensity of blue colour is lower for pure goat milk, it has the highest L^* and lowest b^* value which implies that it is lighter in colour with reduced degree of blueness. Cow milk was more intense in colour with

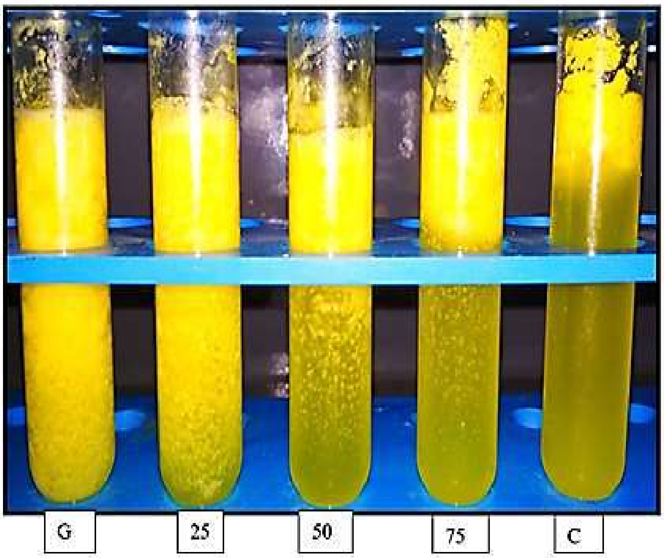


Fig. 3 Decreasing order of rate of coagulation after addition of Bromocresol purple and Seliwanoff's reagent

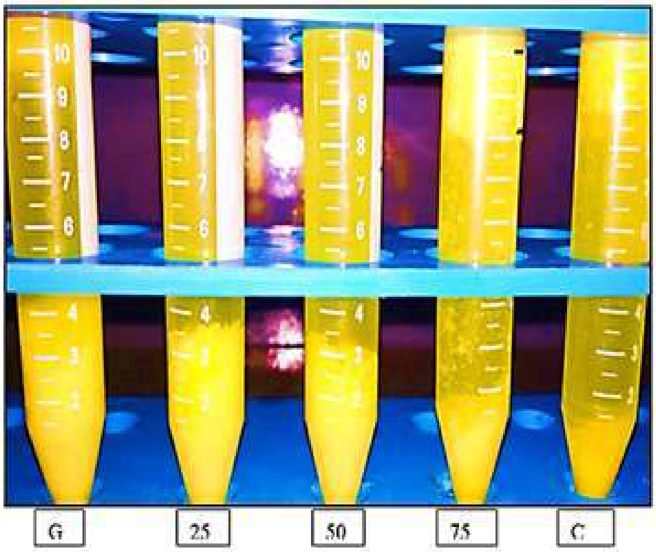


Fig. 4 Sediment formed after centrifugation of coagulum

maximum b^* value for it. A significant decrease and increase were observed in the L^* and b^* values respectively with the addition of cow milk to goat milk.

Difference in reaction with Bromocresol purple and Seliwanoff's reagent

The purplish-blue colour developed upon the addition of Bromocresol purple reagent changed into yellow colour when Seliwanoff's reagent was added to it. All the samples were almost the same in their appearance by visual observation before heating. Coagulation takes place in the samples once they are kept in boiling water for about 5 minutes. The nature and structure of the coagulum developed varied according to the samples. While a completely dispersed coagulum was observed in goat milk, pasty compact cement like coagulum which settles to the top developed in case of cow milk. The rate of settlement of coagulum to the top portion increases when the quantity of cow milk mixed with goat milk increases (Figure 3).

Seliwanoff's reagent is used in sugar test to differentiate between aldose and ketose sugars which consists of concentrated HCl and resorcinol as the major components. The addition of Seliwanoff's reagent causes the decrease in pH and at lower pH (below 5.2) value bromocresol purple developed yellow colour and higher acidity leads to the development of coagulum. The presence of resorcinol gives the solution a bright yellow colour. The smaller size of casein micelles in case of cow milk caused the entire coagulum to settle to the top. The relatively lower content of αs1 casein together with greater casein micelle size made the goat milk coagulum to get completely dispersed.

The amount of sediment produced when the coagulum was subjected to centrifugation at 4000 rpm for 5 minutes also varied for the samples (Figure 4). The coagulum does not completely settle down in case of pure cow milk with some part of it remaining at the top portion while a completely settled coagulum was observed in case of pure goat milk after centrifugation. The amount of settled coagulum decreased with the increase in quantity of cow milk .The smaller size of casein micelles in case of cow milk caused the entire coagulum to settle to the top. The relatively lower content of αs1 casein together with greater casein micelle size made the goat milk coagulum to get completely dispersed.

Difference in structure of milk coagulum

The coagulum developed in each sample after the addition of 70 %ethanol was viewed microscopically by means of Scanning Electron Microscopy (SEM). The difference in the structure and nature of coagulum observed under SEM is given in Plate 1, Plate 2 and Plate 3.

The SEM view of the coagulum of the samples indicated a near clear cut difference in their nature and structure. This may be attributed to their compositional variation such as mineral balance, protein contents etc. A dispersed less firm and non-pasty structure was developed in case of goat milk (Plate 1) while a pasty firm and compact cement like one in cow milk (Plate 2). The 50 %admixture of both goat milk and cow milk had a structure that lies in between both (Plate 3). The weaker scattered coagulum developed in goat milk may be due to the presence of large irregular void spaces in the protein matrix that leads to greater destruction and deformation of the coagulum structure.According to Park et al. (2007) goat milk forms a weaker

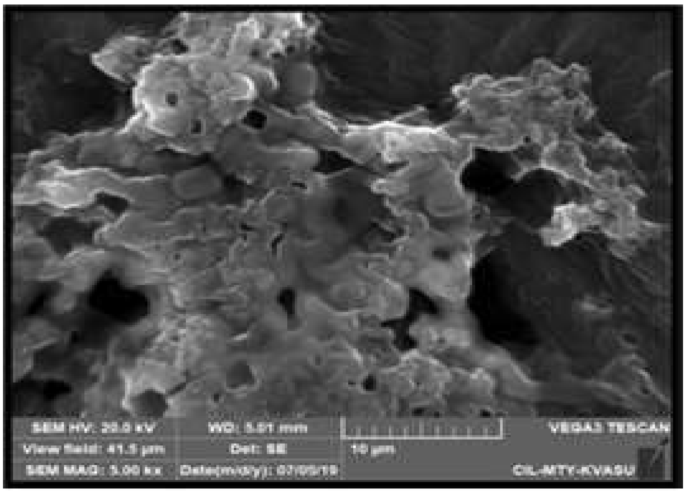


Plate 1: Coagulum of pure goat milk

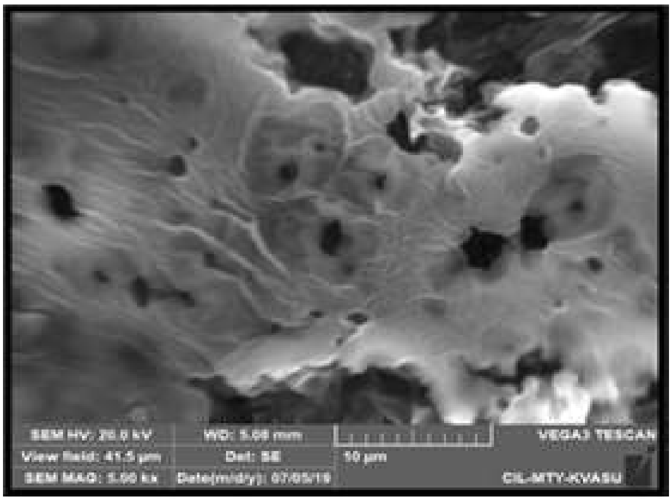


Plate 2: Coagulum of pure cow milk

texture of coagulum due to the lower concentration and ratio of casein fractions and relatively larger size of casein micelles compared to bovine milk. The former also consists of higher free calcium in the serum which may also serve to be a reason for its structure difference with cow milk.

Difference in Fatty acid profile

The variations in the contents of individual fatty acids in terms of fatty acid profile of pure goat milk, pure cow milk and a 50 %mix of both was found out using Gas Chromatography- Mass Spectrometry (GC- MS). The contents of short and medium chain fatty acids such as caproic, caprylic, capric and lauric acid is greater in goat milk whereas cow milk had higher contents of myristic, palmittic, palmitoleic and stearic acids(Vieitez et al. 2016).Butyric acid is present in more amounts in cow milk..The results were in accordance with the standard contents of fatty acids stated by Runowska et al (2013). A proportionate decrease in the short and medium chain fatty acids (C6: 0 caproic acid to C12: 0) present in goat milk was observed when cow milk being mixed with it at 50 percent level. Similarly, the level of long chain fatty acids increased in goat milk upon admixture. This could be used as a tool for indicating whether cow milk has been mixed with goat milk.

Conclusion

A blue colour which varies proportionately in intensity from light blue for pure goat milk to dark purple by increased addition of cow milk was developed by the addition of Bromocresol purple and the same was verified using colorimeter.The addition of Seliwanoffs reagent to the abovesaid samples led to the development of coagulum with significant differences in appearance such as a completely dispersed coaguilum in case of goat milk and a clearly settled coagulum on top portion for cow milk. The rate of settlement of coagulum to the top increases with increase in content of cow milk in goat milk. The above stated differences may be considered as the basis for detection of presence of cow milk in goat milk. Further the Scanning Electron Microscopic studies of coagulum was carried out and fatty acid profile of samples was analyzed using GC- MS. These data finally confirmed our results.

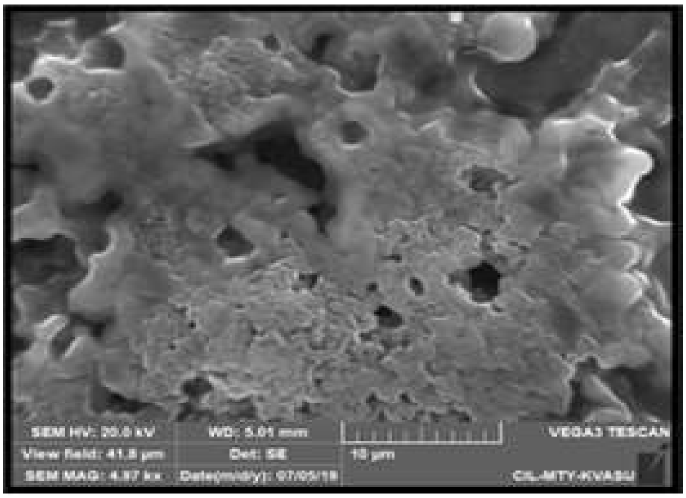


Plate 3: Coagulum of 50 percent mix

Acknowledgements

The study acknowledged Department of Dairy Chemistry, Verghese Kurien Institute of Dairy and Food Technology under Kerala Veterinary and Animal Sciences University, Pookode, Kerala. All the support rendered by the same acknowledged here.

Reference

AOAC(2000)Official Methods of Analysis, (Volume 2) 17th Edition. Arlington, Virginia, USA

BonomiF,IamettiS, PagliariniE, Peri E (1988) A spectro-fluorimetric approach to the estimation of the surface hydrophobicity modifications in milk proteins upon thermal treatment. Milchwissenschaft 43: 281–285

Feligini M, BonizziI, Buffoni JN, Cosenza G, Ramunno L(2009) Identification and quantification of αs1, αs2, β and κ-caseins in water buffalo milk by reverse phase-high performance liquid chromatography and mass spectrometry. J Agric Food Chem 57: 2988–2992

Horne DS, Parker TG (1982) Some aspects of the ethanol stability of caprine milk. J Dairy Res 49: 459- 468

IS 1479 (Part 4): 2009. Methods of Test for Dairy Industry Determination of Freezing point of milk- Thermistor Cryoscope method (Reference Method). Bureau of Indian Standards, Manak Bhavan, New Delhi

IS:10083-1982.Method of Test for Determination of SNF(Solids- Not-Fat) in milk by the use of the lactometer. Bureau of Indian Standards, Manak Bhavan, New Delhi

IS:SP:18 (Part XI). 1981. Hand book of food analysis: Dairy Products. Bureau of Indian Standards, Manak Bhavan, New Delhi

Kang M, Wang H, Shi X, Chen H, Suo R (2022) Goat milk authentication based on amino acid ratio and chemometric analysis. J Food Compos Anal 111(1): 104636

Li JY, Ge Q, Liu B, Gao ZP, Guo CF (2023) Quantification of cow milk in adulterated goat milk by HPLC-MS/MS using N- acetylglucosamineas a reliable biomarkerof cow milk. J Food Compos Anal 123:105583

Mellema M, Leermakers FAM, de Kruif CG (1999) Molecular mechanism of the renneting process of casein micelles in skim milk, examined by viscosity and light-scattering experiments and simulated by model. SCF calculations. Langmuir 15: 6304–6313

Park YW, Juarez M, Ramos M, Haenlein GFW(2007) Physico-chemical characteristics of goat and sheep milk. Small Ruminant Res 68: 88-113

PradeepD J,Dave K(2013) A novel, inexpensive and less hazardous acid-base indicator. J Lab Chem Educ1 (2): 34- 38

Runowska G. C,Keszycka MM, Lipinska P, Wojtowsk J(2013) Fatty acid profile of milk- A review. Bull Vet Inst Pulawy 57: 135- 139

Stocco G, Pazzola M, Dettori ML, Paschino P, Bittante G, Vacca GM (2018) Effect of composition on coagulation, curd firming, and syneresis of goat milk. J Dairy Sci 101: 1-10

Sweetsur AWM, Muir DD (1983) Effect of homogenization on the heat stability of milk. J Dairy Res 50: 291- 300

Vieitez I, Irigaray B, Callejas N, Gonzalez V, Gimenez S, Arechavaleta A, Grompone M, Gambaro A (2016) Composition of fatty acids and triglycerides in goat cheeses and study of the triglyceride composition of goat milk and cow milk blends. J Food Compos Anal 48: 95-101

Indian J Dairy Sci 77(3): 231-235
https://doi.org/10.33785/IJDS.2024.v77i03.006

RESEARCH ARTICLE

Standardization and evaluation of physico chemical properties of jackfruit based bio-yoghurts

[1] Remya PR (✉), [2] Sharon CL and [3] Rammya Molu K

Received: 12 June 2023 / Accepted: 04 October 2023 / Published online: 23 June 2024

Abstract: Jackfruit, the state fruit of Kerala, is often regarded as a wonder fruit due to its nutrient profile, nutraceutical and health benefits and the immense scope for value addition. However, it is mostly underutilized due to lack of knowledge and technical facilities. It can be better utilised, if consumer acceptable, simple as well as nutritious products are prepared out of the fruit. The growing demand for "healthy food" is stimulating the innovation and development of new products nationally and internationally. Yoghurt, a milk based product, is a simple, healthy and nutritious food which offers high amounts of protein, carbohydrates and fat. The added value of yoghurt over milk lies on the presence of beneficial bacteria as well as certain bioactive components. The study was aimed at developing jackfruit based bio-yoghurts and to evaluate its physico- chemical properties.*Lactobacillus acidophilus* was used as the probiotic organism in this study. Hence in this study, yoghurts were prepared by incorporating varying proportions of blanched jackfruit pulp (10%, 20% and 30%) along with equal volumes of homogenized milk. Both *koozha* and *varikka* variety of jackfruits were used for the preparation of bio-yoghurts. The jackfruit bulbs were steam blanched for five minutes and pulped to prepare the bio-yoghurt. Plain yoghurt served as the control. The prepared bio-yoghurts were organoleptically evaluated using a 9 point hedonic scale with a panel of 15 judges. The selection of best sample was done on the basis of Kendall's coefficient of concordance. Among the fruit based bio-yoghurts, the yoghurt with 30% jackfruit pulp scored maximum for the sensory attributes. Physico-chemical parameters like synerisis, water holding capacity, curd tension, viscosity, pH, moisture, fat and protein of the bio-yoghurts were determined.

[1]Family Health Centre, Kondazhy, Thrissur, Kerala

[2] Department of Community Science, College of Agriculture, Kerala Agricultural University, Vellanikkara, Kau P.O. 680656

[3] Department of Community Science, College of Agriculture, Kerala Agricultural University, Vellanikkara, Kau P.O. 680656

Remya PR (✉)
Email: remyapr.remya@gmail.com; Mobile: 9526289881

Introduction

Being the largest tree borne fruit in the world, the jackfruit is a treasure of nutrients and several bioactive components. There are two main varieties of jackfruits: the fibrous, soft, and mushy, *koozha*and the crisp and crunchy, *varikka*. The fruit is a rich source of vitamins, minerals and many other nutrients. Despite these benefits, most of them remain wasted during the season due to improper utilization and lack of technical skills. The raw jackfruit underwent several value addition trials and product diversification processes, whereas, the ripe jackfruit has limited scope and we have products like jackfruit halwa, candies and *chakkavaratty (*a traditional food item*)* out of it.To boost the marketability of ripe jackfruit, simple as well as novel products have to be developed and popularized.

Yoghurt is one of the popular fermented milk products around the world. It is healthy and offers concentrated amount of protein, carbohydrate, fat and so it is considered superior to milk in a nutritional point of view. Yoghurt is produced by the fermentation of milk and the bacterial strains used are *Lactobacillus bulgaricus* and *Streptococcus thermophillus*. The added value of yoghurt over milk lies on the presence of these bacteria and the bioactive components produced during fermentation. Apart from all these, the probiotic strain *Lactobacillus acidophilus* was also added during the process to make the product probiotic. The bio-youghurts are more beneficial than the ordinary yogurts as they boost the gut micro flora.

Yoghurt is a part of regular diet in south Eastern Europe and Middle East for centuries. In India, it is prepared in the eastern region by adding sugar and small amount of starter culture and kept overnight for fermentation (Meenakshi et al. 2018). The term *dahi* was used for the product developed in such a manner. Yoghurt is enjoyed all over the world for its beneficial properties. For many years, plain yoghurt was available in the world market. Recently, the sugar and fruit fortified yoghurts became available

in the market and resulted in the enhanced popularity of the product. The sweet, fruit yoghurt is preferred by children, adolescents and elderly. There is an immense scope for popularizing the fruit based yoghurts.
Hence, the present study is an attempt to assess the suitability of jackfruit pulp for the development of the quite unfamiliar jackfruit based bio-yoghurt using a combination of homogenized milk and to evaluate its physic-chemical properties.

Materials and Methods

Selection and collection of jackfruit: For the present study, *koozha* and *varikka* cultivar of jackfruits were (raw and mature but not yet riped 90-110 days) procured from the local households and allowed to ripe. The soft and fibrous bulbs were of *koozha* and the firm bulbs were of *varikka* jackfruit

Processing of jackfruit pulp

The ripe fruits were cleaned thoroughly under running water before processing. After cutting the fruit, bulbs and seeds were separated. The bulbs were cut into small pieces and steam blanched for five minutes. Once the blanched bulbs came to room temperature, they were pulped by making puree with the help of mixer grinder. The smooth pulp thus obtained was used for the preparation of jackfruit yoghurt.

Procurement of other ingredients

Other ingredients namely homogenised milk, skimmed milk powder and sugar were purchased from the local market. The yoghurt cultures were (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) obtained from the department of Dairy Microbiology, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala. The probiotic strain, *Lactobacillus acidophilus* was obtained from IMTECH, Chandigarh.

Preparation of yoghurt

The yoghurts were prepared as per the method described by Varga (2006) after slight modifications (Figure1). The plain bio-yoghurt served as the control. The various treatments used for the preparation of jackfruit yoghurts are given in Table. 1.

Organoleptic evaluation

A series of acceptability trials were carried out using simple triangle test at the laboratory level and a panel of fifteen judges was selected between the age group of 18-35 years as suggested by Jellinek (1985). The bio-yoghurts were evaluated organoleptically by the judges using a 9 point hedonic scale.

Physico- chemical analysis of the yoghurt: Synerisis, water holding capacity, curd tension, viscosity, pH, moisture, fat, protein, crude fibre and total ash contentof theselected samples were determined according to the standard methods of AOAC (1994). pH of the samples were analysed using a pH meter(Infra digi).

Statistical analysis

The data were statistically analysed using Kendall's co efficient of concordance, DMRT (Duncan's Multiple Range Test) and independent t test

Results and Discussion

Organoleptic evaluation of yoghurts:Sensory evaluation is the expression of an individual like or dislike for a product as a result of biological variation in man and what people perceive as appropriate sensory properties. It is a unique source of product information not easily obtained by other means (Iwe, 2003).

All the prepared yoghurts were organoleptically evaluated by the panel of selected judges. The mean score obtained for the organoleptic qualities of each treatment were statistically analysed

Table 1: Mean scores of organoleptic qualities of jackfruit bio-yoghurts *koozha* variety

Treatments	Sensory Attributes					
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
T_0	9.00 (3.50)	9.00 (3.50)	9.00 (3.50)	9.00 (3.50)	9.00 (3.50)	9.00 (3.50)
T_1	7.62 (130)	7.53 (1.70)	7.60 (1.83)	7.22 (1.43)	7.28 (1.27)	7.35 (1.27)
T_2	7.95 (2.07)	7.71 (1.87)	7.62 (1.73)	7.77 (1.93)	7.82 (1.97)	7.82 (2.00)
T_3	8.62 (3.23)	8.26 (2.90)	8.37 (2.9)	8.64 (3.27)	8.66 (3.37)	8.66 (3.37)
Kendalls W	0.68**	0.52**	0.54**	0.64**	0.74**	0.72**

Figures in parenthesis indicates mean rank scores
**- Significant at 1% level

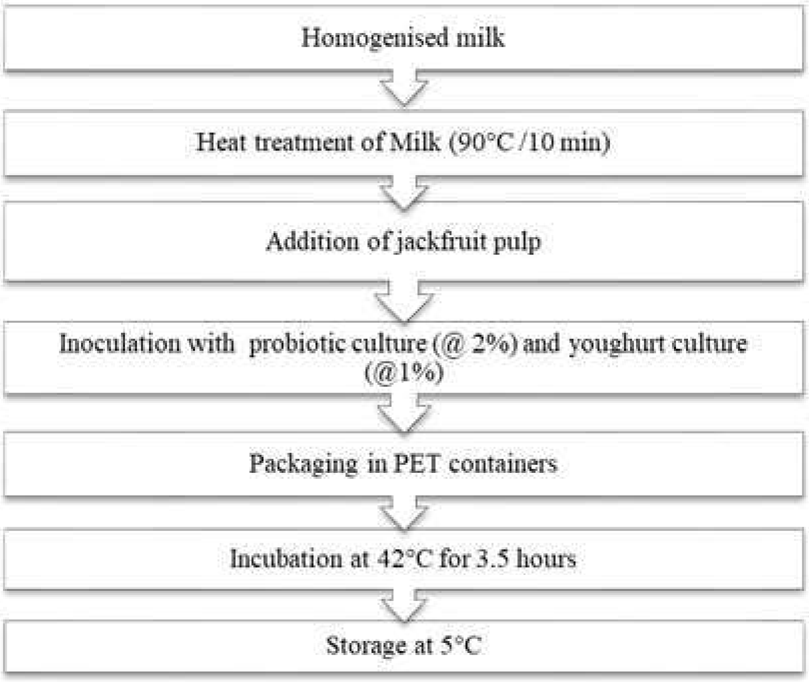


Fig. 1 Flow diagram for the preparation of yoghurt

using Kendall's coefficient of concordance and the mean scores were worked out. The mean score for different treatments are represented in Table2.

Treatment T_3 got the highest mean score for overall acceptability among the jackfruit yoghurts and hence selected as the best treatment. The mean scores for the sensory parameters of the treatments showed a trend to increase from T_1 to T_3. This may be due to the fact that as the amount of jack fruit pulp increased, it provided body and consistency to the final products which inturn increased the acceptability.

The same procedure was repeated with the *varikka*variety also. The organoleptic qualities of which are given in Table 3.

As seen in the *koozha* variety, in *varikka* variety also the treatment with 30% incorporation of jackfruit pulp ~~has~~ got maximum scores for the organoleptic parameters. Here also, the organoleptic properties tended to increase from T_1 to T_3.

Findings of the present study were in agreement with those of Kumar and Mishra (2003) who dealt with the preparation of mango pulp fortified yoghurt. They reported that, the overall acceptability of the product increased as the concentration of fruit pulp increased. The results of the study conducted by Ndife et al. (2014) also reported a similar result. They prepared functional yoghurt with 10%, 20% and 30% incorporation of coconut milk slurry and the one with 30% got the maximum overall acceptability. Gad et al. (2015) prepared functional yoghurts fortified with carrot juice and cantaloupe juice and the fruit yogurts were subjected to sensory evaluation. They came to a conclusion that fruit juice incorporation increased the acceptability of the yoghurts when compared to the plain yoghurts.

Comparison of organoleptic scores of selected fruit yoghurts

Sensory attributes of the selected jackfruit bio-yoghurts (*koozha* and *varikka*) were compared statistically and the results are given in Table 4.

From the table, it is clear that there is no significant difference among the sensory attributes of the two varieties except for flavour and texture. The *varikka*variety was the one with high scores for flavour and texture.

The bio-yoghurt prepared with the *varikka* variety was more acceptable with reference to flavour and texture. Increased flavour of *varikka* yoghurts can be attributed to the presence of increased volatile compounds in the *varikka* variety. Maia et al. (2004) identified the major aroma concentrates of the jackfruit varieties i.e. isopentyl isovalerate and butyl isovalerate. Isopentylisovalerate of *varikka* variety was found to be 28.4% and butyl isovalerate was 25.6% whereas that of *koozha* variety was 18.3% and 12.9% respectively. *Varikka* variety got good texture than the *koozha* variety and this may be due to the increased water content and juiciness of *koozha* (soft fleshed) jack fruit. Gad et.al. (2015) opined that increased water content of fruit juice would lead to pronounced decrease in the body and texture of fruit enriched yoghurts.

Table 2: Mean scores of jackfruit *varikka* variety

Treatments	Sensory Attributes					
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
T_0	9.00 (3.50)	9.00 (3.50)	9.00 (3.50)	9.00 (3.50)	9.00 (3.50)	9.00 (3.50)
T_1	7.93 (1.77)	8.20 (1.80)	8.13 (1.80)	7.67 (1.73)	7.40 (1.57)	7.40 (1.57)
T_2	8.67 (2.37)	8.67 (2.33)	8.33 (2.20)	7.93 (1.77)	7.93 (1.90)	8.13 (2.00)
T_3	9.00 (2.93)	9.00 (2.93)	8.47 (2.47)	8.67 (3.00)	8.67 (3.03)	8.67 (2.93)
Kendalls W	0.33	0.39	0.50	0.64	0.65	0.61

Figures in parenthesis indicates mean rank scores

**- Significant at 1% level

Table 3: Comparison of sensory attributes of selected yoghurts

Treatments	Sensory Attributes					
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
T_3 *(koozha)*	8.62	8.26	8.37	8.64	8.66	8.66
T_3 *(varikka)*	9.00	9.00	8.47	8.67	8.67	8.67
t value	0.44	0.22	2.50	2.78	1.91	1.91
Significance	NS	NS	S	S	NS	NS

Table 4: Physico-chemical properties of jackfruit based bio-yoghurt

Parameters	Control (T_0)	*Koozha*(T_3)	*Varikka*(T_3)
Synerisis	4.25^a	3.70^b	3.55^c
Waterholding capacity	95.75^a	96.30^b	96.45^c
Curd tension	0.115^c	0.128^b	0.157^a
Viscosity	27200^c	28800^b	29200^a
pH	4.11^b	4.61^a	4.63^a
Moisture (%)	75.29^c	78.52^a	76.03^b
Fat (%)	3.42^a	2.40^b	2.39^b
Protein (%)	3.60^a	3.03^b	3.05^b

DMRT row wise comparison.

Values with different super script differ significantly at 5% level

Physio-chemical analysis of the yoghurts

The selected jackfruit bio-yoghurts along with the plain bio-yoghurt (control) were subjected to physic-chemical analysis.

Synerisis of bio-yoghurts was found to follow the order of control>*koozha*>*varikka*bio-yoghurts. The observed rank of bio-yoghurtsin the descending order of water holding capacity was *varikka* based >*koozha* based>control bio-yoghurts. As the water holding capacity of yoghurt gel network increases, the percentage of synerisis decreases.

Study also revealed that the addition of jackfruit pulp was found to increase the curd tension, and viscosity of the yoghurts.

Meenakshi et al. (2018) developed fruit based banana yoghurts with the incorporation of sapota and banana pulp and the study reported an improvement in the physical properties of the fruit based probiotic yoghurts than the control sample The synerisis of control plain yoghurt was 30% whereas that of banana and sapota yoghurts were 6.2 and 21.4% respectively. In the present study the syneris of control yoghurt was 4.25% and that of jackfruit yoghurts were 3.70 and 3.55 % respectively for *koozha* and *varikka* varieties. Viscosity was also improved by the addition of fruit pulp and the viscosity of plain yoghurt of present study was 27200cP and that of *koozha* and *varikka* yoghurts were 2800cP and 29200 cP , respectively. Meenakshi et al. (2015) reported the viscocity of plain, banana and sapota yoghurts as 4450, 11400 and 5200Cp, respectively.

Lowering of pH happened due to the production of lactic acid during fermentation (Elke et al. 2016). The plain yoghurt was more acidic than the fruit yoghurts and this result is in accordance with the findings of Ndife et al. (2014). They reported low pH value (4.32) for plain yoghurt and higher (4.50) for the coconut enriched yogurt. Nazni and Komathi (2014) compared the physicochemical properties of plain as well as fruit yoghurts and found out that the fruit yoghurts were less acidic (6.3) when compared with the plain yoghurts (4.50).

The *koozha* jackfruit based yoghurts were having more moisture than the *varikka* due to the higher moisture content in *koozha* pulp. Pandey and Ukkuru (2004) reported the moisture content of *varikka* jackfruit pulp as 77.98 per cent and that of *koozha* as 79.03 per cent.

The findings of the present study are in line with several other authors. Hossain et al. (2012) reported that the incorporation of 15 per cent strawberry juice during preparation increased the moisture content of yoghurt from 74.03 to 74.29 per cent. Matter et al. (2016) developed cactus pear and papaya yoghurts and the moisture content of the plain, cactus pear and papaya yoghurts were 84.21, 89 and 85.12 per cent respectively.

Fat as well as protein content of the plain yoghurt was higher than that of the fruit yoghurts. This can be attributed to the incorporation of jackfruit, which is not a good source of fat. Same kind of observations were made by Roy et al. (2015) while analyzing different fruit yoghurts and control yoghurt. The control yoghurt was found to contain 3.75 per cent fat while banana, papaya and watermelon incorporated yoghurts were containing 3.56, 3.44 and 3.37 per cent of fat respectively. The papaya yoghurt containing 5, 10 and 15 per cent fruit pulp had a protein content of 3.76, 3.73 and 3.68 per cent respectively. A similar result was also reported by Jayalalitha et al. (2016) during the development of soymilk and mango pulp incorporated yoghurt. Protein content of the yoghurt with 10% mango pulp was 7.12% which decreased to 6.58% with 15% incorporation of mango pulp.

Conclusion

The study put forward the possibility of producing bio-yoghurt enriched with jackfruit pulp. Both the *koozha* and *varikka* varieties were found to be suitable for the product preparation. Incorporation probiotic organism into the product was a novel idea for bringing up a healthier product without compromising the product quality. Both the yoghurts were organoleptically highly acceptable as well as nutritious.

References

AOAC (Association of Official Analytical Chemists) (1994) Official Method for Analysis of the Association of Official Analytical Chemists, 14thedn. Association of Official Analytical Chemists, Washington DC.1141p

Gad AS, Ghita EI, El-Din HMF, Badran SMA, El-Messery TM (2015) Evaluation yogurt fortified with vegetable and fruit juice as a natural sources of antioxidant. Int J Food Nutr. Sci 4(3): 21-28

Elke KA, Peyer L, Zannini E (2016) Lactic acid bacteria as sensory biomodulators for fermented cereal based beverages. Trends Food Sci Technol 54: 17-25

Hossain N, Fakruddin M, Islam N (2012) Quality comparison and acceptability of yoghurt with different fruit juices. J Food Processing Technol 3(8): 1-5

Iwe MO (2003) The Science and Technology of Soya bean, Rejoint communications Ltd,Enugu

Jayalalitha V, Palanidorai R, Manoharan A, Elango A, Pugazhenthi TR, Balasundaram B (2016) Formulation of novel value enriched yoghurt and its quality evaluation. Indian J Dairy Sci 69(2): 143-147

Jellinek G (1985) Sensory Evaluation of Food: Theory and Practice, Ellis Horwood, Chichester, England

Kumar P, Mishra HN (2003) Optimization of mango soy fortified yogurt formulation using response surface methodology. Int J Food Properties 6(3):499-517

Maia JGS, Andrade EHA, Zoghbi MGB (2004) Aroma volatiles from two fruit varieties of jackfruit (*Artocarpusheterophyllus* Lam.).Food Chem 85(2): 195-197

Matter AA, Mahmoud E.AM. Zidan N S (2016) Fruit flavored yoghurt: chemical, functional and rheological properties. Int J Environ Agric Res 2(5): 57-6

Meenakshi V, Suganya, Umamaheswari T (2018) Formulation of value enriched probiotic fruit yoghurt. Int. J. Curr. Microbiol. Appl Sci 7(3): 1440-1450

Nazni P, Komathi K (2014) Quality evaluation of the fruit pulp added yoghurt. Int J Nutr Agric Res 1(1): 48-54

Ndife J, Idoko F, Garba R (2014) Production and quality assessment of functional yoghurt enriched with coconut. Int J Nutr Food Sci 3(6): 545-550

Pandey S, Ukkuru M (2004) Value added products and by products from jack fruit, Ph. D. (FS&N) thesis, Kerala Agricultural University, Vellayani, Kerala

Roy DKD, Saha T, Akter M, Hosain M, Khatun H, Roy MC (2015) Quality Evaluation of Yogurt Supplemented with Fruit Pulp (Banana, Papaya, and Water Melon). Int J Nutr Food Sci 4(6): 695-699

Varga L (2006) Effect of acacia (Robino pseudo acacia) honey the characteristics microflora of yoghurt during refrigerated storage. Int J Food Microbiol 108(2): 272-275

Indian J Dairy Sci 77(3): 236-242
https://doi.org/10.33785/IJDS.2024.v77i03.007

RESEARCH ARTICLE

Aflatoxin M1 and shelf-life analysis of goat and cow milk samples

Jyoti and Namita Ashish Singh(✉)

Received: 07 July 2023 / Accepted: 24 September 2023 / Published online: 23 June 2024

Abstract: Due to its medicinal benefits in mouth ulcers, dengue fever, etc. raw goat milk is widely used but ingestion of raw goat milk may have severe side effects. Aflatoxin M1 is produced by *Aspergillus* species and under long-term exposure, these are mutagenic, genotoxic, and carcinogenic. This study examined the shelf life of raw goat and cow milk at different storage temperatures (including ambient temperature, 5°C, and -19°C), via analysis of microbiological quality by standard plate count method. Incidence of aflatoxin M1 was also studied in raw goat and cow milk samples by lateral flow system. Cow milk showed a shelf life of 12 days while goat milk had up to 48 days at 5°C. The total plate count, coliform count, and spore count of goat milk was reported 5.89 log cfu/mL, 2.80 log cfu/ml, and 2.3 log cfu/ml in 75 days of storage while in cow milk samples it was 7.93 log cfu/ml, 5.64 log cfu/ml and 3.6 log cfu/ml, respectively in 45 days at -19°C. All the goat milk samples were found negligible for Aflatoxin M1 while cow milk samples showed a 40% incidence at 0.5 ppb. Our study showed that the quality of goat milk is commendable compared to cow milk in terms of aflatoxin M1 and bacterial count but due to less production and other factors goat milk is not widely used at the commercial level.

Keywords: Aflatoxin M1, Shelf-life, Goat, Milk, Microbial count

Applied Microbiology and Food Safety Laboratory, Department of Microbiology, Mohanlal Sukhadia University, Udaipur 313001, Rajasthan, India

Namita Ashish Singh (✉)
E-mail: namitas541@ gmail.com

Introduction

Goats are a vital component of the livestock business and the socioeconomic framework of India's smallholder farmers. India has the second-largest goat population, with 148.88 million of them (Basic Animal Husbandry Statistics, 2019). India produces 187.75 million tonnes of milk annually, with goat milk accounting for 3% of that total (Basic Animal Husbandry Statistics, 2019). Goat milk has a high protein and fat content of approximately 4.09% and 6.6%, respectively (Cyrillaa et al. 2015), is easy to digest, and is a good source of potassium, a mineral that is crucial for maintaining healthy blood pressure and heart function (Getaneh et al. 2016).

Large-scale industrialization of goat milk is frequently constrained by low individual production, seasonality, and transportation challenges, forcing farmers to keep raw milk refrigerated for longer than one day before processing. This practice affects the industry's quality since psychrotrophic bacteria can proliferate when raw milk is stored at 4 to 7°C for a few days prior to processing. By producing enzymes like lipases and proteases, psychrotrophic bacteria can change the flavor of milk and make it less appealing to customers. Lipases hydrolyze milk fat into smaller molecules known as free fatty acids, giving dairy products their sour and soapy flavors. Both native milk lipases and microbial lipases have the ability to start the lipolytic rancidity of milk (Delacroix and Lamberet. 2000). Psychrotolerants cannot withstand pasteurization, however, the extracellular enzymes mainly proteases and lipases of psychrotolerants continue to function normally and remain active, degrading the product's quality and subsequently shortening its shelf life (Hantsis-Zacharov et al. 2007). A study reported to analyse bacterial composition of goat milk at different storage conditions i.e. 4^0C and -80^0C showed that the milk bacterial diversity differed between different storage conditions (Kamilari et al. 2020).

Aspergillus flavus and *A. parasiticus* are considered to be the principal producers of aflatoxins (AFs), which are furanocoumarin derivative mycotoxins (Frisvad et al. 2019). Aflatoxins can develop in crops before and after harvesting as well as during storage if they are not kept in ideal circumstances (Kiswii et al. 2014). Aflatoxin B1 and B2 are produced by *Aspergillus flavus* which is changed into aflatoxin M1 (AFM1) when animals eat feed contaminated with aflatoxin B1. The maximum permissible amount of AFM1 in the European Union (EU) for milk and dairy products is 0.050 g/kg. The Codex Alimentarius Commission (CAC) and the Food and Drug Administration (FDA) in the USA both determined an action level for AFM1 in the milk of 500 ng/L (FDA 2005, CAC 2001). Aflatoxins can tolerate pasteurization and they are linked to stunting in children and have been reported to inhibit children's immune systems (Jalili 2015, Raduly et al. 2020). According to the Food and Agriculture Organization of the United Nations (FAO), 25% of the world's food crops are contaminated with mycotoxins. Under long-term exposure, AFs are mutagenic, teratogenic, genotoxic, and carcinogenic (CAC, 2001). AFs can enter into the feed and food chain at any stage from pre-harvest to human consumption. In both animals and humans, these toxins are normally absorbed from the gut and transported to other bodily areas where they can form chemical bonds or undergo chemical modifications (Peleset al. 2019).

Goat milk production is not widely commercialized, despite its compelling prospects. Additionally, it has been discovered that the overall milk output of small-scale dairy farms may not be sufficient to meet the local market's needs for milk. So, the objective of the current study is to evaluate the AFM1 contamination levels and the effect of extended storage on the microbiological quality of raw goat and cow milk in the Udaipur region, India at different storage temperatures for the exploration of goat milk at commercial level.

Materials and Methods

Materials

All the media and reagents were procured from Hi Media Pvt. Ltd. Maharashtra, India. Aflatoxin M1 analysis was done by Rapid one-step assay (ROSA) kit manufactured by Charm Sciences USA. A shelf-life study was done in Biosafety Cabinet-level II, Waiometra, Associated Scientific Technology, Delhi. Incubation was done in MAC BOD incubator (India). Raw goat (n-55) and cow milk samples (n-20) were collected from nearby villages (Amarpura, Kheroda, Khemali, Chinawar, Mudiaphala, Amarpura, and Udaipur city) of Udaipur district, Rajasthan, India. Samples were collected in sterilized bottles and delivered to the laboratory by maintaining the temperature at less than 4°C during transport.

Aflatoxin M1 analysis

Aflatoxin M1 in raw goat (n-55) and cow (n-20) milk samples were analyzed by Rapid one-step assay (ROSA) kit manufactured by Charm Sciences USA which is based on lateral flow mechanism along with positive and negative controls. Dilution buffer (300 µl) was added to the labeled microtube, followed by the addition of raw goat/cow milk sample (300 µl) and mixing. The test strip was labeled with sample identification, placed in Charm EZ system, and incubated at 56°C for 8 minutes and results were observed.

Shelf-Life analysis

For the shelf-life analysis, raw goat and cow milk samples (n=16) were distributed into three fractions and kept at controlled temperatures i.e. ambient temperature, 5°C, and -19°C respectively, and analyzed for the total plate count, coliform count, and spore count during storage.

Total plate count

Total plate count was performed on all goat and cow milk samples by the standard plate count method. One ml of each sample was added in 9 ml sterile 0.85% saline and an aliquot of 1 ml of each dilution was then transferred to petriplates followed by the plating of plate count agar medium. The plates were incubated at 37° C for 24±2 hrs and bacterial colonies were counted and expressed in log colony forming unit (CFU) per milliliter.

Coliform count

Coliform count (CC) analysis of all milk samples was done on violet-red bile agar medium following the standard plate count method mentioned above.

Spore count

Isolation of *Bacillus* spores from milk was done by heating the milk at 80°C for 10 minutes in a heating mantle followed by rapid cooling with water for the elimination of vegetative cells. The samples (1ml) were serially diluted by the above method and plated on nutrient agar media. After incubation, a number of colonies were counted and expressed in log colony forming unit (CFU) per ml (Sharma and Singh 2017).

pH analysis

The pH of the samples was determined using Thermo Scientific pH Electrode ECO Testr pH 1 (Eutech USA). The pH meter was calibrated with pH 4.0, 7.0, and 9.0 buffer solution before analysis.

Statistical Analysis

All collected experimental data were statistically analyzed using the PAST v3.02 software (Hammer et al. 2001). Two-sample t-test was used for analysis of variance for unequal sample size of goat and cow milk. The differences between samples were assessed for effects of storage temperature, and storage time on total viable bacterial count. The mean counts of raw goat and cow milk samples were also calculated considering their storage temperature and storage period in goat farms and compared by Kruskal-Wallis test.

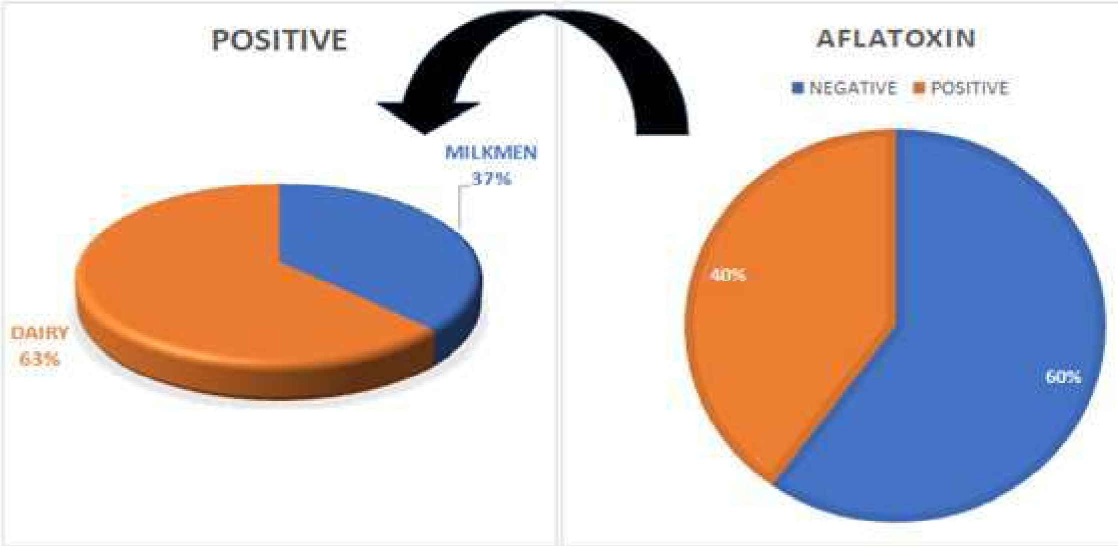


Fig 1. Occurrence of Aflatoxin M1 in raw cow milk samples

Results and Discussion

Aflatoxin M1 analysis

All of the raw goat milk samples (n=55) were found negative for Aflatoxin M1 at a detection limit of 0.5 ppb. In the case of cow milk eight out of twenty samples were found positive showing 40% incidence for Aflatoxin M1 occurrence, while twelve samples were found negative. Among the eight positive samples, 37% samples were from milkmen and the remaining 63% were collected from local dairies as depicted in Fig. 1.

The obtained results for AFM1 in goat milk are in accordance with a survey focused on the incidence of AFM1 in goat milk performed in Lebanon, and all the samples were below the detection limit of the testing methods (Assem et al. 2011). The comparatively high incidence exceeding the EU limit of AFM1 in goat milk was reported by previous studies. In a recent study, 76.0% of goat milk samples were tested AFM1-positive, and 6.7% of them were over the EU limit (Zheng et al. 2022). The concentrations of AFM1in goat milk samples were in a range of 0.0056 to 0.0482 g/L from Brazil (De Matos et al. 2021). AFM1 contamination was positive in 33.3% of 150 goat milk samples in India, of which 10.0% were above the limit of European Union (Nile et al. 2016). The results of the present study revealed lower values of aflatoxin M1 compared to those reported earlier globally however the aflatoxin M1 analysis of the Udaipur region has been done for the first time.

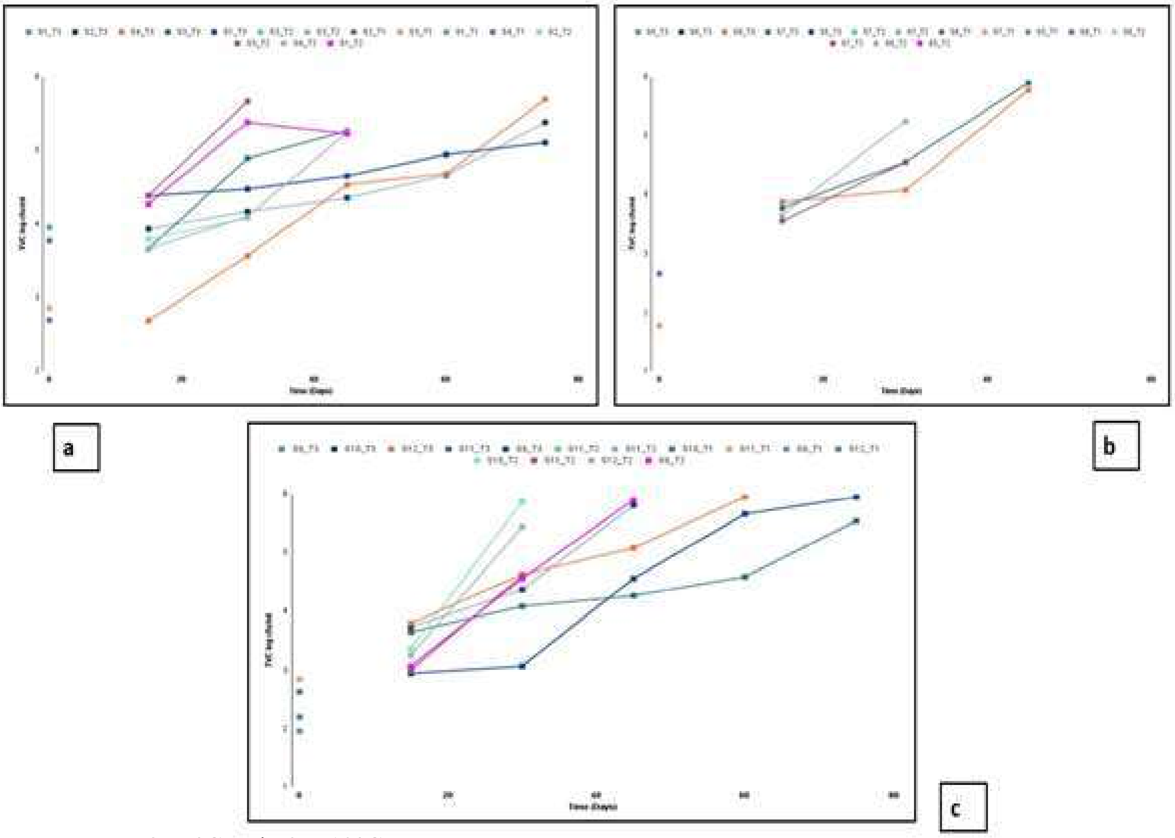
Our study revealed that the percentage of AFM1 contamination found in cow's milk was 40%, which was above the detection limit i.e. 0.5 ppb. Our results were supported by Nile et al. (2016) who reported AFM1 contamination in 45.3% of cow milk samples in India and Zheng et al. (2022) who found AFM1 in 65.7% of cow milk samples from 0.005 to 0.191 g/L. According to an Iranian investigation, 35.9% of cow milk samples were found to be over the EU MRL limit (Bahrami et al. 2016). Mohammedi-Ameur *et al.* reported that 46.42% of samples were positive for AFM1 contamination (toxin levels higher than the 0.050 g/kg EU standard (Mohammedi-Ameur et al. 2020). The cows are maintained in nearby dairy farms and fed compound rations or silage stored in substandard circumstances may be linked to the high prevalence of AFM1 in cow milk samples. This may result in the development of aflatoxin and heavily contaminated areas with toxic *Aspergillus* fungus (Asi et al. 2012).

These results showed that milk contamination levels with AFM1 differ between nations. These variations could be attributed to variations in forage and feed quality, cow diet, geographic location, climatic and seasonal variations, genetic variations in dairy cows, farming systems, and feed storage (Eskandari and Pakfetrat 2014; Sahin et al. 2016). According to Paterson and Lima, the high temperature brought on by climate change promotes mycotoxin contamination (Paterson and Lima 2010). The fact that goats in India are mostly fed by grazing and are only fed on stored grains for three to four months is likely the cause of the reduced presence of AFM1 in goat milk as compared to buffalo and cow milk (Nile et al. 2016).

Microbiological analysis

Total plate count

The initial total plate count ranged from 1.77-3.95 log cfu/ml for goat milk and 4.65-4.94 log cfu/ml for cow milk respectively at zero days. The samples kept at ambient temperature got spoiled within 24 hours in the case of cow milk, while for goat milk the spoilage time was 24-48 hours. The total plate count for the goat milk samples increased up to 5.94 log cfu/ml at -19°Cduring prolonged storage of 75 days and exceeded the EU limits at the end of the shelf-life. The initial total plate count noted ranged from 3.0-4.38 log cfu/ml at 5°C. The samples kept at 5°C showed



Where T1=ambient temperature, T2= 5°C and T3= -19°C
S1=Sample 1, S2= Sample 2, S3=Sample 3, and S4= Sample 4, onwards upto S12

Fig 2. Total plate count of raw goat milk during storage at different temperatures. a) Sample no.1-4, b) Sample no.5-8, c) Sample no.9-12.

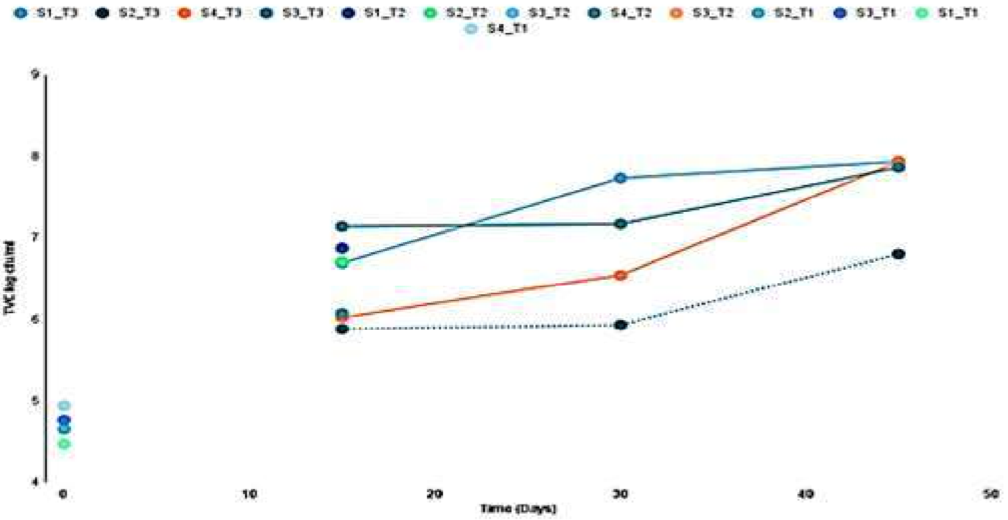


Fig 3. Total plate count of raw cow milk during storage at different temperatures

Where T1=ambient temperature, T2= 5°C and T3= -19°C
S1=Sample 1, S2= Sample 2, S3=Sample 3, and S4= Sample 4

maximum variability in spoilage time where few samples got spoiled at 32 days only, some samples were safe up to 48 days with TPC value 5.86 log cfu/ml and later got spoiled (Fig.2). The total plate count of cow milk samples kept at 5°C was observed ranging from 6.07-6.87 log cfu/ml with a shelf-life of 10-15 days. In cow milk samples the total plate count values reached up to highest 7.93 log cfu/ml at the end of shelf-life i.e. 45 days at -19°C (Fig. 3).

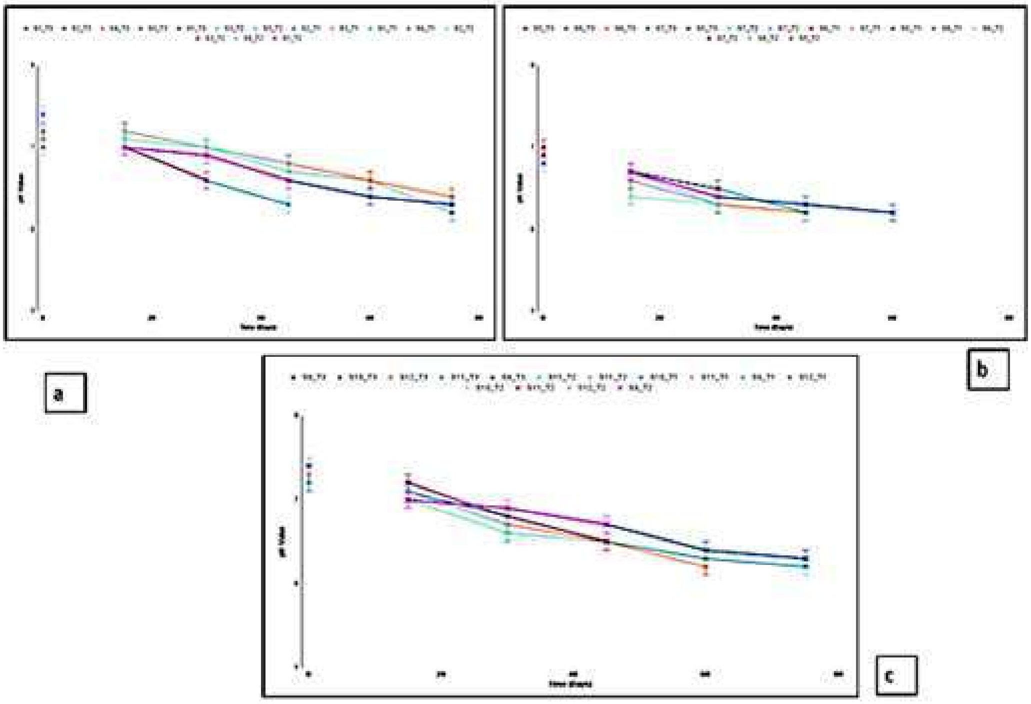


Fig 4. pH analysis of goat milk during storage at various temperatures. a) Sample no.1-4, b) Sample no.5-8, c) Sample no.9-12
Where T1=ambient temperature, T2= 5°C and T3= -19°C
S1=Sample 1, S2= Sample 2, S3=Sample 3, and S4= Sample 4, onwards upto S12

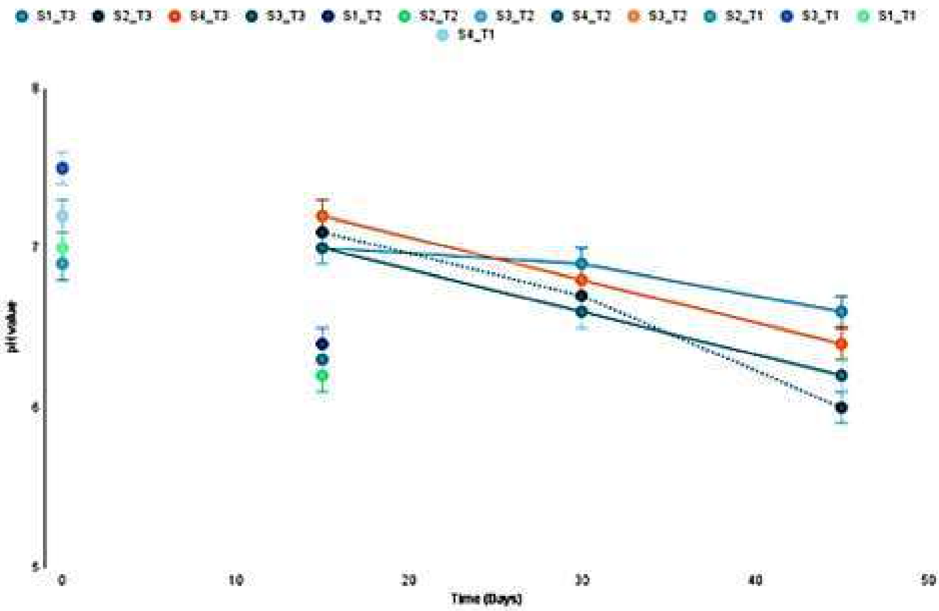


Fig 5. pH analysis of cow milk during storage at various temperatures
Where T1=ambient temperature, T2= 5°C and T3= -19°C
S1=Sample 1, S2= Sample 2, S3=Sample 3, and S4= Sample 4

In our study, the TPCs for the goat milk samples reached up to 5.94 log cfu/ml at -19°C within 75 days while cow milk samples showed the same trend of rise but count was more i.e. 7.93 log cfu/ml in 45 days of storage. These results are in accordance with many previous studies. According to a study by Nile et al. (2016) the bulk tank goat milk's 2007 SPCs kept rising and by day 6 had surpassed the grade A goat milk's limit of 100,000 CFU/ml. A preliminary bacteria count indicates the degree of contamination and quality of the milk samples. The total concentration of aerobic bacteria was 3.44 log cfu/ml (Lai et al. 2016). According to the Malaysian Food Act 1983 and Food Regulations 1985, the total aerobic bacteria concentration in milk, which is safe for consumption should not exceed 5.0 log cfu/ml (USFDA 2005).

In most of the samples including goat and cow milk, the spore count was found nil. During the prolonged storage at -19°C, the spore count in raw goat and cow milk samples grew steadily and reached 2.3 log cfu/ml and 3.6 log cfu/ml respectively. In all three categories, bacterial concentration showed an increasing trend throughout storage. The longer shelf-life of goat's milk as observed in this experiment (more than 60-70 days as compared to 30-45 days for cow's milk) may be explained by the presence of natural bacterial inhibitor in goat's milk.

Evidently, when milk samples were stored at low temperatures (5°C and -19°C), the growth of aerobic bacteria was considerably slower. Lafarge et al. discovered that 24 hours of cold storage dramatically increases the number of psychrotrophic bacteria (Lafarge et al. 2004). One of the main groups of spoilage bacteria found in raw goat milk samples or other dairy products is the psychrotrophic bacteria. *Pseudomonas fluorescens* is the most often isolated species from milk and is the dominant member of the bacterial community at the time of spoiling, making up the majority of the psychrotrophic microorganisms. One of the useful markers for the shelf life and degree of deterioration of raw goat milk samples might be the proteolytic count (Mcphee and Griffiths, 2006). The impact of variables on the composition of the raw milk microbiota and spoilage activity has already been described, mostly in cow milk (Ercolini et al. 2009; Perin et al. 2009).

Coliform Count

In most of the samples of both raw goat and cow milk, the coliform count was found nil. The coliform count (CC) in goat milk continuously increased with time spent in storage. On day zero, the CC was 2.44 log CFU/ml at ambient temperature and at the end of storage, it had risen to 2.80 log CFU/ml at -19°Cwhile CC noted 2.56 log cfu/ml at 5°C. The initial coliform count determined from the raw cow milk sample at zero-day was 1.36 log cfu/ ml at ambient temperature and 4.84 log cfu/ml at 5°C at 15 days storage period. The coliform count in raw cow milk reached 5.64 log cfu/ ml with the end of shelf-life of 45 days -19°C.

The coliform count (CC) was observed as nil in most of the goat and cow milk samples while the increasing trend was followed by the remaining samples. When the storage duration was extended, there was a progressive increase in the concentration of coliform bacteria. The microbiological standard's limit for the concentration of coliform bacteria was exceeded in samples of raw cow milk and reached up to 5.64 log cfu/ml. Although coliform bacteria may grow more slowly in colder temperatures, many of them, including *E. coli* and *Klebsiella* spp., are temperature tolerant and quickly recover under warm conditions. As a result, these coliform groups provide a special risk for the spoiling and contamination of a sample of raw goat milk stored at a low temperature (Lai et al. 2016).

Spore Count

Spore count was found nil in most of the samples of both goat and cow milk. The initial spore count noted at ambient temperature was 1.97 log cfu/ml and 1.51 log cfu/ml for goat and cow milk respectively at zero days. The count was observed as nil at 5°C as the samples got curdled on heating in both the cases of goat and cow milk. The final spore count reached up to 2.3 log cfu/ml for goat milk and 3.6 log cfu/ml for cow milk at -19°C till the final spoilage of samples.

There was a significant difference found between goat and cow milk sample medians in reference to temperature variance by Kruskal-Wallis test and sample variance by t-test. The *H* (chi^2) values for goat and cow milk samples were found as 11 and 6.038 respectively. The p -values (p<0.005) for goat and cow milk were noted 0.003 and 0.048 respectively, represented a significant value.

pH analysis

The initial pH for raw goat and cow milk samples ranged from 7.4±1 to 6.8±1 and 7.5±1 to 6.9±1 respectively at zero days. However, the pH of the milk samples decreased during the storage time and fall up to 6.3±1 for goat milk and 6.2±1 in the case of cow milk at 5°C. The final pH observed at -19°C was 6.2±1 and 6.0±1 for goat and cow milk respectively with the end of the shelf-life of samples (Fig. 4 & 5).

The initial value of pH which ranged from 7.4-6.8 at ambient temperature decreased significantly, and the final pH noted was 6.2 for goat milk while for cow milk it is 6.0 at -19°C. Both lactose fermentation and/or microbial alterations in the product during storage can cause a reduction in the pH values of raw milk. Growing casein and phosphate concentrations during late lactation increased the milk's intrinsic acidity and may be the source of a pH reduction Singh and Sengar 1990). The recent study by Kamilari et al. (2020) provided the information that keeping goat milk overnight in the refrigerator may lead to alterations in the bacterial composition.

Conclusions

Based on our findings, a direct relationship between raw goat milk storage practices and its microbiological quality was found. The study represented that goat milk has a low count and high/ more shelf-life in comparison to cow milk. The shelf-life of raw goat milk was observed more (75 days) in comparison to cow milk (45 days) at -19°C. The total bacterial count, coliform, and spore count in raw goat and cow milk samples increased gradually after prolonged storage at various temperatures. The high bacterial count in local raw goat and cow milk samples highlights the tendency of the bacteria to grow and multiply when stored at low temperatures. A high total plate count and the coliform count were not advisable for raw milk consumption proceedings due to food contamination concerned. Goat milk was found safe for consumption in reference to Aflatoxin M1 while it is a matter of concern that in cow milk incidence is 40%. These findings on aflatoxin M1 level and shelf-life analysis on raw goat and cow milk samples from the Udaipur region, India will be helpful for dairy plants during taking decisions regarding product development using goat milk.

Acknowledgments

The authors are thankful to the authorities of Mohanlal Sukhadia University for providing the infrastructure facilities. Namita Ashish Singh is thankful to the University Grants Commission (UGC), New Delhi for providing the UGC-BSR Research Start-Up-grant letter No.F.30-476/2019 (BSR).

References

Asi RM, Iqbal SZ, Ariño A, Hussain A (2012) Effect of seasonal variations and lactation times on afla-toxin M1 contamination in milk of different species from Punjab, Pakistan. Food Control 25(1):34-38

Assem E, Mohamad A, Oula EA (2011) A survey on the occurrence of aflatoxin M1 in raw and processed milk samples marketed in Lebanon. Food Control 22:1856–1858

Bahrami R, Shahbazi Y, Nikousefat Z (2016) Aflatoxin M1 in milk and traditional dairy products from west part of Iran: occurrence and seasonal variation with an emphasis on risk assessment of human exposure. Food Control 62:250–6

Basic Animal Husbandry Statistics (2019) https://dahd.nic.in/sites/default/filess/BAHS%20%28Basic%20Animal%20Husbandry%20Statistics-2019%29_0.pdf 29 June 2023

Codex Alimentarius Commissions (2001) Comments Submitted on the Draft Maximum Level for Aflatoxin M1 in Milk. 33rd ed. Codex Committee on Food

Cyrillaa L, Purwantob BP, Atabanya A, Astutic DA, Sukmawatid A (2015) Improving Milk Quality for Dairy Goat Farm Development. Media Peternakan 38(3): 204-211

De Matos CJ, Schabo DC, Nascimento YMD, Tavares JF, Lima EDO, da Cruz PO, de Souza EL, Magnani M, Magalhães HIF (2021) Aflatoxin M1 in Brazilian goat milk and health risk assessment. J Environ Sci Health Part B 56:415–422

Delacroix-Buchet A, Lamberet G (2000) Sensorial properties and typicity of goat dairy products 559–563 in Proc 7th Intl Conf Goats INRA Paris France

Ercolini D, Russo F, Ferrocino I, Villani F (2009) Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk. Food Microbiol 26: 228–231

Eskandari MH, Pakfetrat S (2014) Aflatoxins and heavy metals in animal feed in Iran. Food Addit Contam Part B Surveill **7**(3):202-207

Food and Drug Administration. (2005) Sec. 527. 400 Whole Milk, Low Fat Milk, Skim Milk Aflatoxin M1 (CPG 7106.10) FDA/ORA Compliance Policy Guides

Frisvad JC, Hubka V, Ezekiel CN, Hong SB, Nováková A, Chen AJ, Arzanlou M, Larsen TO, Sklenáˇr F, Mahakarnchanakul W (2019) Taxonomy of Aspergillus section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. Stud Mycol 93:1–63

Getaneh G, Mebrat A, Wubie A, Kendie H (2016) Review on Goat Milk Composition and Its Nutritive Value. J Nutr Health Sci 3(4):401

Hammer Ø, Harper DA, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol Electron. 4:9

Hantsis-Zacharov E, Halpern M (2007) Culturable psychrotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits. Appl Environ Microbiol 73:7162–7168

Jalili M (2015) A review on aflatoxins reduction in food. Iran J Health Saf Environ 3:445–459

Kamilari E, Anagnostopoulos DA, Papademas P, Efthymiou M, Tretiak S, Tsaltas D (2020) Snapshot of cyprus raw goat milk bacterialdiversity via 16s rDNA high-throughputsequencing; impact of cold storage conditions. Fermentation 6:100

Kiswii TM, Monda EO, Okemo PO, Bii C, Alakonya AE (2014) Efficacy of Selected Medicinal Plants from Eastern Kenya against Aspergillus flavus. J Plant Sci 2(5): 226

Lafarge V, Ogier JC, Girard V, Maladen V, Leveau JY, Gruss A, Delacroix-Buchet A (2004) Raw cow milk bacterial population shifts attributable to refrigeration. App Env Microbiol 70, 5644-5650

Lai CY, Fatimah AB, Mahyudin NA, Saari N, Zaman MZ (2016) Physico-chemical and microbiological qualities of locally produced raw goat milk. Int Food Res J 23(2): 739-750

Mcphee JD, Griffiths MW (2007) Psychrotrophic bacteria, Pseudomonas spp. In John (Ed). Encyclopedia of Dairy Sciences 2nd Edition, 2007. pp. 379–383. San Diego: Academic Press

Mohammedi-Ameur S, Dahmane M, Brera C, Kardjadj M, Ben-Mahdi MH (2020) Occurrence and seasonal variation of aflatoxin M1 in raw cow milk collected from different regions of Algeria. Vet World 13(3):433-439

Nile SH, Park SW, Khobragade CN (2016) Occurrence and analysis of aflatoxin M1 in milk produced by Indian dairy species. Food Agric Immunol 27: 358–366

Paterson RRM, Lima N (2010) How will climate change affect mycotoxins in food. Food Res Int 43(7):1902-1914

Peles F, Sipos P, Gy˝ori, Z, Pfliegler WP, Giacometti F, Serraino A, Pagliuca, G, Gazzotti T, Pócsi I (2019) Adverse effects, transformation, and channeling of aflatoxins into food raw materials in livestock. Front Microbiol10:2861

Perin LM, Moraes PM, Almeida MV, Nero LA (2012) Intereference of storage temperatures in the development of mesophilic, psychrotrophic, lipolytic and proteolytic microbiota of raw milk. Semina Cien Agr 33:333–342

Ráduly Z, Szabó L, Madar A, Pócsi I, Csernoch L (2020) Toxicological and medical aspects of *Aspergillus*-derived mycotoxins entering the feed and food chain. Front Microbiol 10:2908

Sahin HZ, Celik M, Kotay S, Kabak B (2016) Aflatoxins in dairy cow feed, raw milk and milk products from Turkey. Food Addit Contam Part B Surveill 9(2):152-158

Sharma S, Singh NA (2017) Isolation and characterization of mesophilic spores in different types of milk during winter. Trends in Biosciences 10 (2):754-757

Singh SN, Sengar OPS (1990) Studies on the combining ability of desirable characters of important goat breeds, Final technical report PL-480. RBS College, Bichpuri, Agra, India. 1990, 217–267

U.S. Food and Drug Administration, Public Health Service, Departmentof Health and Human Services (2005) Grade "A" pasteurized milk ordinance. Washington, D.C. http://www.foodscience.cornell.edu/cals/foodsci/extension/upload

Zheng N, Min L, Li D, Tan S, Gao Y, Wang J (2022) Occurrence of Aflatoxin M1 in Cow, Goat,Buffalo, Camel, and Yak Milk in China in 2016. Toxins 14, 870

Indian J Dairy Sci 77(3): 243-251
https://doi.org/10.33785/IJDS.2024.v77i03.008

RESEARCH ARTICLE

Development of convolutional neural network models for evaluation of body condition scores of Holstein Friesian crossbred cows

Shriramulu[1], Heartwin A Pushpadass[1] (✉), Magdaline Eljeeva Emerald Franklin[1], Manimala Kanagaraj[2], Jeyakumar Sakthivel[3], Mukund A. Kataktalware[4], Sivaram Muniandy[5] and Ramesha P. Kerekoppa[6]

Received: 20 September 2023 / Accepted: 17 October 2023 / Published online: 23 June 2024

Abstract: Body condition scoring (BCS) is an efficient tool to monitor the nutritional status of cows by subjective assessment of the amount of fat or stored energy in them. This method of scoring is cumbersome, laborious and inefficient because it takes more time to score due to increasing number of animals in modern dairy farms, besides involving high cost. Therefore, this study proposed a system based on convolutional neural network (CNN) models to automate BCS of cows by image analysis. The digitally-captured images were processed using GIMP software to subtract the background from the captured images of cows. The background-subtracted images were used to detect the edges and contours using fuzzy logic edge detection method in Matlab software. Finally, the images with body contours and edges were employed as input dataset for the development of CNN models. The image dataset was classified into two groups based on the incremental BCS system of 0.25 (CNN model 1) and 0.5 (CNN model 2). The classification accuracy of the first model for 0.25 and 0.50 error ranges was 61.41% and 80.31%, respectively. Similarly, the second model achieved classification accuracy of 81.45% and 93.54%, for the same error ranges. The CNN model 2 was relatively better as compared to the first model owing to the wider BCS range of classification.

[1]Dairy Engineering Section, ICAR-National Dairy Research Institute, Southern Regional Station, Adugodi, Bengaluru-560030, India

[2]Department of Information Science and Technology, Anna University, College of Engineering, Gunidy Campus, Chennai - 600 025, India

[3] Livestock Research Centre, ICAR-National Dairy Research Institute, Southern Regional Station, Adugodi, Bengaluru-560030, India

[4] Livestock Production and Management, ICAR-National Dairy Research Institute, Southern Regional Station, Adugodi, Bengaluru-560030, India

[5] Dairy Economics and Statistics Section, ICAR-National Dairy Research Institute, Southern Regional Station, Adugodi, Bengaluru-560030, India

[6] Dairy Production Section, ICAR-National Dairy Research Institute, Southern Regional Station, Adugodi, Bengaluru-560030, India

Heartwin A. Pushpadass (✉)
Email: heartwin1@gmail.com; P.Dhass@icar.gov.in; Mobile: +91 9448927370

Keywords: Convolutional neural network; Body condition scoring; Classification accuracy; Image processing; Deep learning

Introduction

Assessing the body condition score (BCS) of cows is important to measure the changes in subcutaneous body fat throughout the stages of lactation. This is because the anatomical characteristics of cows such as external shape and tissue cover with frame size can be related to their current nutritional status and productivity. The extremely fat or skinny cows give reduced milk yield, pose risk of metabolic diseases and have low reproductive performance (Albornoz et al. 2021). During early lactation, depletion of their fat reserves takes place, while in late lactation, accumulation of fat takes place. Therefore, it is crucial to manage the BCS of cows at various stages of lactation so as to improve their health condition and productive performance. The most common approach for evaluating the BCS is the subjective method that uses a standardized scale based on the amount of tissue coverage at the hindquarters of the cow.

Body condition scoring is visually done by experts by assessing the rear regions of cows such as the loin, tail and pelvis, and providing a score ranging from 1 to 5. This technique is vulnerable to bias in many situations and requires the animal to be confined during evaluation. It is also time-consuming, restricting the total number of animals that can be examined as well as the frequency with which they can be evaluated. Although the significance of BCS has been evidenced in numerous studies, more than half of the farm producers never record the BCS of cows in their herds regularly (Caraviello et al. 2006). Similarly, the survey conducted by German fresh cow management found that only 36% of farmers measured the BCS of their herd regularly (Heuwieser et al. 2010).

The lack of implementation of regular BCS measurement is due to practical difficulty in scoring and management of BCS scores by the experts and the farmers (Song et al. 2019). To incorporate high-quality manual scoring into farm management, farmers must employ experienced assessors on a regular basis or obtain training to score their own cows, both methods are time-consuming and costly. Hence, there is a need to develop modern hybrid and machine learning computing tools using the assistance of image processing to determine the BCS in an automated way. Such a system will be a user-friendly technique to estimate the BCS of large herds, analyze the BCS data set, and facilitates in easy identification of the variation in BCS of cows so as to take timely and appropriate management decisions.

Image processing is the important step for training and testing the dataset as it extracts the features that will be utilized for estimation of BCS. Developing a predictive BCS model using conventional feature extraction using manual method is less effective and less precise owing to the small amount of samples and feature points, which result in a poor fit (Fischer et al. 2015). In general, 3D vision-based systems achieved more accurate results (Li et al. 2019; Song et al. 2019; Alvarez et al. 2018). This is because the concavity information of the cows' body surface, provided by the 3D image characteristics, is more closely related to the fat accumulation beneath the skin.

In recent years, the area of deep learning has made great strides in the field of computer vision and image classification. Feature extraction is a key aspect of machine learning techniques. Deep learning algorithms fix the problem of feature extraction by automatically extracting relevant features from the raw input data rather than requiring pre-selected features. A deep learning model with various processing layers may learn complex input data attributes at various levels of abstraction (Cao et al. 2018). The convolutional neural network (CNN) is a deep learning method that is used to solve the complexity of developing, training and implementing machine learning models at any scale. It is a biologically-inspired concept of a deep network for feature detection, capable of learning purely complex features, and proves to be more effective in identification of objects (Zhang et al. 2017). In comparison to fully-connected networks with an equal number of hidden units, CNN architecture has the advantage of being simpler to train and has fewer parameters.

The major attraction of CNN is the concept of weight sharing to reduce the number of parameters that need to be trained to achieve greater generalization and only a few parameters are needed for training to avoid over-fitting (Arel et al. 2010). Secondly, the classification stage is combined with the feature extraction stage, both based on the learning process (LeCun et al. 1998). Thirdly, implementing large networks by use of general models in artificial neural networks (ANN) is more complex than implementation in CNN. In this study, a computer-aided CNN model was developed to evaluate the BCS of cows in order to minimize the error and bias that might arise during from interpretation of BCS by experts. The automatic BCS system was developed using various network architecture and general models used in deep-learning techniques, by selecting the body features of cows and extracting them from multiple viewpoints, so that the image-based BCS classification could be accurate and relevant.

Materials and Methods

Data collection

To develop and evaluate the CNN model, five dairy farms were visited and 503 images of Holstein Friesian cows with varying body muscle and fat in the hindquarters were acquired. A digital SLR camera (Nikon D5100, Nikon Corporation, Japan) was used to capture the images manually at ISO speed of 100, exposure time of 1/100 s and resolution of 4928×3264 pixels by ensuring the same distance and angles during image acquisition such that consistency could be maintained and error could be minimized. The images were acquired after cleaning and milking the animals. The vital areas of the cows were imaged at two suitable viewing angles for evaluation of BCS. The first dorsal view provided information on spinal parameters such as hook bones, pin bones and tail head of the cow. The second view was the side image, which gave the edge information on short and long ribs, and the area between pin and hook bones.

Dataset preparation

A scorecard containing images from the two viewing angles and detailed physiological information of the cows was developed. The images were printed and manually evaluated by three veterinary experts and their BCS were obtained on a 5-point scale. This scale is not breed specific, where score 1 represented thin cows and score 5 represented obese cows as explained by Gillund et al. (2001). The dataset of scores by experts was divided into 'training', 'testing' and 'validation' sets. Additionally, the image dataset was classified into two groups based on the incremental BCS system of 0.25 and 0.5 and each CNN model architecture was applied for prediction. This classification helped in evaluating the model performance for each increment of BCS. For development of CNN model, 70% of images were used, while 30% of them were used for testing and validation. Figs. 1 (a) and (b) show the percentage distribution of body condition scores over training and test set for (a) model 1 and (b) model 2.

- Model 1: Increment of 0.25 unit class of BCS
- Model 2: Increment of 0.5 unit class of BCS

System information and software tools

A 2.0 GHz CPU running on 64-bit Windows 10 was used to train the model. The model implementation program for BCS estimation was written in Python (v. 3.7.0, Python Software Foundation, Wilmington, Delaware, United States). The 'Keras' library in Python was used to build the CNN-based image classification. It is an advanced application programming interface (API) that operates on the basis of Tensorflow and offers advanced building blocks for creating deep learning models (Manaswi, 2018). Computer unified device architecture deep neural network

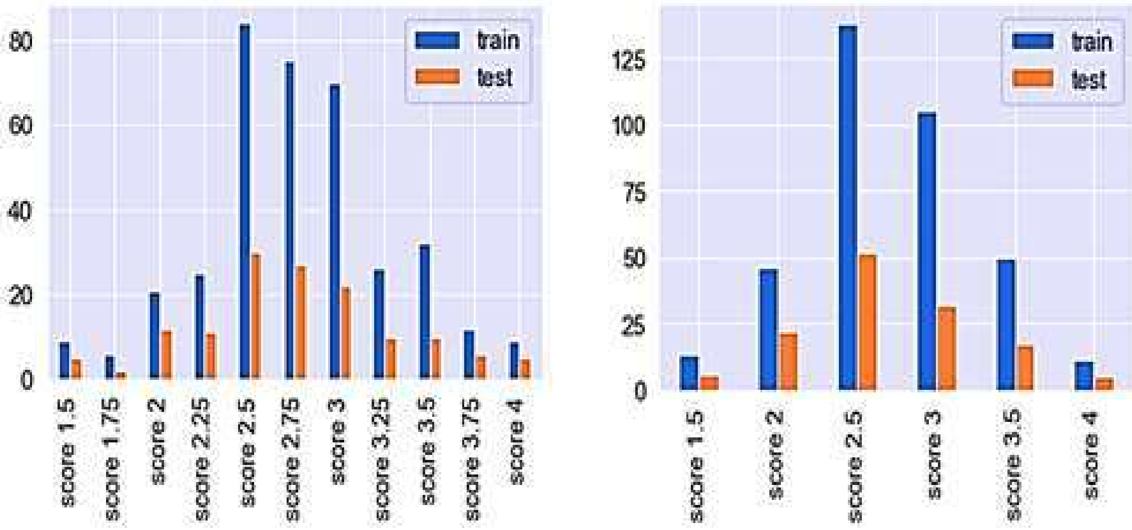


Fig. 1 Percentage distribution of body condition scores over training and test set for (a) model 1 and (b) model 2

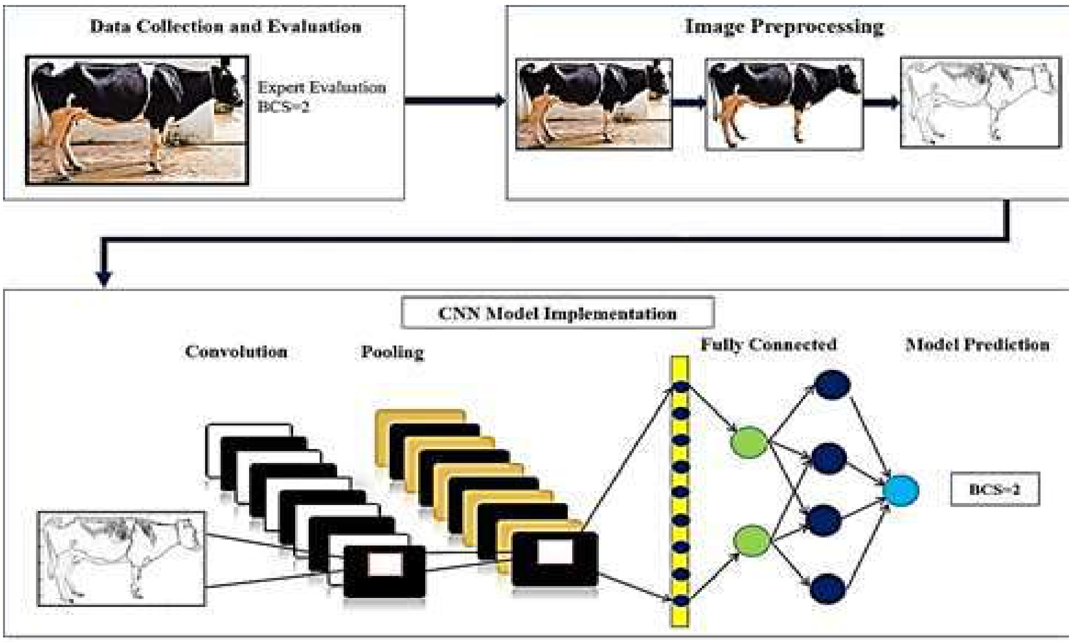


Fig. 2 Overview of the developed convolutional neural network system for estimation of body condition scores

(CuDNN) was used to run the Keras models for superior performance GPU acceleration. CuDNN is a library that offers fine-tuned implementations like forward and backward convolution, normalization, pooling and activation layers (Chetlur et al. 2014).

Background subtraction and fuzzy logic edge detection of images

The segmentation between background and digitally-captured images was subtracted using the GNU image manipulation program (v. 2.10.22, GIMP Development Team, Charlotte, North Carolina, USA). It is an open-source software for performing image-feature tasks such as modifying, organizing and programming images. The background-segmented images thus obtained were converted to edge images using the image processing toolbox in Matlab (v. R2022a, MathWorks, Natick, Massachusetts, USA). A Matlab code was implemented to locate the sharp changes in intensity and to identify the boundaries or contours of the captured images using fuzzy logic edge detection method. In this method, the cow images were converted to grayscale, and then a gradient image was produced using gradient filters. A fuzzy inference system was used to obtain edge images. It used fuzzy set theory, IF-THEN rules, and a fuzzy reasoning process to determine the output corresponding to crisp inputs. The image processing steps for digitally-captured images are shown in Fig. 2. The edge of a digital image is a collection of pixels with change in gray value as well as the area where the brightness of the local area of the image changes significantly.

Edge detection was used as a pre-processing step to obtain low-level boundary features that were passed on to subsequent processing steps like object detection and recognition. The edge-detected images thus obtained were used as input dataset for the development of CNN models.

Model implementation

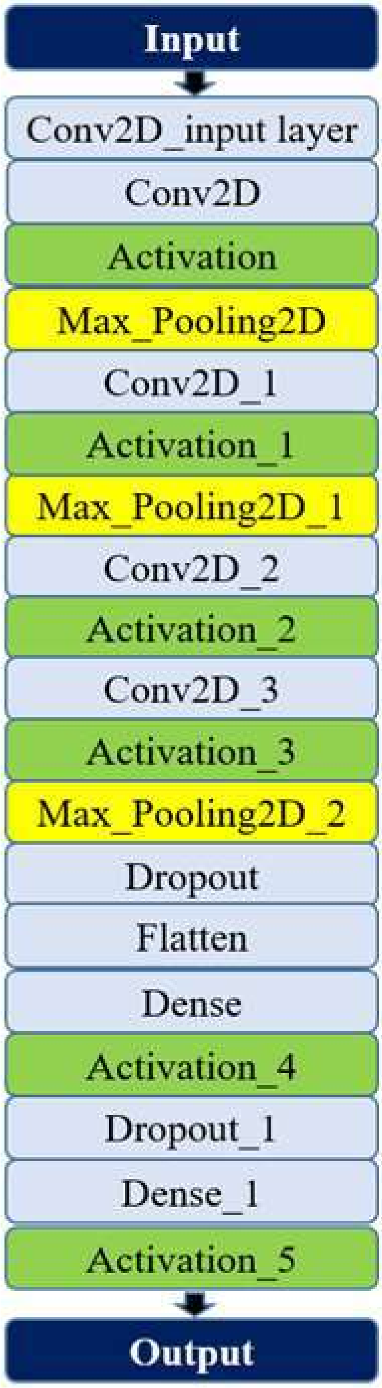
The CNN architecture was designed to evaluate the BCS of the image dataset. Fig. 2 shows the overview of the developed CNN-based BCS estimation system. There were three types of layers in the CNN architecture namely, convolutional, pooling and fully-connected. The convolutional layer computes the convolution operation of the input images using kernel filters to extract various features using spatial filters such as edges, lines and corners. After the convolutional layer, the pooling layer was placed. The pooling layers are effective to minimize the spatial resolution of input volume for the next convolutional layer, making the features robust against noise and distortion (Hasan et al. 2022). The pooling layers do not affect the dimension of volume. The pooling layer applied non-linear down sampling on the activation maps. As reduction in size results in loss of information, the process done by this layer is often referred to as sub-sampling.

Several convolutional and pooling layers are stacked on top of each other to extract more abstract features in the neural network through fully-connected layers. Fully-connected layers contain a variety of classification techniques depending on the structure and type of network. As their name suggests, the neurons in a fully-connected layer have complete connections to all activation functions in the previous layer (Hasan et al. 2022; Hiary et al. 2018). The function of fully-connected layer was to converge the attributes obtained from convolutional and pooling layers to the number of classes the dataset was intended to be classified.

Proposed architecture

The designed CNN architecture used the edge-detected images as input with size of 256×256 px (pixels). The first layer in the CNN applied 32 filters on the input images with size of 3×3 px, producing 32 feature maps of 254×254 px size. The convolved layer then passed through a max-pooling layer of 2×2 px size and produced image size of 127×127 px. The second layer applied 32 filters, each of 3×3 px size, producing 32 feature maps of 125×125 px size and passed through a max-pooling layer of 2×2 px size, producing an image size of 62×62 px. The third layer applied 64 filters, each of 3×3 px size, producing 64 feature maps of 60×60 px size. Subsequently, it passed through the max-pooling layer of size 2×2 px, producing image size of 30×30 px. In this architecture, rectified linear unit (ReLU) function was used as the activation function for both convolutional and fully-connected layers. Dropout was used to prevent overfitting by reducing the correlation between neurons. The architecture of the CNN model for estimation of BCS is shown in Fig. 3.

Fig. 3 Convolutional neural network model for estimation of body condition score



Statistical analysis

The classification performance of each CNN model was assessed using a set of metrics, which measured the accuracy of prediction besides comparing the predictive performance of different models. The confusion matrix, commonly used in classification problems (Çevik, 2020; Tripathi, 2021), is a tool to recognize different classes and show the details of correct and incorrect classification for each class. In a confusion matrix, the rows depict true observations and the columns depict predicted data. The diagonal of the confusion matrix represents the number of correctly predicted objects (Tatbul et al. 2018). In fact, for each class, four possible values can be identified. True-positive (TP) indicated that the true example was positive and the predicted example was positive. True-negative (TN) indicated that the true example was negative and the predicted example was negative. Meanwhile, a false-positive (FP) indicated that the true example was negative and the predicted example was positive. False-negative (FN) indicated that the true example was positive and the predicted example was negative (Tatbul, 2018; Chen et al. 2020). The proposed system was evaluated by the following parameters (Eqs. 1 to 4) that were based on the above factors, and were calculated using the information from confusion matrix.

- Classification accuracy (CA), which is the ratio of the number of true observations to the total number of observations, was calculated using Eq. 1.

$$CA = \frac{(True\,Positive + True\,Negative)}{(True\,Positive + False\,Positive + True\,Negative + False\,Negative)} \quad (1)$$

- Precision (P), which is the ability of the classifier to make positive predictions, was estimated using Eq. 2.

$$P = \frac{TP}{(TP + FP)} \quad (2)$$

- Similarly, recall (R), which is used to detect all positive instances, was determined by dividing number of true positives by the total number of actual positives (Eq. 3).

$$R = \frac{TP}{(TP + FN)} \quad (3)$$

- The last parameter namely, weighted average or harmonic mean of precision and recall (F1-score), was calculated using Eq. 4.

$$F_1 = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}} \quad (4)$$

Results and Discussion

Background subtraction and fuzzy logic edge detection of images

Manual BCS scoring is the traditional method to determine the score of each cow by visual assessment. The images of cows from two viewing angles and their physiological information were compiled. The veterinary experts evaluated the images of these cows and classed them into different BCS, varying from 1 to 5 with increment of 0.25. The digitally-captured images were processed using GIMP software to subtract the background from the images of cows. It was accomplished by using the foreground select tool from the GIMP toolbox. The outline of the cow image was created by choosing each point on the images. Subsequently, the background subtraction methodology was used to obtain processed images without background. The background-subtracted images were used to detect the edges and contours of the cow's body using fuzzy logic edge detection method. A Matlab programme was written to identify the edges and contours of the cow images, and the images with body contours and edges were employed as the training and testing dataset for development of the CNN models.

Convolutional neural network models

Figs. 4 (a) and (b) depict the training and validation accuracy after 10 epochs of training for CNN models 1 and 2, respectively. The training and validation accuracy improved as the number of epochs increased. Similarly, Figs. 4 (c) and (d) depict the training and validation loss after 10 epochs of training for CNN models 1 and 2, respectively. It was observed from Figs. 4 (a) and (b) that the training accuracy of the models was greater than its validation accuracy. This was due to the fact that a model's training accuracy was its performance on examples used in its construction, but its validation accuracy was its performance on unknown data. The rapid increase in accuracy for initial epochs suggested that the model learned quickly. Subsequently, the accuracy curve flattened, indicating that not many epochs were further needed to train the model (Fig. 4 a). Junayed et al. (2021) developed and validated the CNN model, and the findings revealed that training and validation accuracy improved during early epochs and the curve flattened thereafter. In case of model 2 (Fig. 4 b), the training and validation accuracies were improved with number of epochs, suggesting that the model was well-fitted.

Fig. 4 (c) shows that the training loss had a flat line, and it had no chance of learning the training dataset due to insufficient data for extreme BCS classes. In Fig. 4 (d), the validation dataset did not give enough information to assess the ability of model to generalize. This was due to less number of images in the validation dataset as compared to the training dataset. Therefore, the curve for validation loss showed the noisy movements, while the curve for training loss showed linear reduction in loss with increasing number of epochs during training.

Precision of fit of convolutional neural network models

Tables 1 and 2 show the confusion matrix of the test dataset for each model. To consider various error ranges, the confusion matrix was represented by different colour scales. The yellow cells represented accurate predictions, which were consistent with the assessment of experts. The upper triangular portion of the

Fig. 4 Training and validation accuracy for (a) model 1 and (b) model 2 & Training and validation loss for (c) model 1 and (d) model 2

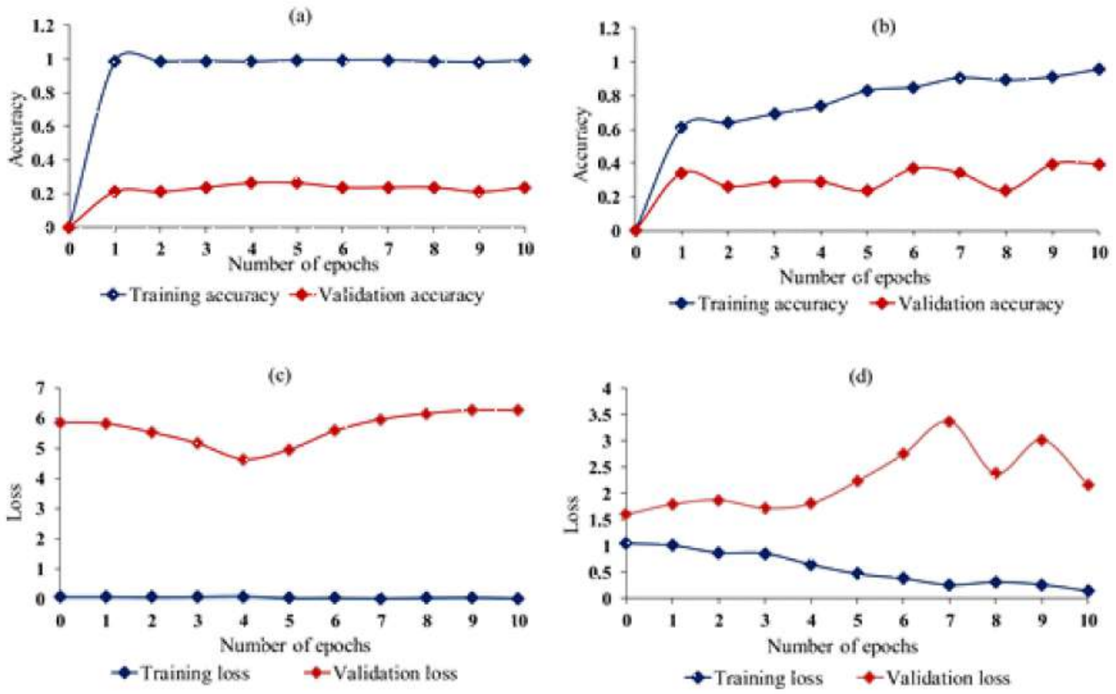


Table 1 Confusion matrix of convolutional neural network model 1

		Confusion matrix										
	1.50	0	0	1	1	0	1	0	0	0	0	0
	1.75	0	1	0	0	0	1	0	0	0	0	0
	2.0	0	0	4	0	6	1	1	0	0	0	0
	2.25	0	0	0	4	3	1	2	0	0	0	1
	2.50	0	0	1	0	19	5	4	1	0	0	0
True class	2.75	0	0	0	0	4	15	6	1	1	0	0
	3.0	0	0	0	0	7	2	12	0	1	0	0
	3.25	0	0	1	0	0	3	2	3	0	1	0
	3.50	0	0	0	0	4	1	2	0	3	0	0
	3.75	0	0	0	0	1	0	0	2	1	1	0
	4.0	0	0	0	0	1	1	1	0	0	0	1
		1.50	1.75	2.0	2.25	2.50	2.75	3.0	3.25	3.50	3.75	4.0
		Predicted class										

Table 2 Confusion matrix of convolutional neural network model 2

		Confusion matrix					
	1.5	0	0	1	1	2	0
	2.0	0	9	11	2	1	0
True class	2.5	0	2	39	8	3	1
	3.0	0	0	13	17	4	0
	3.5	0	0	4	3	11	0
	4.0	0	0	2	2	0	0
		1.5	2.0	2.5	3.0	3.5	4.0
		Predicted class					

confusion matrix were indicative of overestimation by the models for the test dataset, while the lower triangular portion signified underestimation by the models. The green cells represented predictions within 0.25 unit of error, while the light orange cells represented predictions within 0.50 unit of error. This approach allowed simplifying the calculation of remaining parameters by using the values of confusion matrix with different error ranges. Majority of data in the whole dataset were represented along the diagonal of confusion matrix, while the remaining elements were near zero. It could be stated that the optimum condition was not entirely achieved. It was also evident that the BCS between 2 and 3 were more accurately predicted than the extreme BCS values owing to the less number of such extreme BCS values (or images) in the dataset, which made the model difficult to recognize the pattern of such extreme BCS during training (Rodríguez et al. 2018).

Classification accuracy and comparison of convolutional neural network models

Table 3 shows the classification accuracy of each model, which was calculated using the values of confusion matrix within various error ranges. The accuracy of the first CNN model for 0, 0.25 and 0.50 error ranges was 46.32%, 63.23% and 85.29%, respectively. Similarly, the second model achieved classification accuracy of 55.88%, 86.02% and 94.85% for the corresponding error ranges. The accuracy of the second model was relatively better as compared to the first model owing to the bigger BCS range of classification (0.5 instead of 0.25) of the dataset. As the BCS increment of the second model was higher, it enabled the model to differentiate the BCS values easily without much misinterpretation. If the increment between the classes of BCS was lower, it would be tough for the model to recognize and classify the dataset for each range. However, the overall accuracy of the second CNN model was better (94.85%) as compared to manual assessment (78%) in evaluating the BCS. It was observed that the models of this study achieved accuracy levels similar to earlier works for different error ranges. Bewley and Schutz (2008) assessed the BCS by manually marking 23 anatomical features from the rear contour of cows, and achieved an accuracy of 92.79% within 0.25 units of error. Similarly, Spoliansky et al. (2016) did

Table 3 Classification accuracy of Convolutional Neural Network models

Classification accuracy (%)		
Error range	Model 1	Model 2
0 (exact)	46.32	55.88
0.25	63.23	86.02
0.50	85.29	94.85

BCS study using a low-cost camera, and achieved an overall accuracy of 71.35% within 0.25 error range. A recent study employing image analysis model for CNN achieved an accuracy of 78% within 0.25 error range (Alvarez et al. 2018). Yukun et al. (2019) developed a CNN-based BCS system to evaluate the back images of 686 cows, and the model obtained average accuracy of 45%, 77% and 98% within 0, 0.25 and 0.50 error ranges, respectively. From these studies, it could be stated that the method of data collection and feature extraction techniques used in image processing influence the prediction accuracy of BCS.

Tables 4 and 5 show the detailed evaluation of metrics such as precision, recall and F1-score for the two models with different error ranges. The last two rows of each table represent the combined results of the weighted and unweighted average metrics of the models for each class of BCS by considering the distribution in the test dataset. The weighted average was included because of the imbalance in the image dataset within each BCS class. In Tables 4 and 5, the zero values signified that the model could not predict positive samples for the BCS class of 1.5 and 4.0 regardless of the error range. It

Table 4 Classification measures of Convolutional Neural Network model 1

	Error range								
BCS score	0 (Exact)			0.25			0.50		
	Precision	Recall	F1-score	Precision	Recall	F1-score	Precision	Recall	F1-score
1.50	0	0	0	0	0	0	1.00	0.33	0.50
1.75	1.00	0.50	0.66	1.00	0.50	0.67	1.00	0.50	0.66
2.0	0.57	0.33	0.41	0.57	0.33	0.42	0.90	0.83	0.86
2.25	0.80	0.36	0.49	0.88	0.64	0.74	0.89	0.73	0.80
2.50	0.42	0.63	0.50	0.56	0.80	0.66	0.83	0.97	0.90
2.75	0.48	0.55	0.51	0.74	0.78	0.76	0.84	0.96	0.87
3.0	0.30	0.54	0.38	0.58	0.64	0.61	0.85	1.00	0.92
3.25	0.42	0.30	0.35	0.55	0.50	0.52	0.90	0.90	0.90
3.50	0.50	0.30	0.37	0.60	0.30	0.40	0.83	0.50	0.62
3.75	0.50	0.20	0.28	0.66	0.40	0.50	1.00	0.80	0.89
4.0	0.50	0.25	0.33	0.50	0.25	0.33	0.50	0.25	0.33
Weighted average per class	0.47	0.46	0.43	0.66	0.60	0.60	0.85	0.85	0.84
Unweighted average per class	0.50	0.36	0.39	0.60	0.47	0.51	0.86	0.71	0.75

Table 5 Classification measures of Convolutional Neural Network model 2

BCS score	0 (Exact)			0.25			0.50		
	Precision	Recall	F1-score	Precision	Recall	F1-score	Precision	Recall	F1-score
1.5	0	0	0	0	0	0	1.00	0.25	0.40
2.0	0.82	0.39	0.52	1.00	0.87	0.97	1.00	0.96	0.98
2.5	0.56	0.73	0.62	0.88	0.92	0.90	0.96	0.98	0.97
3.0	0.52	0.50	0.50	0.87	1.00	0.93	0.97	1.00	0.98
3.5	0.52	0.61	0.56	0.70	0.78	0.74	0.86	1.00	0.92
4.0	0	0	0	0	0	0	0.67	0.50	0.57
Weighted average per class	0.56	0.56	0.53	0.82	0.86	0.84	0.95	0.95	0.94
Unweighted average per class	0.47	0.45	0.37	0.58	0.60	0.59	0.91	0.78	0.80

Error range applies to all columns after BCS score.

occurred because in the image dataset these classes had very less data for adequate training and testing of the models.

In F1-score (the harmonic mean of precision and recall), the classes that included more dataset of images in training and are in the middle of the BCS scale (BCSe”2 and BCSd”3), achieved the best outcome. The mean F1-score was 0.50, 0.78 and 0.93 within the error ranges of 0, 0.25 and 0.50, respectively. Conversely, in case of extreme BCS scores (BCS 1 and 4), the F1 score was 0.26, 0.33 and 0.64 within the error ranges of 0, 0.25 and 0.50, respectively. It was also observed from the results of the confusion matrix and classification measures that the models showed difficulty in classifying the dataset of extreme BCS scores. These issues were attributed due to the lack of adequate distribution of such extreme BCS in the image dataset, and the limited ability of the models to extract features related to extreme BCS scores, which resulted in their average performance for that category. However, it is not a major concern as it is not common to have cows in such poor or obese physical condition in a typical dairy farm. Ferguson et al. (2006) and Rodríguez et al. (2018) reported similar difficulties in differentiating extreme BCS of less than 2 and greater than 3. The classes with more training dataset in the middle of the BCS scale showed good predictive results. The predictive performance of the models for extreme range of BCS could be improved by collecting a larger dataset consisting of extreme BCS. About 66% of images in the training dataset had BCS between 2 and 3. This implied that the majority classes (BCSe”2 and BCSd”3) were important in determining the overall accuracy. As a result, the overall accuracy dropped if the majority of classes were incorrectly categorized. On the contrary, if the minority classes (BCS 1 and 4) were mis-classified, the loss of overall accuracy would not increase considerably. Based on the results, it could be stated that the developed models achieved good results for the middle range of BCS scores and provided strength to models, despite the lack of extreme BCS scores in the dataset. Hence, the developed models would perform effectively for commercial dairy farms, which do not commonly have cows with poor or high BCS as they would not be very productive.

Conclusion

Body condition score (BCS) is an efficient tool to monitor the nutritional status of cows. The quality of manual BCS is decided by the assessor's experience and the standard of scoring technique. As a result, incorporating regular high-quality scoring of BCS of individual cows in a commercial farm as a routine procedure of management is challenging. In this study, two CNN models with two different error ranges were used to estimate the BCS of cows using edge detected images for different increments and error ranges of BCS. The classification accuracy of the models ranged between 85 and 95%, depending on the error range selected. The CNN models performed adequately for the middle range of BCS, wherein the data of most cows lied, but did not predict the extreme BCS classes very well. It is not a major concern as farms do not keep cows with such extreme BCS scores. However, there is further scope to make the system robust for all BCS classes by enlarging the image dataset to train and validate the CNN based system.

References

Albornoz RI, Giri K, Hannah MC, Wales WJ (2021) An improved approach to automated measurement of body condition score in dairy cows using a three-dimensional camera system. Anim 12(1): 72

Alvarez JR, Arroqui M, Mangudo P, Toloza J, Jatip D, Rodríguez JM, Mateos C (2018) Body condition estimation on cows from depth images using convolutional neural networks. Comput Electron Agric 155: 12-22

Arel I, Rose DC, Karnowski TP (2010). Deep machine learning-a new frontier in artificial intelligence research. IEEE Comput Intell Mag 5(4): 13-18

Bewley JM, Schutz MM. (2008) An interdisciplinary review of body condition scoring for dairy cattle. Prof Anim Sci 24(6): 507-529

Cao C, Liu F, Tan H, Song D, Shu W, Li W, Xie Z (2018) Deep learning and its applications in biomedicine. Genom Proteon Bioinform (1): 17-32

Caraviello DZ, Weigel KA, Craven M, Gianola D, Cook NB, Nordlund KV, Wiltbank MC (2006) Analysis of reproductive performance of lactating cows on large dairy farms using machine learning algorithms. J Dairy Sci 89(12): 4703-4722

Çevik KK (2020) Deep learning based real-time body condition score classification system. IEEE Access 8: 213950-213957

Chen W, Li Y, Xue W, Shahabi H, Li S, Hong H, Wang X, Bian H, Zhang S, Pradhan B, Ahmad BB (2020) Modeling flood susceptibility using data-driven approaches of naïve bayes tree, alternating decision tree, and random forest methods. Sci Total Environ 701: 134979

Chetlur S, Woolley C, Vandermersch P, Cohen JM, Tran J, Catanzaro B, Shelhamer E (2014) cuDNN: Efficient primitives for deep learning. *ArXiv, abs/1410.0759*

Ferguson JD, Azzaro G, Licitra G (2006) Body condition assessment using digital images. J Dairy Sci 89(10): 3833-3841

Fischer A, Luginbühl T, Delattre L, Delouard JM, Faverdin P (2015) Rear shape in 3 dimensions summarized by principal component analysis is a good predictor of body condition score in Holstein dairy cows. J Dairy Sci 98(7): 4465-4476

Gillund P, Reksen O, Gröhn YT, Karlberg K (2001) Body condition related to ketosis and reproductive performance in Norwegian dairy cows. J Dairy Sci 84(6): 1390-1396

Hasan N, Ibrahim S, Aqilah Azlan A (2022) Fish diseases detection using convolutional neural network (CNN). Int J Nonlinear Anal 13(1): 1977-1984

Heuwieser W, Iwersen M, Gossellin J, Drillich M (2010) Survey of fresh cow management practices of dairy cattle on small and large commercial farms. J Dairy Sci 93(3): 1065-1068

Hiary H, Saadeh H, Saadeh M, Yaqub M (2018) Flower classification using deep convolutional neural networks. IET Comput Vis 12(6): 855-862

Junayed MS, Islam MB, Jeny AA, Sadeghzadeh A, Biswas T, Shah AS (2021) ScarNet: development and validation of a novel deep CNN model for acne scar classification with a new dataset. IEEE Access 10: 1245-1258

LeCun Y, Bottou L, Bengio Y Hafner P (1998) Gradient-based learning applied to document recognition. Proc IEEE 86(11): 2278-2323

Li WY, Shen Y, Wang DJ, Yang ZK, Yang XT (2019) Automatic dairy cow body condition scoring using depth images and 3D surface fitting. In: 2019 *IEEE* Int Conf Unmanned Syst Artif Intell IEEE pp. 155-159. 22-24 **November** 2019. Xi'an, China

Manaswi NK (2018) Understanding and working with keras. In: Deep Learning With Applications Using Python. pp. 31-43, Apress, Berkeley, CA

Rodríguez AJ, Arroqui M, Mangudo P, Toloza J, Jatip D, Rodrígue JM, Teyseyre A, Sanz C, Zunino A, Machado C (2018) Body condition estimation on cows from depth images using Convolutional Neural Networks. Comput Electron Agric 155: 12-22

Song X, Bokkers EAM, Van Mourik S, Koerkamp PG, Van Der Tol PPJ (2019) Automated body condition scoring of dairy cows using 3-dimensional feature extraction from multiple body regions. J Dairy Sci 102(5): 4294-4308

Spoliansky R, Edan Y, Parmet Y, Halachmi I (2016) Development of automatic body condition scoring using a low-cost 3-dimensional Kinect camera. J Dairy Sci 99(9): 7714-7725

Tatbul N, Lee TJ, Zdonik S, Alam M, Gottschlich J (2018) Precision and recall for time series. In: 32nd IEEE Int Conf Neural Netw Inf Process Syst 31, pp. 1920-1930. 3-8 December 2018. Montréal, Canada

Tripathi M (2021) Analysis of convolutional neural network based image classification techniques. J Innov Image Process 3(2): 100-117

Yukun S, Pengju H, Yujie W, Ziqi C, Yang L, Baisheng D, Yonggen Z (2019) Automatic monitoring system for individual dairy cows based on a deep learning framework that provides identification via body parts and estimation of body condition score. J Dairy Sci 102(11): 10140-10151

Zhang K, Zuo W, Chen Y, Meng D, Zhang L (2017) Beyond a gaussian denoiser: Residual learning of deep CNN for image denoising. IEEE Trans Image Process 26(7): 3142-3155

Indian J Dairy Sci 77(3): 252-257
https://doi.org/10.33785/IJDS.2024.v77i03.009

RESEARCH ARTICLE

Optimum model for prediction of daughters pregnancy rate in Holstein Friesian crossbred cattle of Kerala, India

Jamuna Valsalan, Tina Sadan (✉), Kulangara Anilkumar and TV Aravindakshan

Received: 11 February 2023 / Accepted: 21 August 2023 / Published online: 23 June 2024

Abstract: To improve the overall performances of dairy cattle, fertility should be given due weightage along with milk yield in breeding programmes. The study was initiated to genetic evaluate the crossbred cattle of Kerala based on fertility traits especially daughters pregnancy rate (DPR). An optimum model for the prediction of DPR based on standardized Voluntary Waiting Period (VWP) has been developed. Data pertaining to 1180 crossbred cattle sired by 208 Frieswal bulls, spread over a period of 17 years from 2003 to 2020, maintained at different farms of Kerala Veterinary and Animal Sciences University and field centres of ICAR- Field Progeny Testing Scheme were analysed in the study. VWP is standardized as 55 days as cattle inseminated during 55 -63 days after calving had highest milk yield and pregnancy rate. Average DPR in the study was estimated as 0.34 ± 0.01 with coefficient of variation 74.61%. Low heritability (0.092) for DPR was observed compared to 305 days milk yield (MY) (0.170) and AFC (0.172). It also showed unfavourable genetic correlation with MY(-0.119). Seven simple and multiple regression models were developed using all possible combinations of age at first calving (AFC), service period (SP) and milk yield per day of lactation (MY/LL) for the prediction of DPR. It was observed that model containing only SP as independent variable fulfilled four criteria's *viz.* highest R^2, minimum mean sum of squares due to error (MSSe), lowest Aaike information criterion (AIC), Bayesian information criterion (BIC) values and the optimum model developed was DPR = 0.002(309-SP). The study standardized VWP and developed an optimum model for prediction of DPR in crossbred cattle also emphasis the importance of fertility in genetic evaluation and selection criteria of dairy cattle.

Centre For Advanced Studies in Animal Genetics and Breeding, Kerala Veterinary and Animal Sciences University, Thrissur – 680651,Kerala, India

Tina Sadan(✉)
Email: tinasadan@gmail.com; Tel: +91 9400675241

Keywords: Voluntary Waiting Period, Daughters Pregnancy Rate, crossbred cattle, optimum model

Introduction

According to 20[th] livestock census, India possessed 193.46 million cattle comprising of 142.11 million indigenous/non-descript and 51.63 million crossbred/exotic heads (20th Livestock Census All India Report, 2019). Crossbred cattle constitutes more than 93% of cattle population of Kerala state as crossbreeding is the accepted breeding policy for genetic improvement of cattle in the state. Cross breeding with exotic cattle mainly Holstein Friesian breed was under taken in Kerala state since 1960.

Progeny testing programme for Holstein Friesian crossbred bulls was implemented in the state since 1985 by Indian Council of Agricultural Research (ICAR). Semen of Frieswal bulls is being used for breeding of cattle. During past decades, the breeding values for milk production for crossbred cattle of Kerala increased about 450-500 kg. Despite of the considerable increase in milk production, fertility remains low as the conception rate of cattle is only 40-45% (ICAR- Annual Report, 2019). Genetic evaluation of dairy animals in the country is done mainly on the basis of first lactation milk yield only without considering major functional traits *viz.* fertility, may lead to increased generation interval, decreased genetic gain and reduces overall performance of cattle. Most of the developed countries have reversed this situation by estimating breeding values for fertility along with milk yield and including them with appropriate weightage in a multi-trait selection index for estimating the overall breeding objective of dairy farm (USDA, 2019). In 2003, United States Department of Agriculture introduced 21 days pregnancy rate as a direct measure for evaluation of fertility in dairy animals. Pregnancy rate measures the percentage of non pregnant animals that become pregnant during each oestrous cycle. It is calculated as the ratio of number of animals that become pregnant during oestrous cycle (21 days) to the number of non-pregnant animals passed the voluntary waiting period that were eligible for breeding at least 11 days of 21 day oestrous cycle (Van Raden et al. 2004). Voluntary waiting period (VWP) is the initial phase of lactation during which no insemination is carried out varies from breed to breed in different herds. VWP mainly depends on management practices followed during transition period. VWP in various Indian breeds were standardized as 53 days in Karan Fries cattle (Dash et al. 2016) and 63 days in Murrah breed of buffalo (Patil et al. 2014).

Genetic evaluation of bulls based on DPR measures the genetic ability of his daughters to become pregnant in each oestrous cycle. Evaluations are expressed as predicted transmitting ability for pregnancy rate (Van Raden et al. 2004). About 18 countries already evaluated cow fertility based on non-return rate, conception rate, pregnancy rate, interval from calving to first service and days open (Interbull, 2012). A comparison of pregnancy rate with other fertility traits from 14 countries had been conducted by Interbull in 2007 indicated that pregnancy rate is highly correlated with other fertility traits. These correlations indicate that pregnancy rate can be expected to improve fertility in dairy animals (Jorjani, 2007). Norman et al. (2009) reported that bulls of superior genetic merit for DPR had negative relationship with daughter days open, which resulted in longer productive life and reduced culling for reproduction traits. Keeping this in view, the present investigation was made to develop an optimum predictive model for prediction of Daughters Pregnancy Rate in crossbred cattle of Kerala.

Materials and methods

Source of data

In the present study, breeding records of 1180 crossbred cattle, pertaining to first lactation spread over a period of 17 years from 2003 to 2020, were collected from the history-cum-pedigree sheet and milk yield registers maintained different farms of Kerala Veterinary and Animal Sciences University *viz.* University Livestock Farm, Mannuthy; University Livestock Research station, Thiruvazhamkunnu; Cattle Breeding Farm, Thumburmuzhy and different field centres of ICAR- Filed progeny testing scheme, Thrissur. Semen of Frieswal bulls under Indian Council of Agriculture Research- Field progeny Testing scheme is being used for breeding of cattle in the study.

Data editing and Statistical analysis

The major traits considered were age at first calving (AFC), service period (SP), daughter pregnancy rate (DPR) and 305 days or less milk yield (MY). Data were checked for its normality and outliers as well as abnormal records were eliminated. After normalisation of records with mean ± 2SD, 1005 first lactation records of crossbred cows sired by 208 sires were considered for this study. The data were adjusted for significant non-genetic factors *viz.* different periods, seasons, centres and age group at first calving using fixed linear models. Since the data was non-orthogonal, least squares analysis of variance by SPSS V.21 (SPSS Inc., Chicago, IL, USA) was used to estimate the effect of non-genetic factors, and the means were compared using Duncan's multiple range test.

The model considered was as follows: $Y_{ijklm}=\mu+S_i+P_j+A_k+C_l+e_{ijklm}$

where, Y_{ijklm} is the observation of mth crossbred cattle born in the lth centre, kth age group, jth period, and ith season; μ, the overall mean; S_i, the fixed effect of ith season of calving (winter (January-February), summer (March-May), rainy (June-September), and autumn (October-December)); P_j, the fixed effect of the jth period of calving (1 to 15); A_k, the fixed effect of the kth age group of calving (1 to 10); C_l, the fixed effect of the lth centre (1-10) and e_{ijklm}, the random error~NID (0, σ^2e)

Genetic parameters *viz*. heritability, genetic and phenotypic correlations of different traits were estimated with "Average Information" restricted maximum likelihood (AI-REML) procedure with various multi-trait models, using WOMBAT software (Meyer 2008).

Standardization of Voluntary Waiting period

The waiting period (WP) or days to first service is the initial phase of lactation, during which no insemination occurs. It was estimated as

$$WP = DOFAI - DOFC$$

Where, DOFAI is the date of first AI after first calving and DOFC is the date of first calving. The crossbred cattle having a WP of 43, 63, 84, and 105 days were considered for this study. In order to standardize the VWP period, 1005 cattle were classified into four groups based on the minimum WP of 43, 63, 84, and 105 days and they were 341,220, 329, and 115, respectively. Before standardization of VWP, in order to find the significant effect of WP on milk production traits, the group having maximum number of cattle i.e 43- 62 group was further classified into 3 subclasses with five days class intervals. The major production trait considered was total milk yield per day of lactation length (MY/LL). Averages of MY/LL corresponding to each WP subclasses were also calculated. Voluntary waiting period for crossbred was standardized based on the groups having highest number of observations and having comparatively high MY/LL and DPR.

Estimation of Daughter Pregnancy Rate

Daughter pregnancy rate (DPR) measures how quickly cows become pregnant again after calving. DPRs were estimated as

$$DPR = 21 / (\text{Service Period} - \text{Voluntary Waiting Period (55)} + 11)$$

The constant factor 11 centralizes the measure of possible conception within each 21-day time period (Van Raden et al. 2004). DPR measures expected differences in 21-d pregnancy rate when comparing the daughter of a sire to a population (AIPL, 2013).

Development of optimum model for prediction of DPR in crossbred cattle

Multiple regression analysis was performed using R software version 3.4.3 (https://www.r-project.org/) for the prediction of DPR:

$$Y_i = a + b_1X_1 + b_2X_2 + b_3X_3 + e_i$$

Where, Y_i is the DPR of the i^{th} cattle; a is the intercept, X_1, X_2 and X_3 are AFC, SP and MY/LL respectively; b_1,b_2 and b_3 are the partial regression coefficients of AFC, SP and MY/LL respectively; and e_i is the random error of the i^{th} observation, normally and independently distributed with zero mean and variance (σ^2e). Seven simple and multiple regression models were developed using all possible combinations of the two independent reproduction traits (AFC,SP) and production trait (MY/LL) for the prediction of DPR. The coefficient of determination (R^2), mean sum of squares due to error (MSSe), Aaike information criterion (AIC), and Bayesian information criterion (BIC) were used for predicting the optimum model for DPR of crossbred cattle. The best model to predict DPR was the model with the lowest AIC value (Akaike, 1974), BIC value (Schwarz, 1978), highest R^2, and minimum mean sum of squares due to error (Kebede and Gebretsadik, 2010) using various combinations of the traits.

Results and Discussion

The data was adjusted for significant non-genetic factors. The overall least square mean of AFC, DFS, SP and MY were 1052.35±10.68 days, 84.46±0.89 days, 119.80±2.51 days and 2596.07±32.05 kg, respectively. Season of calving had significant effect (P<0.01) on both DFS and SP where, winter and post monsoon were favourable for fertility traits in crossbred cattle of Kerala. Period of calving significantly influenced (P<0.01) all fertility traits and MY in the present study. Age group at first calving had significant effect only on MY (P<0.05). Different centres significantly influenced AFC, SP and FLMY. In the present study DPR had low heritability (0.092 ± 0.029). Heritability of AFC,SP and MY were 0.172 , 0.032 and 0.170 ± 0.094 respectively. Phenotypic (rp), genetic (rg), and residual correlation (re) estimates are detailed in Table 4. MY had negative genetic correlation with DPR (-0.119), AFC (-0.197) and SP (-0.08).

Standardisation of VWP

On the basis of waiting period (WP) observed (days), total cattle were first classified into four classes with an increment of 20 days. Even though higher pregnancy rate was obtained for the class 105-236, practically WP above 85 days cannot be advised for cattle. Therefore, WP group having maximum number of cattle (43-62) was further classified into three classes (Table 1). This was plotted against corresponding mean of MY/LL (litre/day). The WP group (55-62 days) were having maximum number of cattle, highest DPR and highest MY/LL. The crossbred cattle which were inseminated before 55 days after calving (VWP < 55 days) produced relatively lower amount of MY/LL, presented in Table 2 and depicted in Figure 1. Crossbred cattle inseminated during 55–62 days after calving (class-III) produced highest MY/LL and higher pregnancy rate which was 8.56 kg per day and 0.34 , respectively. Therefore, VWP of crossbred cattle was standardized as minimum 55 days and were used for the estimation of daughter pregnancy rate in crossbred cattle of Kerala. The average daughters pregnancy rate (DPR) was estimated as 0.34 ± 0.01 with coefficient of variation 74.61%. DPR in the study ranged from 0.11 to 0.95.

Table 1: Different levels of Waiting period with corresponding number of animals and DPR

Range of WP	No of Animals	DPR
43-62	341	0.30
63-83	220	0.30
84-104	329	0.35
105-236	115	0.41

WP, Waiting period; DPR, Daughters Pregnancy Rate

Optimum model for prediction of DPR

For prediction of optimum DPR in crossbred cattle, seven regression models were developed using different combinations of AFC, SP, and MY/LL traits as independent traits, presented in Table 3. It was observed that Model II, having SP only as independent variable fulfilled four criteria's *viz.* highest R^2, minimum mean sum of squares due to error (MSSe), lowest AIC value and BIC values. Model VII, incorporating three independent variables AFC, SP, and MY/LL had also comparatively high R^2, low AIC value and BIC values, but MSSe values was higher. Therefore, Model II was considered as best model for prediction of DPR in crossbred cattle of Kerala. The model was made as follows:

$$DPR = 0.002(309\text{-}SP)$$

Comparison of average and predicted value

The average daughters pregnancy rate (DPR) in the study was estimated as 0.34 ± 0.01. Using the optimum model, predicted value was 0.36 ± 0.002. The average error, variance was comparatively low for predicted value than estimated DPR.

Fertility of dairy cattle influences genetic improvement as well as financial stability of herd. Fertility traits like Daughters Pregnancy

Table 2: Different levels of Waiting period with corresponding number of animals, DPR and milk yield

Range of WP	No of Animals	DPR	MY/LL
43-49	8	0.29	7.84
50-54	21	0.30	8.42
55-62	312	0.34	8.56

WP, Waiting period; DPR, Daughters Pregnancy Rate; MY/LL, milk yield per day of lactation

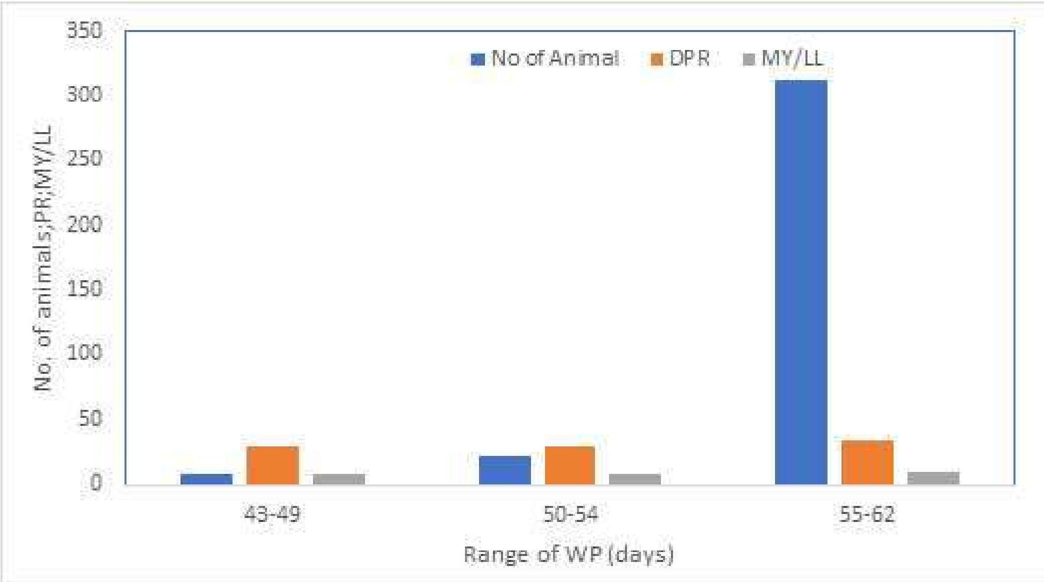


Fig 1. Different levels of Waiting period with corresponding number of animals, DPR and milk yield

rate is highly correlated with all other fertility traits *viz.* service period, non return rate, age at first calving and can improve overall performance of dairy animals (Jorjani, 2007). But selection criteria of elite cattle under field condition of the country are mainly carried out through conventional selection criteria like phenotypic information of animal related to production traits only. It results in declining of overall performance of crossbred cattle, as there exists negative association between production and fertility traits. However, for genetic evaluation of dairy animals, majority of the developed nations have incorporated important performance traits like fertility based on pregnancy rate (Van Raden et al. 2007; De Vries, 2010). In the present study, performance of crossbred cattle was assessed through major reproduction traits with greater economic impact like daughter pregnancy rate (DPR) and standardised VWP on the basis of performance of animals in terms of MY/LL to obtain a better DPR of crossbred cattle of Kerala.

The least-squares means of fertility traits *viz*. age at first calving (AFC) and service period (SP) and production traits *viz*. 305 days or less milk yield (MY) were in consonance with the estimates obtained by many other workers (Naicy and Anilkumar, 2009; Reshmi and Stephen, 2010; Kumar et al. 2011; Girimal et al. 2020). Fertility and production traits were significantly influenced by different non-genetic factors and the results indicated the importance of environmental and management effects on the traits and inclusion of major fixed effects *viz.* season, centre, year and herd in the genetic evaluation of traits. The heritability estimates of fertility traits in the present study were low which was comparable with Van Raden et al. (2007) in Holstein Friesian cattle. Reported heritability estimates of 0.17 for MY in the present study is in agreement with study of Rincon et al. (2015) who showed that heritability for MY were 0.16 and 0.15 for Holstein and Jersey cattle respectively. In consistent with observed negative genetic correlation between DPR and MY in the present study Van Raden et al.(2007) also found that genetic association of pregnancy rate with MY in HF cows as “0.20. The antagonistic relationship between the production traits and fertility traits emphasizes the role of fertility traits as indirect variable in the selection criteria of dairy cattle.

For obtaining better pregnancy rate and to improve reproductive efficiency in dairy cattle, a waiting period after calving should be voluntary left by the management. In the present study, VWP of crossbred cattle of Kerala was standardized as 55 days. Fetrow et al. (2007) suggested that after pregnancy for complete uterine involution, resumption of normal ovarian cyclicity and to improve the rate of successful conception in dairy cattle a minimal VWP of 45 to 60 day post-partum is required. In the present study, maximum numbers of cattle were falling in WP group 55-62 days and total milk yield per day of lactation length (MY/LL) was also found to be higher (8.56 kg) for this group. Hence, minimum VWP of 55 days after calving was standardized as VWP in the studied crossbred cattle population. Present result is comparable with VanRaden et al. (2004) and Chang et al. (2007). They also standardized WP as 60 days for obtaining optimum pregnancy rate in Holstein cows. In contrast Norman et al. (2009) reported WP as 85 days in Holstein cows and 86 days in Jersey cows, respectively which is higher than present result. Scanty reports were available regarding the standardization of VWP in Indian cattle. Divya et al. (2014) standardized VWP as 51 days in Karan Fries cattle. Patil et al. (2014) and Sathwara et al. (2020) reported 63 days of VWP is most appropriate for Murrah and Mehsana buffaloes, respectively. To achieve one calf per year goal in the dairy herd, cows should conceive within a period of 85 days post-partum, for which cyclicity should commence as early as 45 days and should be bred by 60 days. In general, a minimal VWP of 50 to 60 days post-partum breeding interval could be recommended allowing for complete uterine involution in crossbred cattle of Kerala.

Daughters Pregnancy Rate of different cattle breeds ranged from 19.1% in Guernsey cattle to 26.4% in Jersey cattle, reported by Lima et al. 2020. Van Raden et al. 2007 reported average DPR in Holstein Friesian and Jersey as 19.1% and 22%, respectively. Average pregnancy rate of Karan Fries cattle, Indian Murrah and Mehsana buffaloes were reported as 33%, 36% and 31%, respectively (Dash, 2014; Valsalan et al. 2014 and Sathwara et al. 2020). A comparison of pregnancy rate with other fertility traits from 14 countries had been conducted by Interbull in 2007 indicated that pregnancy rate is highly correlated with other fertility traits. These correlations indicate that pregnancy rate can be expected to improve fertility in dairy animals (Jorjani, 2007).

Scanty reports were available about the literature reviewing the development of an optimum model for prediction of DPR in dairy cattle. Van Raden et al. 2004 developed a linear model using days open for prediction of DPR in Holstein Friesian cattle, pregnancy rate = 0.25 (233 - days open). Evaluations were expressed as predicted transmitting ability (PTA) for daughter pregnancy rate. He also reported that each increase of 1% in PTA pregnancy rate, resulted a decrease of 4 days PTA days open. Patil et al. 2014 reported the best prediction model for DPR in Indian Murrah buffaloes was DPR = 0.0033(274-SP). Chebel and Veronese, 2020 developed linear between genomic merit DPR and conception rate as GDPR = (0.7793 × GCCR) " 0.3865; and milk yield as GDPR = ("0.001 × GMY) + 2.589. Gobikrusanth et al. 2020 reported that variability of sires PTA for DPR is strongly due to variation in cow conception rate.

Conclusions

In the present study, an optimum model for prediction of cow fertility based on DPR has been developed in crossbred cattle of Kerala. Voluntary Waiting Period of the cattle has been standardized as 55 days corresponding to pregnancy rate and per day milk yield. DPR had low h^2 (0.092) and exhibited negative correlation with milk yield (-0.119), Seven simple and multiple regression models were developed using all possible combinations of age at First Calving (AFC), service Period (SP) and milk yield per day of lactation (MY/LL) for the prediction of DPR. It was observed that model containing only SP as independent variable fulfilled four criteria's *viz*. highest R^2, minimum mean sum of squares due to error (MSSe), lowest Aaike information criterion (AIC), Bayesian information criterion (BIC) values and the optimum model developed was DPR = 0.002(309-SP). The average daughters pregnancy rate (DPR) in the study

Table 3: Estimates of phenotypic (rp), genetic (rg) and residual (re) correlation between the milk yield and fertility traits in crossbred cattle

Estimates	Milk Yield		
	Phenotypic correlation (r_p)	Genetic correlation (r_g)	Residual correlation (r_e)
Age at first calving	-0.009±0.032	-0.197±0.414	0.025±0.077
Service period	0.052±0.032	-0.08*	-0.025±0.068
Daughters pregnancy rate	0.040±0.003	-0.119±0.029	0.108±0.122

*Indicate failure in approximation of standard error (SE)

Table 4: Estimation of intercept, regression coefficient (s), criterion values for judging the optimum model for DPR

Model	Traits	Intercept	AFC	SP	MY/LL	Number of parameters	R^2	MSSe	AIC	BIC
I	AFC		0.008			2	0.6189	0.05	-8393.92	-4193.97
II	SP			0.002		2	0.6695	0.01	-9761.1	-4877.56
III	MY/LL	0.306			0.037	2	0.6170	0.068	-8132.72	-4063.37
IV	AFC,SP	0.634661	0.015	-0.001		3	0.6548	0.057	-7565.2	-4135.33
V	AFC,MY/LL	0.6295	0.004		0.018	3	0.6280	0.063	-7395.2	-4092.82
VI	SP, MY/LL	0.602		-0.002	0.069	3	0.6607	0.074	-7121.8	-4030.44
VII	AFC,SP, MY/LL	0.624	0.008	-0.002	0.043	4	0.6956	0.071	-9288.1	-4439.05

AFC, Age at first calving; SP, Service period, MY/LL, milk yield per lactation length; R^2 coefficient of determination; MSSe Mean sum of square due to error; AIC Akaike information criterion, BIC Bayesian information criterion was estimated as 0.34 ± 0.01. Using the optimum model, predicted value was 0.36 ± 0.002. The average error, variance was comparatively low for predicted value than estimated DPR. The study suggests that a waiting period of 55 days should be voluntarily left by the management for better pregnancy rate and fertility i.e. DPR would be included in the genetic evaluation of crossbred cattle of Kerala.

Conflict of interest

The authors declare that there is no actual or potential conflict of interest that could inappropriately influence in this work.

Acknowledgement

The authors express their sincere gratitude to Vice Chancellor, KVASU for providing all research facilities for the successful completion of the study. This work was supported by the Department of Science and Technology-SERB, Govt. of India [Grant no. ECR/2017/000455] and Director, ICAR- Central Institute of Research on Cattle, Meerut, India.

References

AIPL. Animal Improvement Programs Laboratory, ARS-USDA. 2013. Daughter pregnancy rate evaluation of cow fertility. https://aipl.arsusda.gov/reference/fertility/DPR_rpt.htm.

Akaike H, (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19(6): 716–723

20th Livestock Census All India Report, DAHD & F (2019) Ministry of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India. https://dahd.nic.in/documents/statistics/livestock-census

Annual Report, 2019-20. ICAR- Central Institute for Research on Cattle. https://circ.icar.gov.in/annualreports.php

Chang YM, Recico OG, Weigel KA, Fricke PM (2007) Genetic Analysis of the Twenty-One-Day Pregnancy Rate in US Holsteins Using an Ordinal Censored Threshold Model with Unknown Voluntary Waiting Period. J Dairy Sci 90:1987–1997

Chebel RC, Veronese A (2020) Associations between genomic merit for daughter pregnancy rate of Holstein cows and metabolites postpartum and estrus characteristics. J Dairy Sci10754-10768

Dash SK, Gupta AK, Singh A, Shivahre PR, Panmei A, Singh M (2016) Comparative assessment of sire evaluation by univariate and bivariate animal model for estimation of breeding values of first lactation traits in HF cross cattle. Indian J Anim Sci 86:177-179

De Vries A (2010) Economic improvements of genetic improvements in milk production, reproduction and productive life. Department of Animal Sciences, University of Florida. http://edis.ifas.ufl.edu

Divya P, Avtar S, Alex R (2015) Standardization of voluntary waiting period and evaluation of production and reproduction traits in Karan Fries cows. Haryana Vet 53:113-116.

Evans RD, Wallace M, Shalloo L, Garrick DJ, Dillon P

(2006) Financial implications of recent declines in reproduction and survival of Holstein-Friesian cows in spring-calving Irish dairy herds. Agric Syst 89:165–183

Fetrow J, Sewart S, Eicker S, Rapnicki P (2007) Reproductive health programs for dairy herds: analysis of records for assessment of reproductive performance. In: Current Therapy in Large Animal Theriogenology (2nd edn.). Youngquist, R. and Threlfall, W.R. (edts.). Saunders Elsevier, St. Louis, Mo

Girimal DG, Kumar D, Shahi BN, Ghosh AK, Kumar S (2020) Studies on some reproduction and first lactation milk yield traits in Sahiwal and crossbred Cattle. J Vet Med Anim Sci 3(1): 1019

Gobikrushanth M, Macmillan K, Hipkin D, Colazo MG (2020) The relationships among sire's predicted transmitting ability for daughter pregnancy rate and cow conception rate and daughter's reproductive performance in Canadian Holstein cows. Theriogenology 149: 117-122

Interbull (2012) Routine genetic evaluation for female fertility traits. https:// interbull.slu.se/Female fert/fert-aug12.html)

Jorjani H (2007) International genetic evaluations for female fertility traits. Interbull Bull 34: 57–64

Kebede K, Gebretsadik G (2010) Statistical modelling of growth performance data on sheep using mixed linear models. Livest Res Rural Dev. doi: http://www. lrrd.org/lrrd22/4/kefe22080.htm

Kumar A, Gandhi RS, Haile A (2011) Estimation of variance components of milk yield and genetic evaluation of Sahiwal cattle using mixed linear models. Indian J Anim Sci 81(6): 328-329

Lima FS, Silvestre FT, Penagaricano F, Thatcher WW (2020). Early genomic prediction of daughter pregnancy rate is associated with improved reproductive performance in Holstein dairy cows. J Dairy Sci 103:3312-3324

Naicy T, Anilkumar K (2009) Evaluation of ten sire families of crossbred dairy cattle of Kerala based on milk production and milk composition traits. Vet World 2(1):10-12

Norman HD, Wrigh JR, Hubbard SM, Miller RH, Hutchison JL (2009) Reproductive status of Holstein and Jersey cows in the United States. J Dairy Sci 92:3517-3528

Patil CS, Chakravarty AK, Singh A, Kumar V, Valsalan J, Vohra V (2014) Development of predictive model for daughter pregnancy rate and standardization of voluntary waiting period in Murrah buffalo. Trop Anim Health Prod 46:279–284

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reshmi RC, Stephen M (2010) Evaluation of lactation milk yield in crossbred cattle. Indian Vet J 87:363-365

Rincón FJ, Zambrano AJ, Echeverri J (2015) Estimation of genetic and phenotypic parameters for production traits in Holstein and Jersey from Colombia. Revista MVZ Córdoba 20:4962-73

Sathwara RN, Gupta JP, Chaudhari, JD, Parmar G, Prajapati BM, Srivastava AK, Chauhan H, Patel PA, Prajapati MN (2020) Analysis of association between various fertility indicators and production traits in Mehsana buffaloes. Trop Anim Health Prod 2585-2592.

Schwarz GE (1978) Estimating the dimension of a model. Ann Stat 6(2): 461–464

USDA (2019) Daughter pregnancy rate evaluation of cow fertility. https://aipl.arsusda.gov/reference/ fertility/DPR_rpt.htm

Valsalan J, Chakravarty AK, Patil CS, Dash SK, Mahajan AC, Vijay K, Vohra V (2014) Enhancing milk and fertility performances using selection index developed for Indian Murrah buffaloes. Trop Anim Health Prod 46:967-974

Van Raden PM, Sanders AH, Tooker ME, Miller RH, Norman HD, Kuhn MT, Wiggans GR (2004) Development of a national genetic evaluation for cow fertility. J Dairy Sci 87: 2285–2292.

Van Raden PM, Tooker ME, Cole JB, Wiggans GR, Megonigal Jr JH (2007) Genetic evaluations for mixed-breed populations. J Dairy Sci 90:2434-2441

Indian J Dairy Sci 77(3): 258-266
https://doi.org/10.33785/IJDS.2024.v77i03.010

RESEARCH ARTICLE

Anionic mixture supplementation and their impact on hemato-biochemical parameters and post-partum performance of buffaloes under field conditions

Sohan Vir Singh[1](✉), Gaurav Kumar[2] and Rajkumar[3]

Received: 17 October 2023 / Accepted: 15 November 2023 / Published online: 23 June 2024

Abstract: In order to demonstrate the beneficial effect of anionic mixture supplementation to the famers, advanced pregnant (nine months) Murrah buffaloes were selected from adopted village (Kathura, Distt. Sonipat) under Farmer FIRST project. Experimental animals were supplementing anionic mixture (@100gm/day/head) from 21 days before expected date of parturition. Blood samples were collected from the control and experimental group of animals at the start of supplementation of the anionic mixture and after 21 days of parturition. Blood samples were analyzed for hematological and biochemical parameters. Experimental animals were also monitored for the udder development, milk fever, retention of placenta and body condition. The overall mean values of liver enzymes i.e. ALT, AST were numerically lower while the mean values of total protein, Albumin, Globulin, Triglyceride and Potassium were higher during postpartum compared to prepartum period in supplemented than control group. The overall mean values of hematological parameters (Hb and PCV) were found significantly ($P<0.05$) higher in supplemented group than control group during postpartum, whereas no significant difference was observed in RBC and WBC concentration among control and treatment group. Based on the farmer's feedback and physical observations, the development of the udder size was more prominent in supplemented group than control. No case of milk fever was observed in supplemented group of buffaloes. Timely expulsion of placenta was significantly ($P<0.05$) higher in supplemented group of buffaloes than control. Significantly ($P<0.05$) higher milk yield was recorded in supplemented group of buffaloes than control. Glossy skin, shiny hair coat and increase in resistance of diseases were also reported in treatment group of buffaloes. Based on the results of the study, it can be stated that supplementation of anionic mixture is helpful in improving the health and reproductive status of buffaloes.

Keywords: Anionic mixture; Buffaloes; Hemato-biochemical; Murrah; Reproduction

[1]ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

[2]ICAR-CSSRI, Karnal -132001

(✉)Animal Physiology Division, ICAR-National Dairy Research Institute, Karnal- 132001.
Email: Sohanvir.singh@icar.gov.in

Introduction

The productive and reproductive performance of dairy animals is influenced by several environmental factors, such as climate and geography (García et al. 2007). Peripartum disorders negatively influences productive and reproductive performance of dairy animals. Therefore, it is important to consider the characteristics and farming background of that area. Giuliodori *et al.* (2013) reported that peripartum management influences subsequent reproductive performance, which is one of the most important factors influencing efficient dairy management. Milk fever, a metabolic disease, and retention of placenta affects high producing dairy animals just after calving are causing the reduction in milk yield. The major reasons for these diseases are low blood calcium levels in these animals. The Incidence of retention of placenta and milk fever varies 4-5% during second lactation and increases with increasing age. Therefore, there is an economic loss due to investment on veterinary services, medicines and reduction in milk yield. The transition from gestation to lactation requires physiological adaptations by the dairy animals, which can significantly affect the following lactation and subsequent reproduction. Nutrition management during the transition period is challenged by reduced DMI during the late gestation period coupled with a drastic increase in nutrient requirements after calving. One of the most significant challenges involves calcium homeostasis and can result in clinical or subclinical hypocalcaemia. Block (1984) reported that cows experiencing clinical hypocalcaemia during the immediate periparturient period produced 14% less milk than cows with normal serum calcium concentrations. In addition to decreased milk yield, cows that experienced clinical or subclinical hypocalcaemia are at greater risk for developing other metabolic disorders (Curtis et al. 1984). Feeding negative DCAD diets prepartum stimulated calcium absorption and mobilization, thus preventing hypocalcaemia and maintained DMI and improved the post partum milk yield (Block, 1984). Therefore, proper feeding and management during the transitional period may improve postpartum productivity and reproductive performance of dairy cows. Additionally, the incidence of common clinical diseases is closely associated with calving, with the high-risk period being within 21 days after calving. Periparturient period (from 3 weeks before parturition to 3 weeks after parturition) is associated with multiple changes including hormonal and biochemical changes, serum levels of major macro minerals etc. (Singh, 2014). In this regard, metabolic profiling, which refers to the analysis of blood biochemical constituents, is an important tool for detecting metabolic disorders in dairy animals before any clinical manifestations appear (Baèiæ et al. 2007 and Rossato et al. 2001). Naher et al. (2020) reported 30% subclinical hypocalcaemia and 25% subclinical ketosis in crossbred lactating cows. Furthermore, it is important to have baseline data on actual serum concentration of different hemato-biochemical and mineral status during periparturient period that will certainly help to prevent metabolic diseases. This study, therefore, was conducted to study the effect of anionic mixture supplementation on hemato- biochemical parameters, mineral status, incidence of milk fever, retention of placenta, milk yield etc during periparturient period in buffaloes under field conditions.

Materials and Methods

Thirty six pregnant Murrah buffaloes (2-3 parity) were selected from the adopted village (Kathura) of Sonipat District under the Farmer FIRST project and equally divided into two groups i.e. control and treatment. Treatment group of animals were started supplementing Anionic Mixture (@100 gm/day/animal before 21 days of expected date of calving and supplemented till the day of calving. All the experimental animals were fed traditionally as per the availability of feed and fodders at farmer's door step and concentrate mixture @ 3kg/day/animal were also fed. Blood samples from all the experimental animals were collected 21 days before expected date of calving (just before supplementation) and after 21 days of calving. Immediately after collection, blood samples were transported to laboratory at ICAR-NDRI, Karnal in an ice box for hematological and biochemical parameters. Blood samples were centrifuged and blood plasma was separated and stored at -20°C for further analysis of biochemical parameters.

Hematological parameters i.e. red blood corpuscles (RBC), hemoglobin (Hb) and packed cells volume (PCV) was estimated using the standard methods.

Biochemical Parameters

Total plasma protein

Total plasma proteins were estimated using commercial diagnostic kit of Recombigen Laboratories Pvt. Ltd., New Delhi.

Assay principle: The peptide bonds of proteins react with cupric ions alkaline solution to form a colored chelate, the absorbance of which was measured at 578 nm (550-580 nm). The biuret reagent contains sodium-potassium tartrate, which helped in maintaining solubility of this complex at alkaline pH.

Calculation:

$$\text{Total Protein concentration (g/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 6$$

Plasma albumin

Plasma albumin was estimated using commercial diagnostic kit purchased from (Recombigen Laboratories Pvt. Ltd., New Delhi).

Assay principle: Plasma albumin in presence of Bromocresol-Green (BCG) under acidic condition forms green colored complex whose absorbance read at 630nm wavelength. Absorbance at this wavelength was proportional to the albumin concentration in plasma.

Calculation:

$$\text{Albumin (gm/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 3$$

Plasma Globulin

Globulin was calculated using following formula:

Globulin=Total protein - Albumin

Liver enzymes

Aspartate aminotransferase and Alanine aminotransferase was estimated using commercial diagnostic kit (Recombigen Laboratories Pvt. Ltd., New Delhi).

Estimation of minerals in blood plasma

The concentration of minerals (calcium, phosphorus and sodium) in blood plasma was estimated using atomic absorbance spectrometer (AA7000F) in Central laboratory of ICAR- CSSRI, Karnal, Haryana.

For preparation of mineral extract, 1 ml plasma sample was taken and 5 ml of tri-acid mixture was added. The samples were digested using Kelplus- KES 12L R digestion system (Pelican India Ltd., Chennai, India). When the digested samples became clear, the tubes were taken out and the samples were allowed to cool. To the digested samples, a few ml of doubled distilled water (DDW) was added and passed through Whatman filter paper No. 42 and final volume was made to 10 ml. Standard curves were prepared for different minerals. The samples were run under same conditions and concentration of a particular mineral in samples was determined using standard curve.

***Calcium*:** Absorbance of standard and test against blank was recorded at 630 nm (600-650).

Sodium and phosphorus: Absorbance of standard and test against blank was recorded at 589 nm and 766 nm respectively

All the experimental animals were monitored for their health, body condition score and udder development regularly throughout the study period. After calving milk fever, retention of placenta daily milk yield and their composition were also monitored.

The data was statistically analyzed for mean and standard error and significance using SPSS software (version 26.0).

Ethical approval

The experiment was approved and carried out according to the established standards of the Institutional Animal Ethics Committee (IAEC) which was formed in accordance with Article 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules ((IAEC Approval No. 42-IAEC-18-7).

Results and Discussion

Hematological parameters

Red and white blood corpuscles

The results of the hematological parameters i.e. red blood corpuscles (RBC), white blood corpuscles (WBC), hemoglobin (gm %) and packed cell volume (PCV%) have been presented in fig.1. The RBC varied from 6.88 to 8.68 millions/ µl with an overall mean of 7.98 ± 0.16 millions /µl in control group of pregnant buffaloes. The respective values of RBC in treatment group of pregnant buffaloes varied from 6.24 to 9.29 millions/µl with an overall mean values 7.84 ± 0.25 millions/µl, 21 days before the expected date of calving. On 21 days postpartum, the RBC varied from 7.03 to 9.19 millions/µl with an overall mean values 7.84 ± 0.17 millions/ µl in control group of buffaloes whereas the respective values of RBC in treatment group of buffaloes varied from 7.04-9.72 millions/µl with an overall mean values 8.37 ± 0.23 millions/µl (Fig.1). The mean values of RBC found during the present study are within the normal range (5.12-8.54 millions/ µl) as reported by Dhillon et al. (2020) in buffaloes.

The WBC varied from 9.53 to 12.32 thousands/ µl with an overall mean of 10.39 ± 0.22 thousands /µl in control group of pregnant buffaloes. The respective values of WBC in treatment group of pregnant buffaloes varied from 7.70 to 15.30 thousands/ µl with an overall mean values 10.35 ± 0.49 µl, on 21 days before the expected date of calving. On 21 days postpartum, the WBC varied from 8.90 to 13.90 thousands/ µl with an overall mean values 11.11 ± 0.35 thousands/ µl in control group of buffaloes whereas the respective values of WBC in treatment group of buffaloes varied from 7.20-13.40 thousands/ µl with an overall mean values 10.17 ± 0.41 thousands/ µl. Kour et al. (2023) also reported significantly ($p<0.05$) higher values of WBC ($x10^3$/µl) during post partum period compared to pre-partum period in buffaloes.

Hemoglobin and Packed cell volume

The overall mean values of Hb and PCV was 11.01±0.28 gm% and 34.82±0.66% respectively during prepartum period (21 days before expected date of calving) in control group of buffaloes. Whereas, the respective values of Hb and PCV was 11.25±0.24 gm% and 35.94±0.77% respectively in treatment group of buffaloes. The Hb and PCV values varied from 10.80 to 12.80 gm% and 31.60 to 42.10 % with an overall mean value of 11.63±0.35 gm% and 35.97±0.86% respectively during prepartum period in control group of buffaloes (Fig.1). Whereas, the respective values of Hb and PCV varied from 11.00 to 15.40 gm% and 37.00 to 51.30 % with an overall mean values of 12.30±0.33 gm% and 40.15±1.18 % respectively during postpartum period in treatment group of buffaloes. Dhillon et al. (2020) also reported almost similar range of Hb from 8.9 to 14.2 gm% in buffaloes

Liver enzymes

Aspartate transaminase (AST):

The AST activity varied from 233.4 to 309.6 IU/ L with an overall activity of 267.52±5.80 IU/ L in control group of pregnant buffaloes. The respective activity of AST in treatment group of pregnant buffaloes varied from 239.9 to 316.6 IU/ L with an overall mean activity 272.65 ± 5.06 IU/ L, on 21 days before the expected date of calving. On 21 days postpartum, the AST activity ranged from 243.6 to 314.7 IU/ L with an overall mean activity 276.22 ± 5.84 IU/ L in control group of buffaloes whereas the respective activity of AST in treatment group of buffaloes varied from 215.7-297.1 IU/ L with an overall mean activity 263.44±6.07 IU/ L (Fig.2) . The activity of AST was found to be significantly ($P<0.05$) lower during post partum period in anionic mixture supplemented group than control. A significant increase ($p<0.05$) in the mean activity of serum glutamic-oxaloacetic transaminase (SGOT) has been observed in buffaloes of prepartum to postpartum period indicating of stressed hepatic metabolism and pronounced catabolism of body reserves (Kour et al. 2022). Similar findings related to levels of SGOT and SGPT were found by Abdulkareem (2013) and Fiore et al. (2017) . The changes in the concentrations of liver enzymes indicate metabolic disorders with involvement of liver even at sub-clinical levels (Fiore *et al.* 2015).

Alanine aminotransferase (ALT)

On 21 days before the expected date of calving, the ALT activity varied from 47.62 to 78.92 IU/ L with an overall mean of 61.28±2.55

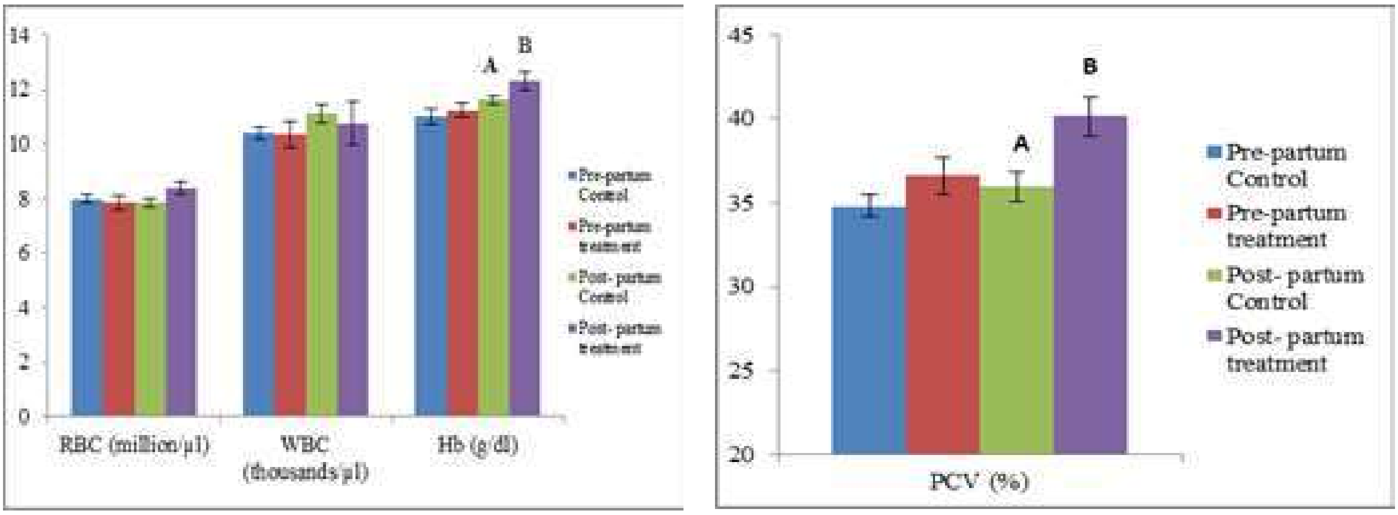


Fig. 1 Effect of anionic mixture supplementation on hematological parameters of periparturient buffaloes

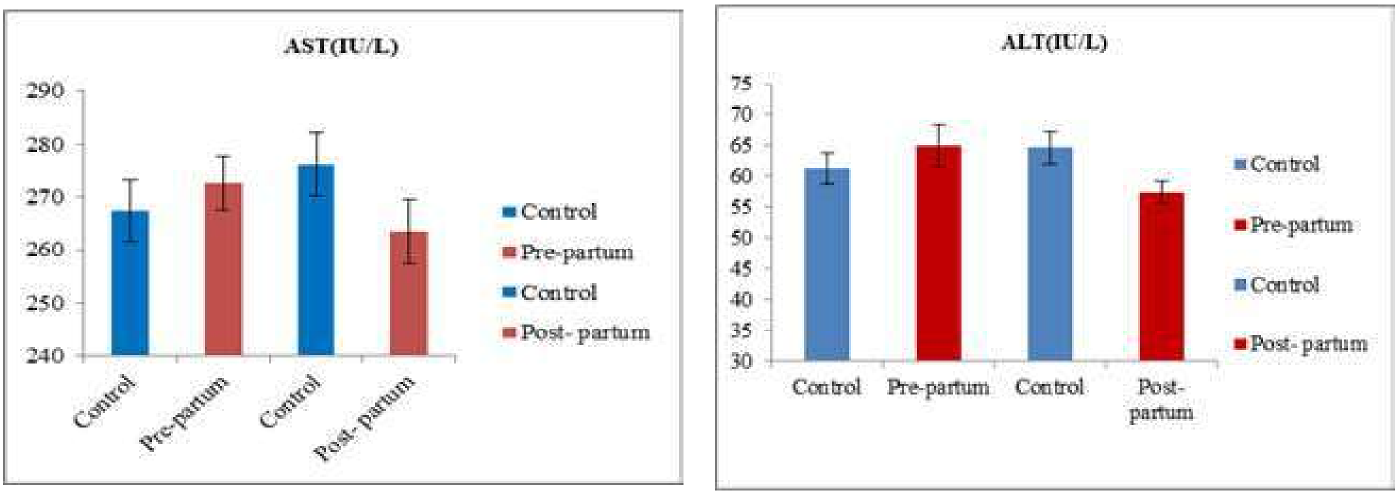


Fig. 2 Effect of anionic mixture supplementation on plasma liver enzymes levels of periparturient buffaloes

IU/ L in control group of pregnant buffaloes. The respective activity of ALT in treatment group of pregnant buffaloes varied from 44.47 to 87.02 IU/ L with overall mean values 64.94±3.41 IU/ L. On 21 days postpartum, the ALT activity varied from 52.64 to 80.54 IU/ L with an overall mean activity of 64.67±2.67 IU/ L in control group of buffaloes whereas the respective activity of ALT in treatment group of buffaloes varied from 43.45-68.62 IU/ L with an overall mean activity of 57.45±1.65 IU/ L (Fig.2) .

The liver as a most important organ in ruminant metabolism and also very sensitive to nutrition fed to the animals. The significant ($P<0.05$) lower activity of AST and ALT in treatment group compared to control group of buffaloes during the present investigation are in accordance with those of Gonzalez et al. (2011) and Kataria and Kataria (2012) who reported that ALT and AST activity in serum/plasma are commonly used as markers of liver damage resulting from metabolic disease or stressors. Higher ALT and AST activities were observed due to the damage of liver, resulting in release of these cellular enzymes into the serum. The ALT and AST activities were increased linearly with decreasing DCAD, suggesting that the cows incurred some degree of liver damage (Gonzalez et al. 2011).

Total Protein

The total protein levels varied from 6.94 to 9.24 g/dl with an overall mean of 8.13±0.19 g/dl in control group of pregnant buffaloes. The respective values of total protein levels in treatment group of pregnant buffaloes varied from 6.44 to 9.66 g/dl with an overall mean values 8.28±0.24 g/dl, on 21 days before the expected date of calving. On 21 days postpartum, the total protein levels varied from 6.96 to 9.62 g/dl with an overall mean values 8.27±0.22 g/dl in control group of buffaloes whereas the respective values of total protein levels in treatment group of buffaloes varied from 7.50 to 9.97 g/dl with an overall mean values

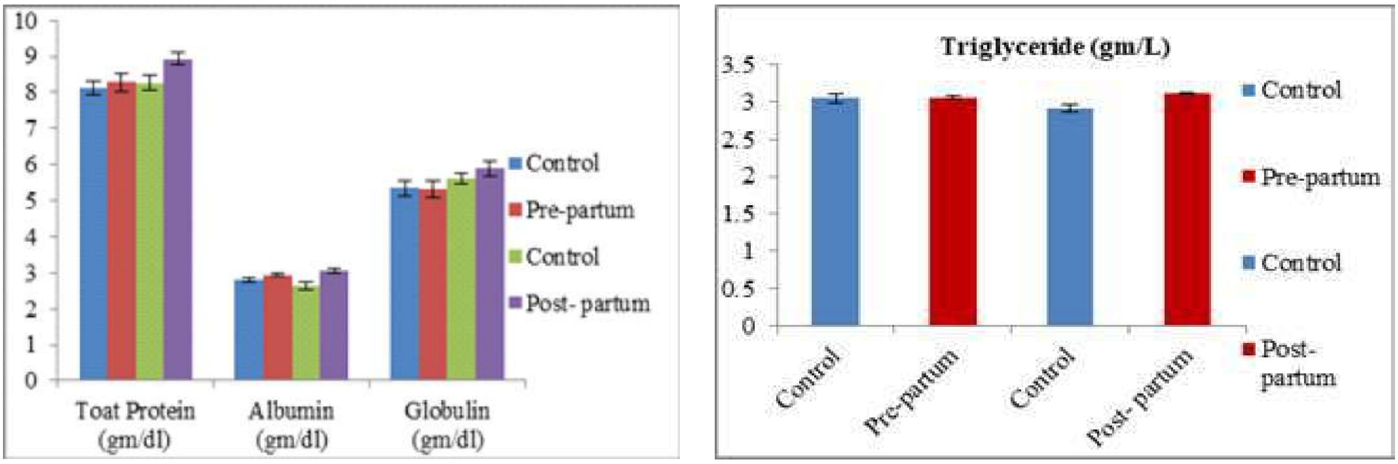
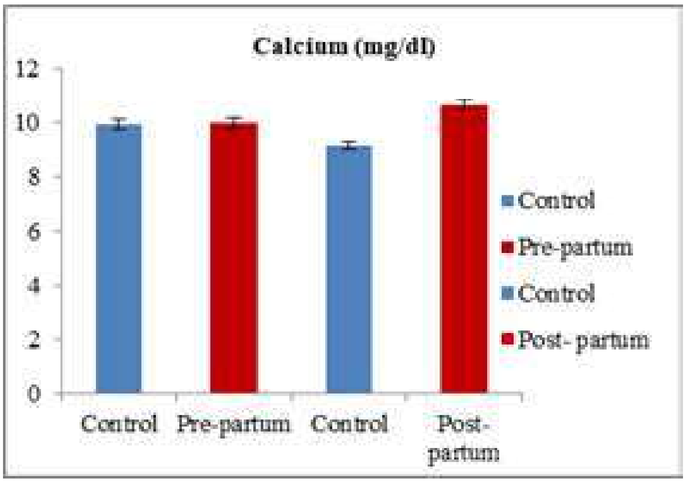
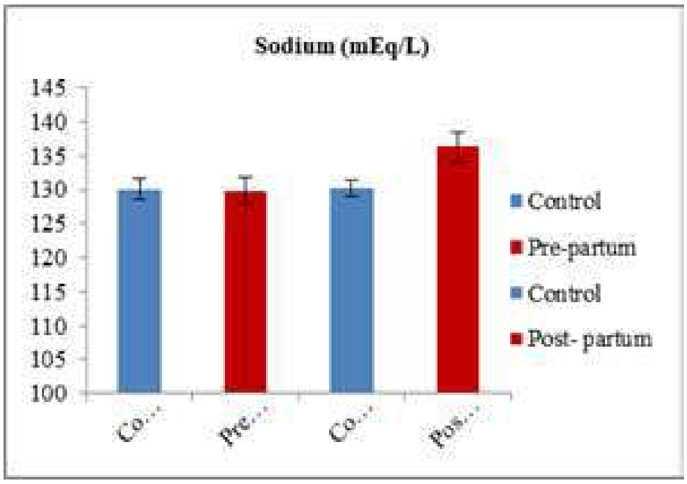


Fig. 3: Effect of anionic mixture supplementation on plasma protein and triglyceride levels of periparturient buffaloes





8.94±0.18 g/dl (Fig.3). The total protein content was non-significantly lower and higher in control and treatment group of buffaloes respectively during post partum than preparation period in buffaloes. Abdulkareem (2013) also found non-significant difference ($p>0.05$) among the mean concentrations of total protein, albumin and globulin in riverine buffaloes during transition period.

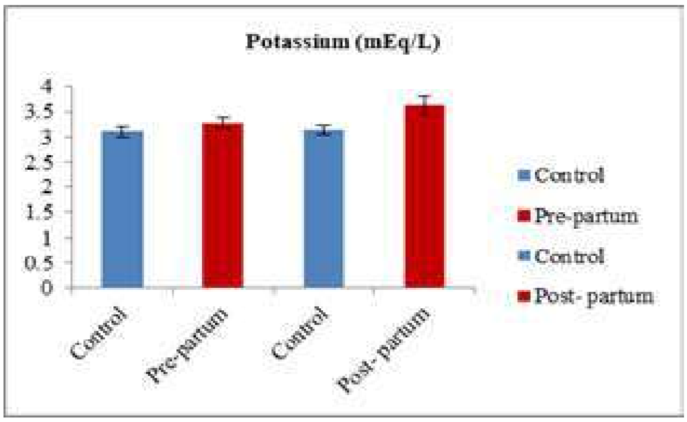


Fig. 4 Effect of anionic mixture supplementation on plasma minerals content of periparturient buffaloes

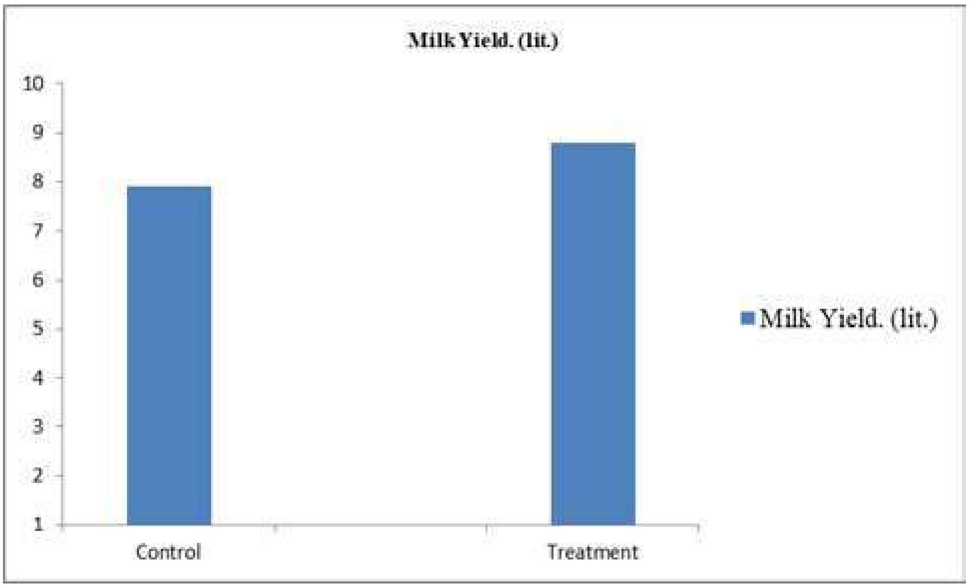
Albumin

On 21 days before the expected date of calving, the albumin content varied from 2.17 to 3.19 g/dl with an overall mean of 2.79±0.07 g/dl in control group of pregnant buffaloes. The respective values of albumin content in treatment group of pregnant buffaloes varied from 2.68 to 3.21 g/dl with an overall mean values 2.95±0.05 g/dl. On 21 days postpartum, albumin content varied from 1.97 to 3.28 g/dl with an overall mean values 2.65±0.11 g/dl in control group of buffaloes whereas the respective values of albumin content in treatment group of buffaloes varied from 2.64 to 3.28 g/dl with an overall mean values 3.04±0.06 g/dl (Fig.3) .

Globulin

The overall mean values of globulin content were 5.34±0.22 and 5.32±0.23 g/dl in control and treatment group of pregnant buffaloes respectively on 21 days before the expected date of

Fig. 5 Effect of anionic mixture supplementation on milk yield of buffaloes



calving. Whereas the respective values of globulin content was 5.61±0.16 and 5.90±0.21 g/dl in control and treatment group of buffaloes respectively during post partum period (Fig.3).

The results obtained during the present investigation i.e. higher albumin content in Anionic mixture supplemented group during post partum period are in accordance with those of Seifi et al. (2005) in dairy cows.

Plasma minerals

Potassium content

Twenty one days before the expected date of calving, the potassium content in blood plasma varied from 2.56 to 4.09 mEq/L with an overall mean value of 3.28±0.10 mEq/L in control group of pregnant buffaloes. The respective values of potassium content in treatment group of pregnant buffaloes varied from 2.59 to 3.80 mEq/L with an overall mean value 3.28±0.10 mEq/L. On 21 days postpartum, potassium content varied from 2.57 to 3.59 mEq/L with an overall mean values 3.23±0.17 mEq/L in control group of buffaloes whereas the respective values of potassium content in treatment group of buffaloes varied from 2.87 to 4.98 mEq/L with an overall mean values 3.63±0.17 mEq/L (Fig.4). No significant difference was observed in potassium content in blood plasma of buffaloes during peripartum period in control and supplemented group. The values of potassium was found during the present investigation are in accordance with those of Dhillon et al. (2020) who reported the reference values of plasma potassium 3.55–5.9 mmol/L in Murrah buffaloes. Mahanrao et al. (2016) also did not find any difference in the potassium content in blood plasma of cows among pre and post partum period. These results of the present study are consistent with those of Tucker et al. (1988b) who observed non-significant effect of altering DCAD levels on plasma K+ concentrations in lactating cows.

Sodium content

Prior to parturition (21 days), the sodium content in blood plasma varied from 123 to 140 mEq/L with an overall mean values of 130.07± 1.59 mEq/L in control group of pregnant buffaloes. The respective values of sodium content in treatment group of pregnant buffaloes varied from 118 to 146 mEq/L with an overall mean values 129.87±2.03 mEq/L (Fig.4). Dhillon et al. (2020) also reported the reference values of plasma sodium of 125.7–164 mmol/L in Murrah buffaloes. On 21 days postpartum, sodium content varied from 123 to 137 mEq/L with an overall mean values 130.27±1.17 meq/L in control group of buffaloes whereas the respective values of sodium content in treatment group of buffaloes varied from 122 to 149 mEq/L with an overall mean values 136.27±2.09 meq/L. The higher plasma sodium content in treatment group of buffaloes during the present study during post partum period are in accordance with those of Mahanrao et al. (2016) who also found positive correlation with dietary DCAD supplemented group and plasma sodium level was found maximum in +31 meq/100 g of DM DCAD fed cows. Tucker et al. (1988b) also reported higher plasma sodium at high DCAD levels. These results are in consistent with previous reports in lactating cows and calves (Tucker et al. (1988b) and buffaloes (Shahzad et al. 2011). The findings of this study are in line with Tucker et al. (1988b) who reported higher plasma sodium at high DCAD levels. The plausible explanation of this might be increased uptake of sodium at high DCAD level. Serum potassium concentration remained unaffected (4.22 to 4.24 meq/L) by DCAD levels.

Calcium content

The calcium content in the blood plasma varied from 8.72 to 11.14 mg/dl on 21 day prior to calving with an overall mean of 9.91±0.20 mg/dl in control group of pregnant buffaloes. The respective values of calcium content in treatment group of pregnant

Photo 1: Effect of anionic mixture supplementation on udder development and body condition of buffaloes under farmer's field

Buffalo without supplementation

Buffalo supplemented with anionic mixture

buffaloes varied from 9.16 to 11.67 mg/dl with an overall mean values 9.98±0.18 mg/dl (Fig.4). On 21 days postpartum, calcium content in the blood plasma varied from 8.29 to 10.06 mg/dl with an overall mean values 9.17±0.14 mg/dl in control group of buffaloes whereas the respective values of calcium content in treatment group of buffaloes varied from 9.49 to 11.88 mg/dl with an overall mean values of 10.66±0.19 mg/dl. The calcium content in blood plasma of supplemented group of buffaloes was significantly ($P<0.05$) higher than control group. The values found during the present study are well within the normal range reported by Dhillon et al. (2020) i.e. 8.72–12.3 mg/dl plasma calcium in Murrah buffaloes. In the periparturient period from advanced pregnancy to early lactation, the clearance of calcium to the placenta ceases, but the lactation calcium demand increases rapidly (Ramberg et al. 1984). The reduction of body fluid's pH by addition of low dietary cation-anion difference (DCAD) diet can help in calcium mobilization (Bushinsky et al. 1993; Schonewille et al. 1994). The results of the present study are in accordance with those of Mahanrao et al. (2016) who reported the higher levels of calcium after parturition in Hariana cows. Blood calcium level was higher for the lowest DCAD (+11 meq/100g of DM), suggesting that low DACD helps in mobilization of stored calcium in periparturient cows. A negative DCAD diet tends to increase the serum calcium, directly by calcium mobilization from bones and indirectly through increased absorption from the intestine due to increased synthesis of 1,25(OH) 2D3 (Block,1984).

Triglycerides (gm/L)

The triglycerides content of blood plasma varied from 2.58 to 3.42 g/dl with an overall mean of 3.04±0.06 g/dl in control group of pregnant buffaloes on 21 day prior to calving. The respective values of triglycerides content of blood plasma in treatment group of pregnant buffaloes varied from 2.81 to 3.11 g/dl with an overall mean values 3.05±0.02 g/dl. The plasma content of triglycerides was almost similar at the start of the experiment. On 21 days postpartum, triglycerides content varied from 2.58 to 3.21 g/dl with an overall mean values 2.90±0.05 g/dl in control group of buffaloes whereas the respective values of triglycerides content in treatment group of buffaloes varied from 2.99 to 3.16 g/dl with an overall mean values 3.11±0.01 g/dl (Fig. 3). The basal values of triglycerides in blood plasma of both the group of buffaloes were higher whereas upper limit remained within the normal physiological range (1.44 – 3.60g/dl) as reported by Mamun et al. (2013) and Cozzi et al. (2011) in cattle.

Reproductive performance

Deficiency of minerals like calcium, magnesium, phosphorus, copper, selenium, zinc, manganese etc. in the blood plasma of dairy animals have been linked with occurrences of milk fever, retained placenta, abortion, dystocia, vaginal prolapse, downer cow syndrome and ultimately reduced reproductive performance (Amen and Muhammad, 2016; Mokolopi, 2019; Yatoo et al. 2018). During the present study, supplementation of anionic mineral mixture to buffaloes enhanced the udder development visibly much faster compared to control group of buffaloes (photo 1). No case of milk fever was reported in anionic mineral mixture supplemented group, whereas two animals from control group showed the symptom of milk fever. The results of Joyce et al. (1997) also showed no case of retained placenta in cows fed with DCAD diets. After parturition, due to higher milk yield and colostrum production in dairy cows and buffaloes, calcium requirement may increase up to 10 times than the dry period (Patel et al. 2011). If this additional requirement of calcium is not fulfilled, calcium deficiency leads to a condition known as hypocalcaemia or milk fever. It is a chronic incident notably observed in high yielding dairy animals. Milk fever is a first step for several other reproductive problems (Buragohain and Kalita 2016). Milk fever is an important production disease occurring most commonly in adult dairy animals within 48-72 hours after parturition, which is characterized clinically by hypocalcemia, general muscular weakness, circulatory collapse and depression of consciousness. Higher risk of mastitis, retained placenta, dystocia, prolapsed uterus, metritis, delayed uterine involution, retained fetal membranes, displaced abomasum, reduced feed intake, poor rumen and intestine motility was reported in the bovines affected with mastitis (Goff 2008; Oetzel and Miller 2012; Reinhardt et al. 2011). During the present investigation, all the buffaloes of supplemented group expelled the placenta in time whereas three animals from control group took more time for expulsion of placenta. Razzaghi et al. (2012) also reported the similar results. Generally expulsion of placenta ranged from 2.5 to 7 hours. In the present study, placenta expulsion time of buffaloes supplemented with anionic mixture was within reported ranges i.e. around 5 hours.

The health status, body condition score and shining of the skin was improved with supplementation of Anionic mixture compared to control group of buffaloes (photo 1). Milk fever is a nutritional disorder which could be prevented with proper feeding of dairy animals during prepartum. The prepartum supplementation of anionic diets prevent milk fever as reported by several researchers (Shahzad et al. 2008; Charbonneau et al. 2006; Lean et al. 2019; Melendez et al. 2019; Wu et al. 2008).

When milk fever results due to imbalance in blood Ca, P and Mg levels, it is known as "Milk fever complex". Generally the milk fever is sporadic but on individual farms the incidence may rarely reach 25-30% of susceptible cows and increases with age and yield. During the present investigation no case of milk fever was observed in the anionic supplemented group of buffaloes.

Milk Yield

The supplementation of anionic mixture is economical for dairy farmers which improves the milk yield (by 10%), fat content of the milk and the immunity of the animals apart from preventing various diseases (Charbonneau et al. 2006). The milk yield of control and anionic mixture supplemented group was recorded weekly and data has been presented in Fig. 5. The average milk yield was 7.90±0.11 litres/day (ranged 6.5 to 9.5 litres/day) and 8.80±0.11 litres/day (7.25 to 10.30 litres/day) in control and supplemented group of buffaloes respectively. Supplementation of anionic mixture prior to 21 days of expected date of calving to buffaloes increased the milk yield by 10.13 % over the control group. Milk yield was found significantly higher ($P<0.05$) in treatment than control group of buffaloes. Cariappa et al. (2021) reported 12% increase in milk yield of buffaloes under field conditions by supplementation of anionic mixture. Tucker et al. (1988b) also reported 9% increase in milk yield of early lactating cows. Moore et al. (2000) also reported that feeding of negative DCAD to periparturient dairy cows proved a useful nutritional practice; it enhanced blood calcium and postpartum milk production. Several other authors also demonstrated that cows fed higher DCAD level produced more milk during early lactation (Hu and Murphy, 2004; Hu et al. 2007a). The potential effect of DCAD on lactating dairy cows has also been explored, and results indicate that DCAD and production are related possibly through acid-base regulation (Hu and Murphy, 2004).

Conclusion

The supplication of anionic mixture to advanced pregnant buffaloes improved the hematological, plasma protein and their fractions, mineral status and lowered the levels of liver enzymes during postpartum compared to prepartum period in supplemented than control group. Udder size was more prominent and no case of milk fever was observed in supplemented group. Higher ($P<0.05$) milk yield was recorded in supplemented group of buffaloes than control. Glossy skin, shiny hair coat and increase in resistance of diseases were also reported in treatment group of buffaloes. Based on the results of the study, it can be stated that supplementation of anionic mixture is helpful in improving the health and reproductive status of buffaloes.

Acknowledgements

The authors would like to thank the Director of ICAR-NDRI, Karnal and Director, ICAR-CSSRI, Karnal for providing the facilities for carrying out the research work. Fund received from ICAR, New Delhi under Farmer FIRST project is highly acknowledged.

References

Abdulkareem TA (2013) Some hematological and blood biochemical attributes of Iraqi riverine buffaloes (*Bubalus bubalis*) around calving and postpartum periods. Al-Anbar J Vet Sci 6: 143-150

Amen MHM, Muhammad SS (2016) Effect of zinc supplementation on some physiological and growth traits in local male rabbit. Vet J 6: 151–155

Baèiæ G, Karadjole T, Maèešiæ N, Karadjole M (2007) A brief review of etiology and nutritional prevention of metabolic disorders in dairy cattle. Vet *arhiv* **77:**567–77

Block E (1984) Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J Dairy Sci 67:2939-2948

Buragohain R, Kalita G (2016) Milk fever: Prevention strategies with special emphasis on nutritional management. CAU Farm Mag 6: 41–43

Bushinsky DA, Wolbach W, Sessler N, Mogilevsky R, Levi-Setti R (1993) Physicochemical effects of acidosis on bone calcium flux and surface ion composition. J Bone Min Res 8: 93–102

Cariappa A A, Chande BS, Sankhala G, Mani V, Sendhil R, Dixit A, Meena B S (2021) Estimation of economic losses due to milk fever and efficiency gains if prevented: evidence from Haryana, India. https://arxiv.org/abs/2105.09782

Charbonneau E, Pellerin D, Oetzel GR (2006) Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows/ : A Meta-Analysis. J Dairy Sci 89: 537–548.

Cozzi G, Ravarotto L, Gottardo F, Stefani AL, Contiero B, Moro L, Brscic, M, Dalvit P (2011) Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. J Dairy Sci 94: 3895–3901

Curtis CR, Erb HN, Sniffen CJ, Smith RD (1984) Epidemiology of parturient paresis: predisposing factors with emphasis on dry cow feeding and management. J Dairy Sci 67:817-825

Dhillon KS, Randhawa CS, Gupta K, Singh RS, Chhabra S (2020) Reference values for haematological and biochemical profile in adult Indian buffaloes. Buffalo Bull 39:145–154.

Fiore E, Barberio A, Morgante M, Rizzo M, Giudice E, Piccione G, Lora M, Gianesella M (2015) Glucose infusion response to some biochemical parameters in dairy cows during the transition period. Animal Sci Papers and Reports 33(2): 129-136.

Fiore E, Giambelluca S, Morgante M, Contiero B, Mazzotta E, Vecchio D, Vazzana I, Rossi P, Arfuso F, Piccione G, Gianesella M (2017) Changes in some blood parameters, milk composition and yield of buffaloes (*Bubalus bubalis*) during the transition period. Anim Sc J 88: 2025-2032.

García-Ispierto I, López-Gatius F, Santolaria P, Yániz JL, Nogareda C, López-Béjar M (2007) Factors affecting the fertility of high producing dairy herds in northeastern Spain. *Therio* 67**:** 632–638

Giuliodori MJ, Magnasco RP, Becu-Villalobos D, Lacau-Mengido IM, Risco CA, de la Sota RL (2013) Clinical endometritis in an Argentinean herd of dairy cows: risk factors and reproductive efficiency. J Dairy Sci 96**:** 210–218

Goff JP (2008) The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet J 176: 50–57

Gonzalez FD, Muino R, Pereira V, Campos R, Benedito JL (2011) Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. J Vet Sci 12:251–255

Hu W, Murphy M R (2004) Dietary cation anion difference effects on performance and acid base status of lactating dairy cows: a meta-analysis. J Dairy Sci 87: 2222 –2229

Hu W, Murphy MR, Constable PD, Block E (2007a) Dietary cation-anion difference and dietary protein effects on performance and acid-base status of dairy cows in early lactation. J Dairy Sci 90:3355–3366

Joyce PW, Joyce WK, Sanchez JP, Goff (1997) Effect of anionic salts in prepartum diets based on alfalfa. J Dairy Sci 80: 2866-2875

Kataria N, Kataria AK (2012) Use of serum gamma glutamyl transferase as a biomarker of stress and metabolic dysfunctions in *Rathi* cattle of arid tract in India. J Stress Physiol and Biochem 8:23–29

Kour S, Sharma N, Kour S (2022).Transition period in dairy animals; physiological and molecular insights. Transition. 10: 237-246.

Kour S, Sharma N, Zul-I-Huma, Ahemd T, Kour S, Pathak AK (2023) Haemato-biochemical Profiling in Buffaloes in Relation to Metabolic Changes during Transition Period. Indian J Anim Res doi10.18805/IJAR.B-4992

Lean IJ, Santos JEP, Block E, Golder HM (2019) Effects of prepartum dietary cation-anion difference intake on production and health of dairy cows/ : A meta-analysis. J Dairy Sci 102: 1–31

Mamun M, Hassan M, Shaikat A, Islam S, Hoque M, Uddin M, Hossain M B (2013) Biochemical analysis of blood of native cattle in the hilly area of Bangladesh. Bangladesh J Vet Med 11**:** 51–56

Melendez P, Zaror V, Gaul P, Poock S E, Goff J P (2019) Effect of diets containing sulfate or chloride-based anionic salts, fed to grazing prepartum dairy cows, on concentrations of Ca in plasma, disease incidence and milk yield. New Zealand Vet J 67**:** 79–85

Mohanrao B A, Kumar V, Roy D, Kumar M, Srivastava M, Gupta V P (2016) Influence of dietary cation-anionic difference on hematobiochemical profile, mineral metabolism, post-partum reproductive and productive performance of Hariana cows. Indian J Anim Res 50: 497-504

Mokolopi B G (2019) Phosphorus, calcium, and magnesium contents of pasture and their effect on body condition scores and body mass of communal cattle depending on natural pasture of Mogosane Village, of the North West Province, South Africa. J Trop Animal Hlth and Prod 51**:** 1295–1787

Moore SJ, Vandehaar MJ, Sharma K, Pilbcam TF, Beede DK, Bucholtz F, Liesman JS, Horst RL, Goff JP (2000) Effect of altering dietary cation-Anion difference on calcium and energy metabolism in peripartum cows. J Dairy Sci 83:2095–2104.

Naher L, Samad M, Siddiki S, Islam M (2020) Prevalence and risk factors of subclinical milk fever and ketosis in lactating cross-bred dairy cows with their therapeutic management in Bangladesh. J Vet Med Res 2:139–182

Oetzel GR, Miller BE (2012) Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds. J Dairy Sci 95: 7051–7065

Patel VR, Kansara JD, Patel BB, Patel PB, Patel SB (2011) Prevention of Milk fever: Nutritional Approach. Vet World 4**:** 278–280

Ramberg CF, Mayer GP, Kronfeld DS, Phang JM, Berman M (1984) Calcium homeostasis in cows, with special reference to parturient hypocalcaemia. Am J Physiol 246:698–704

Razzaghi A. Aliarabi H. Tabatabaei MM, Saki AA, Valizadeh R, Zamani P (2012) Effect of Dietary Cation-Anion Difference during Prepartum and Postpartum Periods on Performance, Blood and Urine Minerals Status of Holstein Dairy Cow. Asian-Australas J Anim Sci 25: 486–495

Reinhardt TA, Lippolis JD, Mccluskey BJ, Goff JP, Horst RL (2011) Prevalence of subclinical hypocalcemia in dairy herds. Vet J 188: 122–124

Rossato W, Gonzalez FH, Dias MM, Riccó D, Valle SF, Rosa VL, Conceição T, Duarte F, Wald V. (2001) Number of lactations affects metabolic profile of dairy cows. Archive Vet Sci 6:83–88

Schonewille JT, Van't Klooster AT, Dirkswager A, Bayen A (1994) Stimulatory effect of an anion (chloride)-rich ration on apparent calcium absorption in dairy cows. Livestock Prod Sci 40: 233–24

Seifi HA, Mohri M, Ehsani A, Hosseini E, Chamsaz M (2005) Interpretation of bovine serum total calcium: Effects of adjustment for albumin and total protein. Comp Clin Pathol 14: 155-159

Shahzad AM, Sarwar M, Mahr-un-Nisa (2008) Influence of varying dietary cation anion difference on serum minerals, mineral balance and hypocalcemia in Nili Ravi buffaloes. Livestock Sci 113**:** 52–61

Shahzad M, Sharif M, Nisa M, Sarwar M, Khalid MF, Saddiqi HA (2011) Changing certain dietary cationic and anionic minerals: Impact on blood chemistry, milk fever and udder edema in buffaloes during winter. African J Biotech 10: 13651-13663

Singh G (2014) Metabolic profiling, therapeutic and prophylactic studies on metabolic diseases in crossbred cows from Punjab [PhD]: Guru Angad Dev Vet. and Animal Sciences University.

Tucker WB, Xin Z, Hemken R W (1988b) Influence of dietary calcium chloride on adaptive changes in acid-base status and mineral metabolism in lactating dairy cows fed a diet high in sodium bicarbonate. J Dairy Sci 71**:** 1587–1597

Wu WX, Liu J X, Xu G Z, Ye JA (2008) Calcium homeostasis, acid-base balance, and health status in periparturient Holstein cows fed diets with low cation-anion difference. *Livestock Sci* 117: 7–14

Yatoo M, Parray O, Ashraf T, Ashraf S, Nisar M, Taifah S (2018) Longitudinal study on evaluation of serum micromineral and hormone interrelation in cattle. J Pharmaco Phytochem 7: 1201–1203

Indian J Dairy Sci 77(3): 267-274
https://doi.org/10.33785/IJDS.2024.v77i03.011

RESEARCH ARTICLE

Time series analysis and ARIMA models for milk yield data

Navneet Kaur and Vinod Kumar(✉)

Received: 24 September 2023 / Accepted: 16 December 2023 / Published online: 23 June 2024

Abstract: The present study is concerned with the time series analysis of milk yield data collected from Instructional Dairy Farm (IDF), Pantnagar, India. ARIMA models have been constructed for milk yields of Crossbred and Sahiwal cows and Murrah buffaloes. It is found that ARIMA models (0,1,1) $(1,1,1)_{12}$ for Crossbred cows, (1,1,0) $(0,1,1)_{12}$ for Sahiwal cows and (0,1,1) $(2,1,0)_{12}$ for buffaloes are more appropriate for predicting milk production.

Keywords: Trend analysis, stationarity, forecasting, white noise, autocorrelation

Introduction

Dairy is a growing industry in India and a large part of the country's population is engaged in dairy production, making it a source of financial benefits to the agricultural population and an important contributor to the country's economy. India ranks first in terms of milk production and milk is the second most important commodity after rice in our country, accounting for 20% of overall production and contributing about 5.3% of India's agricultural GDP (Belhekar and Dash, 2016). The National Dairy Development Board (NDDB) estimates that demand for milk is expected to reach 180 million tons by 2022 and to meet this demand, production must be increased by an average of 5 million tons per year over the next 15 years, otherwise, India will have to depend on milk imports from the world market.

Suresh and Priya (2011) forecasted the sugarcane area, production and productivity of Tamilnadu through fitting of univariate Auto Regressive Integrated Moving Average (ARIMA) models. Using the Augmented Dickey Fuller unit root test, Etuk & Mohamed (2014) found that seasonal series of precipitation data are stationary for one year. To predict future sustainable milk production and its policy implications, Deshmukh and Paramasivam (2016) used vector autoregression (VAR) and autoregressive integrated moving average (ARIMA) models. Model reliability was assessed using the Akaike Information Criterion (AIC), Schwarz-Bays Criterion (SBC), The Mean Absolute Percentage Error (MAPE), R-square and RMSE. Thakur and Gupta (2020) used ARIMA time series (p,d,q) to estimate monthly milk production over time. The study showed that climatic factors and weather conditions were responsible for the year-to-year variation in monthly milk production for all crossbred cows. Kamble et al. (2020) also published their paper on growth and performance of dairy sector in India and emphasized the need for increasing milk production to meet out the needs of the people. Taye et al. (2021) used inference statistics to test hypotheses, estimate trends, fit models and make predictions. They found that milk production was time-dependent and that the average amount of milk produced was decreasing.

Milk production is an integral part of Instructional Dairy Farm (IDF) Nagla, Pantnagar of the College of Veterinary and Animal Science, G.B. Pant University of Agriculture and Technology. Initially, the Tarai State Dairy Farm was established in 1948 with a herd of Murrah buffaloes and Haryana cows. Later, in 1956, Sahiwal cows were also included in the farm. As the population and consequently the demand for milk increased, more cows and buffaloes were introduced to the farm. Therefore, the future milk production trend needs to be monitored so that preventive measures can be taken to meet the future demand and hence the study was undertaken.

Department of Mathematics, Statistics and Computer Science, G.B. Pant University of Agriculture & Technology, Pantnagar-263 145, Uttarakhand, India

Vinod Kumar(✉)
E-mail: vinod_kumarbcb@yahoo.com

Materials and Methods

Data collection

The daily milk yield data of dairy cattle namely crossbred and Sahiwal and Murrah buffaloes for a period of 10 years (2011-2012 to 2019-2020) were collected from the Bureau of records maintained at Instructional Dairy Farm (IDF) Nagla, Pantnagar. Daily data were converted to monthly data and the averaged monthly milk yield data for a period of 2011-2018 were used for the development of the model and data of the period 2019 and 2020 were used for prediction and testing the efficiency of the model.

Tools

Statistical techniques commonly used by researchers, statisticians and meteorologists to assess the past trend and predict the future accordingly, include multiple regression analysis, analysis of variance (ANOVA), auto-regression, moving average, regression analysis and several other advanced techniques available nowadays, such as single-equation regression models, exponential smoothing method, simultaneous equation regression, autoregressive integrated moving average (ARIMA) models, vector autoregression, artificial neural network (ANN), fuzzy logic etc. which are frequently used in data analysis in various fields of science and technology using the latest software like SAS, SPSS, STATISTICA, JMP, EViews, GRETL, Rstudio etc. The present study focuses on ARIMA models for the analysis of milk productivity and forecasts future supply based on past trends to meet the forthcoming demands so that necessary actions can be taken.

Trend analysis

The Mann-Kendall test is a non-parametric test used to observe a trend in a data series, even if the data series has a seasonal component.

The Mann-Kendall test statistic is calculated as follows;

$$\tau = \frac{S}{n(n-1)/2}$$

It ranges from -1 to +1 and corresponds to the correlation coefficient in regression analyses. The null hypothesis of no trend is then rejected if S and τ are significantly non-zero.

Or Compute the M-K test statistic, Z_{MK} as: $Z_{MK} = \frac{S-1}{\sqrt{VAR(S)}}$

The statistical value of the standard Z-test is compared to the normal variance (within ±1.96) in the table at the 5% significance level for the null hypothesis of no trend and the alternative hypothesis of trend.

Stationarity detection by unit root test

KPSS test considers null hypothesis (H_0) that time series is stationary, and tests such as ADF and P-P, for which null hypothesis is on the contrary, is that the series possesses a unit root and data is not stationary. In the interpretation of the KPSS test, if the p-value of its test statistic is greater than the critical value of say 0.463, then we reject the null hypothesis of having a level stationary series and we accept the alternate hypothesis that it has a unit root. The P-P and ADF tests, on the other hand, test for the null hypothesis of unit root against an alternative hypothesis of stationary by rejecting the null hypothesis, if its *p*-value is less than the critical value.

Model-identification

Model-identification can be done by examining the significant spikes of the autocorrelation function (ACF) and partial autocorrelation function (PACF) plots. Software packages like GRETL and SPSS are used for the easy calculations of autocorrelation functions and partial autocorrelation functions.

Estimation of model parameters

By comparison, the residuals of the models with the lowest AIC and BIC values resemble white noise.

AIC= 2m-2 log L

Where, m= p+q (Estimated parameters)

L= the maximized value of the likelihood function of the model.

BIC is calculated as -2 ln(L)+ln(n)k, where L is the likelihood, n is the number of residuals and k is the number of free parameters.

Diagnostic check

The Ljung-Box Q statistic (LBQ) tests the null hypothesis that autocorrelation is zero up to lag k, which means that the data values are random and independent up to a certain number of lags. If the LBQ statistic is greater than a certain critical value, the autocorrelation of one or more lags may deviate significantly from zero, indicating that the values are not random and independent over time. Because the samples are finite, the autocorrelations for each sample may not be exactly zero. White noise ACFs are said to have a sampling distribution that can be approximated by a normal curve with zero mean and standard error. In the white noise process, 95% of all autocorrelation values in the sample must lie within the range defined by the mean ±1.96 standard error. In this case, since the process has a mean of zero and a standard error of about 95% of all autocorrelation values in the sample should be expected to fall within this range.

Ljung-Box test statistic

$$Q = T(T+2)\sum_{K=1}^{m}\frac{\hat{\tau}_K^{\ 2}}{T-K} \sim \chi_m^{\ 2}$$

T= Sample size

τ_K= Autocorrelation at lag K

K= lags, 1st lag, 2nd lag…………

Critical region

If $\chi_m^{\ 2}$ cal $\geq \pm 1.96$ the null hypothesis is accepted, otherwise rejected.

If this condition is not met, the fitted model indeed follows a process with white noise, or the residuals are not white noise.

The quantitative performance of developed models may also be evaluated by applying various statistical indices viz. Root Mean Square Error (RMSE), Coefficient of determination (R^2), Coefficient of efficiency (CE) and Mean Absolute Error (MAE)

Statistical analysis

The time series analysis of milk yield data was carried out by using statistical tools described above and ARIMA models were developed for the milk yield data for different breeds of cows and buffaloes.

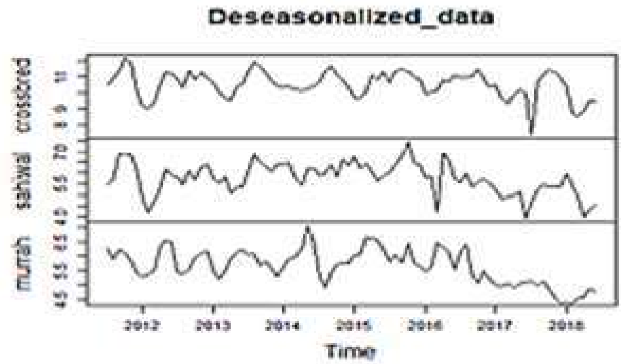


Fig 1: Seasonally differenced time series plot of monthly milk yield

Results and Discussion

The trend component of this time series was confirmed by Mann-Kendall's test. The values of the Mann-Kendall statistical test (τ) were -0.264986,-3.182073e-01 and -5.159664e-01 and Z values were -4.2892, -5.1512 and -8.3539 for Sahiwal and Murrah crosses, respectively. The calculated value was found to be not within the range of +1.00 and -1.00 and +1.96 and -1.96 for the calculated τ and Z value respectively, so the null hypothesis of the existence of a trend in the series was rejected at a 5% significance level. So, the data exhibits a trend component in the correlation function.

When developing any time series model, the first step is to determine stationarity. Since there is a trend in the data series, it is necessary to differentiate them (i.e. d=1). However, the milk production data are not stationary because the data are seasonal, with a periodicity of 12 months, and a seasonal differential is needed to achieve stationarity (d=1). Figure 1 shows a plot of the seasonally differentiated time series of monthly milk production.

Therefore, the long-term value of the trend component was taken for further analysis. Data without trends were used to test for stationarity, so the data still show non-stationarity. To achieve stationarity, seasonal differentiation is performed. After

Table 1: AIC values of the selected models for Crossbred, Sahiwal and Murrah

Crossbred		Sahiwal		Murrah	
Model	AIC	Model	AIC	Model	AIC
ARIMA (0,1,1) $(1,1,1)_{12}$	238.85	ARIMA (0,1,0) $(3,1,2)_{12}$	201.162	ARIMA (0,1,2) $(2,1,2)_{12}$	103.304
ARIMA (0,1,0) $(1,1,1)_{12}$	255.349	ARIMA (3,1,2) $(0,1,0)_{12}$	234.934	ARIMA (0,1,1) $(2,1,0)_{12}$	98.503
ARIMA (1,1,1) $(0,1,0)_{12}$	263.093	ARIMA (0,1,2) $(3,1,0)_{12}$	196.857	ARIMA (0,1,0) $(2,1,4)_{12}$	108.553
ARIMA (0,1,3) $(1,1,0)_{12}$	251.445	ARIMA (1,1,0) $(0,1,1)_{12}$	189.982	ARIMA (2,1,4) $(0,1,0)_{12}$	129.589
ARIMA (1,1,3) $(0,1,0)_{12}$	267.026	ARIMA (1,1,1) $(3,1,0)_{12}$	192.154	ARIMA (2,1,0) $(0,1,4)_{12}$	102.812

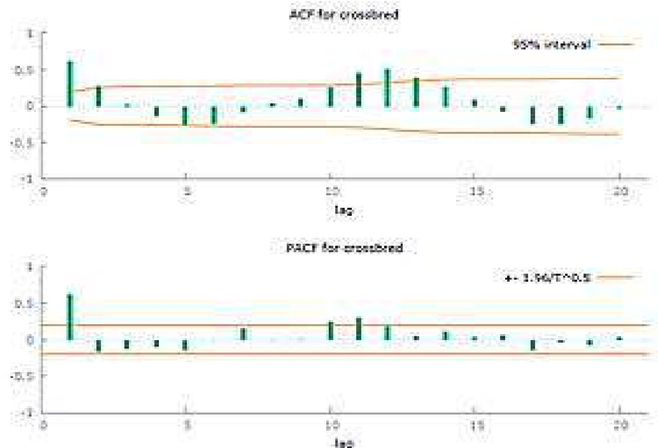


Fig 2: ACF & PACF graphs of de-trended & de-seasonalized data for Crossbred cows

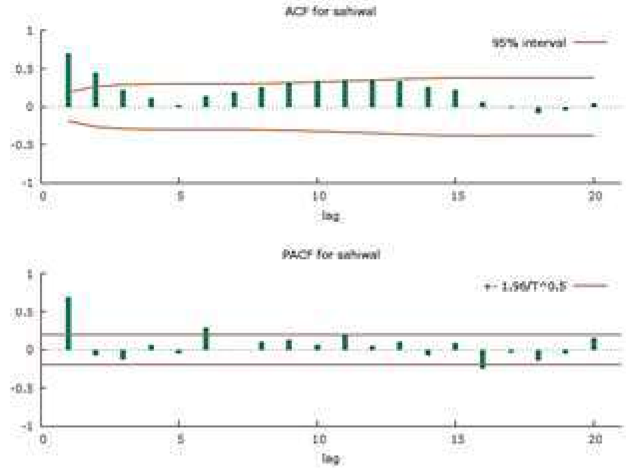


Fig 3: ACF & PACF graphs of de-trended & de-seasonalized data for Sahiwal cows

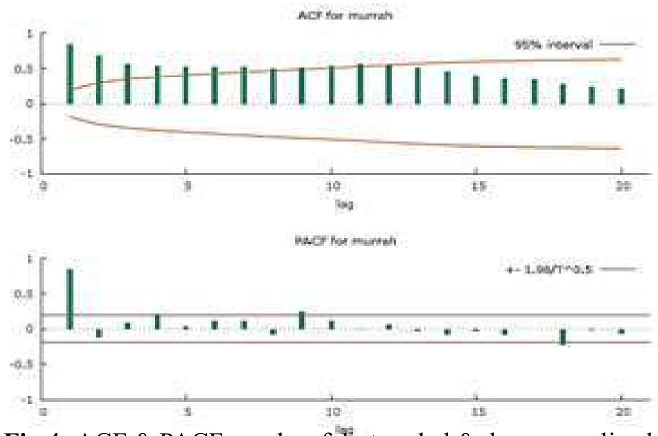


Fig 4: ACF & PACF graphs of de-trended & de-seasonalized data for Murrah buffaloes

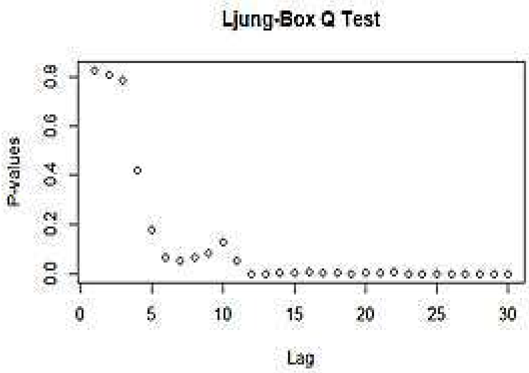


Fig 5: Ljung-Box Q plot for Crossbred

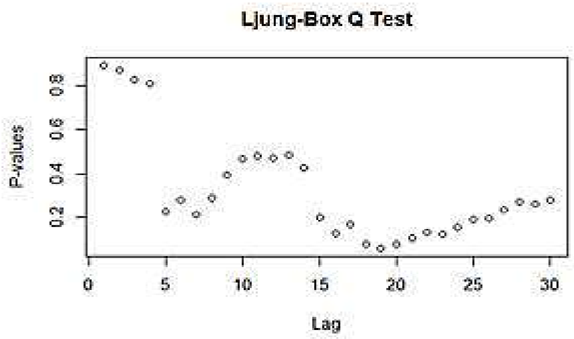


Fig 6: Ljung-Box Q plot for Sahiwal

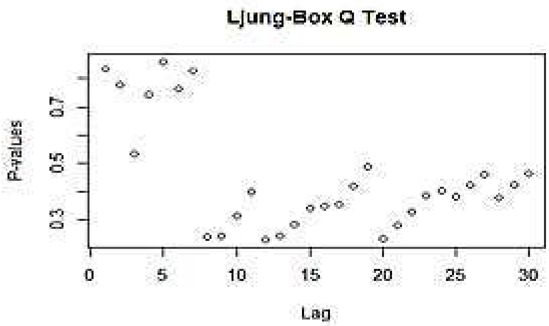


Fig 7: Ljung-Box Q plot for Murrah

Table 2: MAE, RMSE, R^2, MAPE and CE values of Crossbred, Sahiwal and Murrah

Performance indices	Crossbred		Sahiwal		Murrah	
	ARIMA (0,1,1) $(1,1,1)_{12}$		ARIMA (1,1,0) $(0,1,1)_{12}$		ARIMA (0,1,1) $(2,1,0)_{12}$	
	Training	Testing	Training	Testing	Training	Testing
RMSE	0.585	0.664	0.469	0.687	0.330	0.546
MAE	0.416	0.393	0.370	0.414	0.250	0.301
R^2	0.580	0.612	0.649	0.683	0.819	0.725
MAPE	4.04	4.18	6.68	6.01	4.69	4.90
CE	0.654	0.557	0.766	0.620	0.755	0.722

deseasonalization, the data are used again to test for stationarity using unit root tests.

The calculated values of the Augmented Dickey-Fuller (ADF) test for crossbred, Sahiwal and Murrah are 6.84e-010, 4.14e-010 and 2.76e-016, respectively, which are very small than the critical value of 0.05. Also, the critical value of the Kwiatkowski-Phillips-Schmidt-Shini test (KPSS) is 0.462 at the 5% significance level. The calculated p-values are 0.0162417, 0.0583065, and 0.0359997 for crosses, Sahiwal, and Murrah, respectively, which are smaller than the critical range, therefore the null hypothesis is accepted and the critical value of the P-P test is -2.886074 at the 5% significance level. The calculated values of Philips-Perron (PP) test statistics are -17.5211, -15.5328 and -16.4597 for crosses, Sahiwal and Murrah animals respectively, which are not in the critical range. Consequently, the null hypothesis is rejected, which means that the data are stationary.

Tentative models based on the seasonally differenced and non-trended series can be found. Figures 2, 3, and 4 show the ACF and PACF plots of the seasonally differenced and trendless series for crossbred, Sahiwal, and Murrah animals, respectively.

After carefully examining the ACF and PACF plots, the following preliminary models with their Akaike's information criterion (AIC) values were determined. The model with the lowest AIC value is selected and considered the best model.

It is evident from the Table 1 that ARIMA (0,1,1) $(1,1,1)_{12}$ for Crossbred cows, ARIMA (1,1,0) $(0,1,1)_{12}$ for Sahiwal cows and ARIMA (0,1,1) $(2,1,0)_{12}$ for Murrah buffaloes have the minimum value of AIC. Therefore, the ARIMA (0,1,1) $(1,1,1)_{12}$ for Crossbred cows, ARIMA (1,1,0) $(0,1,1)_{12}$ for Sahiwal cows and ARIMA (0,1,1) $(2,1,0)_{12}$ for Murrah buffaloes are found to be the best models for the study.

For the diagnostic check, the Box-Ljung test is applied to the residuals of a fitted ARIMA model, not the original series, and in such applications, the hypothesis being tested is that the residuals from the ARIMA model have no autocorrelation. The p-values for the Ljung-Box Q test all are well above 0.05, indicating "non-significance" (Figures 5, 6 and 7).

The regeneration performance of the ARIMA model was evaluated by the statistical parameters such as mean and standard deviation. The (mean, standard deviation) of regenerated series of Crossbred, Sahiwal and Murrah were found to be (10.37, 0.66), (5.56, 0.63) and (5.40, 0.72) respectively. These values are close to the (10.38, 0.90), (5.59, 0.79) and (5.39, 0.77) of Crossbred, Sahiwal and Murrah respectively of the historical series.

Qualitative evaluation

The observed and predicted values of Milk Yield of Crossbred, Sahiwal and Murrah of IDF Nagla, Pantnagar using the selected ARIMA model for the training period of eight years (2011-2018) are shown in Figures 8, 9 and 10 for Crossbred, Sahiwal and Murrah respectively. Similarly, the observed and predicted values for the testing period of two years (2019-2020) are also presented in the form of bar graphs in Figures 11, 12 and 13 for Crossbred, Sahiwal and Murrah respectively. Based on these figures, it is clear that there is good agreement between the predicted and

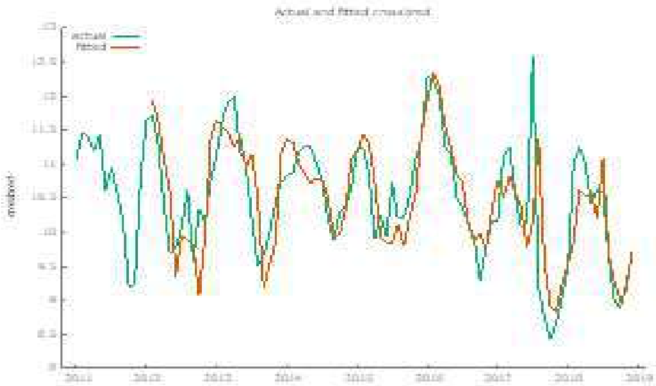


Fig 8: Actual vs Fitted Milk Yield data of Crossbred Cows

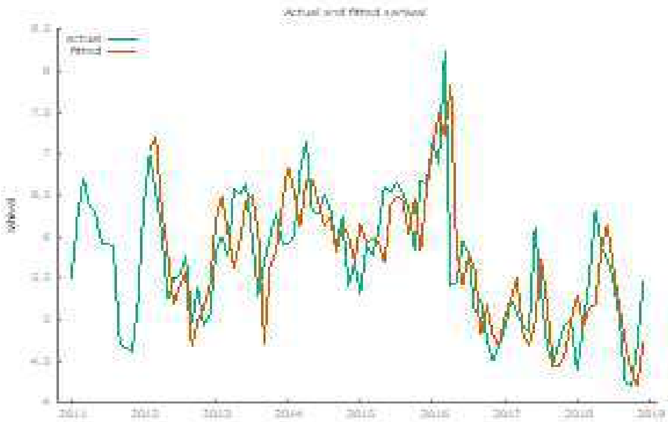


Fig 9: Actual vs Fitted Milk Yield data of Sahiwal Cows

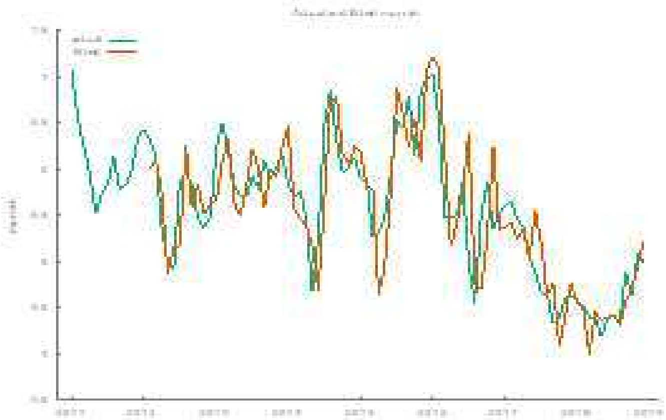


Fig 10: Actual vs Fitted Milk Yield data of Murrah Buffaloes

observed Milk Yield data, and the overall shape of the predicted Milk Yield data of Crossbred is similar to that of the observed Milk Yield.

Quantitative evaluation

For SARIMA based milk yield prediction model, the values of Root Mean Square Error (RMSE), Coefficient of determination (R^2), Coefficient of efficiency (CE), Mean Absolute Error (MAE) and Mean Absolute Percentage Error (MAPE) for Pantnagar during calibration (2011-2018) and validation (2019-2020) periods for the selected models are close to each other.

The developed models were used for the forecasting of monthly Milk Yield data of two years ahead i.e., for the years 2019 and 2020 (Figures 14, 15 and 16).

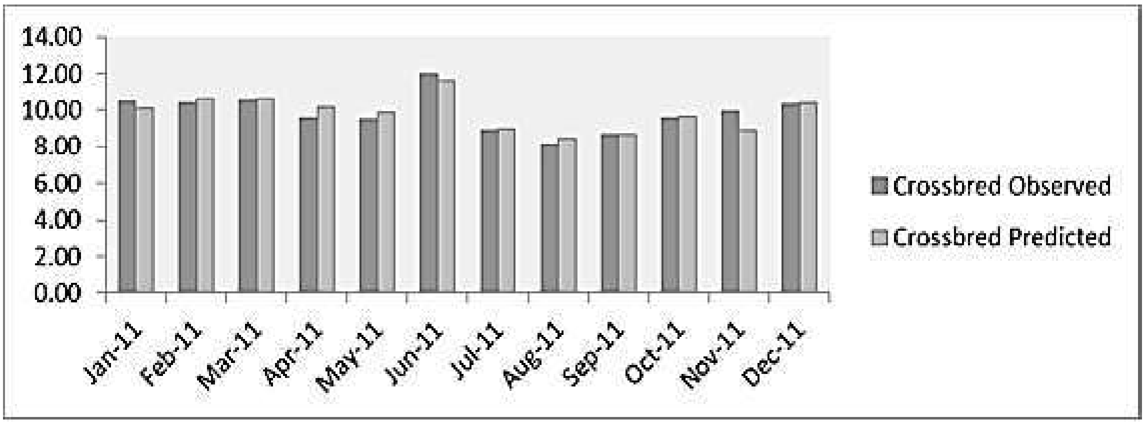


Fig 11: Mean monthly values of historical and regenerated data of Crossbred cow

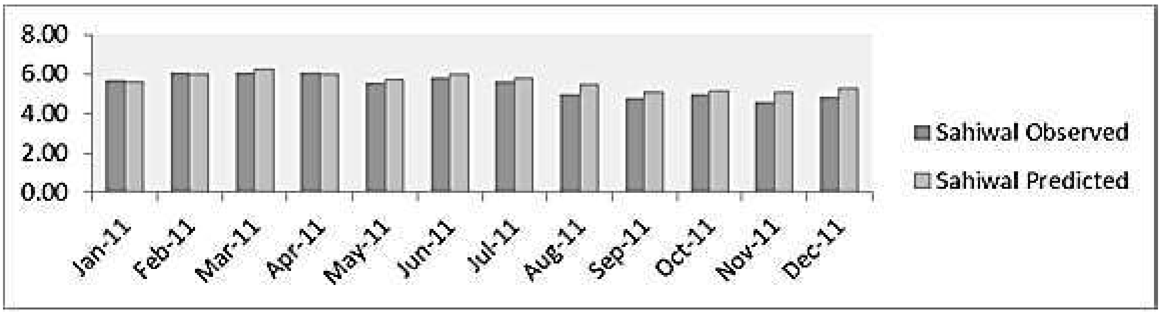


Fig 12: Mean monthly values of historical and regenerated data of Sahiwal cow

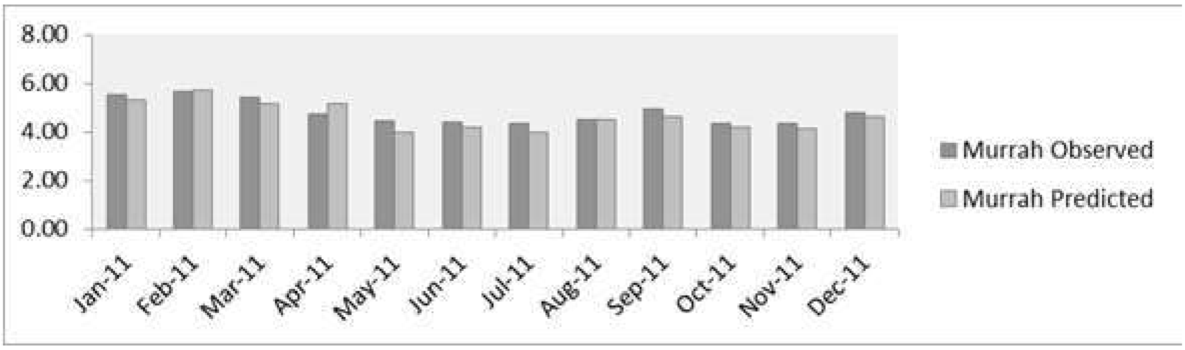


Fig 13: Mean monthly values of historical and regenerated data of Murrah buffalo

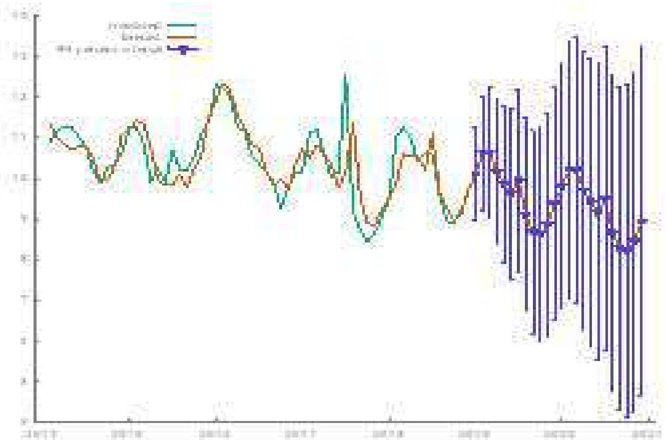


Fig 14: forecasts monthly Milk Yield for the years 2019 and 2020 for Crossbred Cows

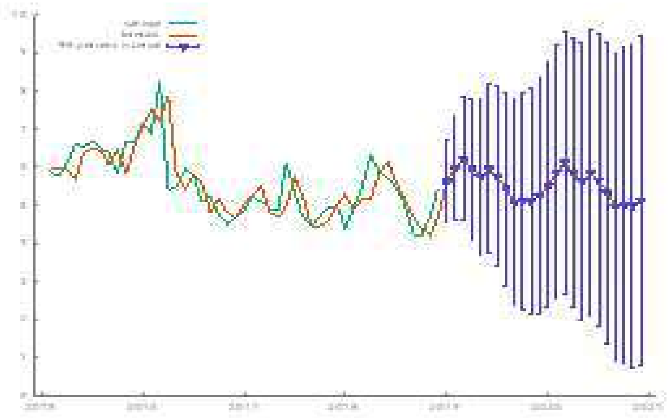


Fig 15: forecasts monthly Milk Yield for the years 2019 and 2020 for Sahiwal Cows

Conclusions

The Auto-Regressive Integrated Moving Average (ARIMA) modelling was carried out by using GRETL, SPSS and Eviews software. The trend component of this time series was confirmed by the Mann-Kendall test. Therefore, the long-term value of the trend component was taken as the unit for further analysis. However, the milk yield data was still non-stationary due to seasonality, as the data had a 12-month periodicity and seasonal differencing (D=1) was required to achieve stationarity.

The seasonally adjusted data were used to check stationarity using unit root tests such as Augmented Dickey Fuller (ADF), Kwiatkowski-Phillips-Schmidt-Shin (KPSS) and Philips-Perron (PP) tests, which showed that the data were stationary. Then, after a thorough examination of the ACF and PACF plots, some tentative models with their Akaike Information Criterion (AIC) values were identified. The models with the lowest AIC value were selected and considered the most appropriate. Thakur and Gupta (2020) used ARIMA time series (p,d,q) to estimate monthly milk production over time and found that ARIMA model (6,0,2) was the best fitted model for prediction of monthly milk yield. However, for the present study we have found different models for different breeds and animals. ARIMA (0,1,1) $(1,1,1)_{12}$ for Crossbred cows, ARIMA (1,1,0) $(0,1,1)_{12}$ for Sahiwal cows and ARIMA (0,1,1) $(2,1,0)_{12}$ for Murrah buffaloes had the lowest AIC value and were considered the most suitable for the study. To verify the applicability of the models, monthly milk yield data for the period 2011-2018 were regenerated. The model also used for short term forecasting or prediction of monthly milk yield for the years 2019-2020.

As discussed above, Taye et al. (2021) estimated trends, fitted models and made predictions, hence, found that milk production was time-dependent and that the average amount of milk produced was decreasing. Similarly for the present study, the milk production for the predicted years (2019-2020) is found to be decreasing with the time as the milk production was time-dependent. This agrees with the findings of Taye et al. (2021).

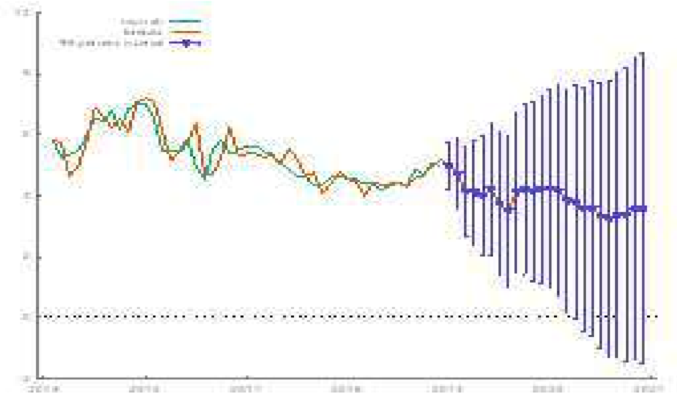


Fig 16: forecasts monthly Milk Yield for the years 2019 and 2020 for Murrah Buffaloes

The performance of developed models was evaluated qualitatively and quantitatively. The quantitative performance of developed models was assessed using statistical indices such as Root Mean Square Error (RMSE), Coefficient of Determination (R^2), Mean Absolute Error (MAE) and Mean Absolute Percentage Error (MAPE).

Acknowledgement

The authors are thankful to the employees and In-charge, IDF, Pantnagar for their help in providing the necessary data for the purpose. The authors are also indebted to the learned referees and Editorial Board of the Indian Journal of Dairy Science for their valuable comments and suggestions which has helped us in improving the quality of this manuscript.

References

Australian Bureau of Statistics. *Time Series Analysis: The Basics.* April 06, 2021
https://www.abs.gov.au/websitedbs/d3310114.nsf/home/time+series+analysis:+the+basics

Belhekar S, Dash S (2016) Role of dairy industry in rural development in India. Indian J Res 5(11): 509-510

Berryman D, Bobée B, Cluis D, Haemmerli J (1988) Nonparametric tests for trend detection in water quality Time Series. J American Water Res Assoc 24(3): 545-556

Box–Jenkins method April 08 (2021) https://en.wikipedia.org/wiki/Box%E2%80%93 Jenkins_method

Department of Statistics *A brief history of time series analysis.* March 21 (2021) https://www.statistics.su.se/english/research/time-series-analysis/a-brief-history-of-time- series-analysis-1.259451

Deshmukh SS, Paramasivam R (2016) Forecasting of milk production in India with ARIMA and VAR time series models. Asian J Dairy and Food Res 35(1): 17-22

Etuk EH, Mohamed TM (2014) Time series analysis of monthly rainfall data for the Gadaref rainfall station, Sudan, by SARIMA methods. Int J Scientific Res Knowledge 2(7): 320

Kamble et al. (2020) “Growth and performance of dairy sector in India”. FnBnews.com. April 16 (2020). http://www.fnbnews.com/Top-News/growth-and-performance-of-dairy-sector-in- india-54646

Mann-Kendall Test for Monotonic Trend. April 04, (2021) https://vsp.pnnl.gov/help/vsample/design_trend_mann_kendall.htm

Prabhakaran S (2014) Augmented Dickey Fuller Test (ADF Test)—Must Read Guide. Machine Learning Plus.

Pranovich M (2021) “Modeling non stationary variables”. Joint Vienna Institute/IMF ICD. April 07 (2021). https://en.ppt-online.org/48550

Sahu PK, Das AK (2009) Agriculture and Applied Statistics-II. New Delhi, Kalyani Publishers. 50 p.

Suresh KK, Priya SK (2011) Forecasting sugarcane yield of Tamilnadu using ARIMA models. Sugar Tech, 13(1): 23-26.

Taye BA, Alene AA, Nega AK, Yirsaw BG (2021) Time series analysis of cow milk production at Andassa dairy farm, west Gojam zone, Amhara region, Ethiopia. Modeling Earth Sys Env 7(1): 181-189.

Thakur R, Gupta RK (2020) Prediction of milk yield using ARIMA. J Entomol Zool Stud 8(3): 1119-1123

Unit Root Tests. April 06, (2021) http://sfb649.wiwi.hu-berlin.de/fedc_homepage/xplore/tutorials/sfehtmlnode53.html#dfstatistik

Indian J Dairy Sci 77(3): 275-281
https://doi.org/10.33785/IJDS.2024.v77i03.012

RESEARCH ARTICLE

Profitability and economic viability of commercial dairy farms in trans-Gangetic plains of India

Arti[1], AK Chauhan[2], Anil Kumar Dixit[3] Chandan Kumar Rai[4] and Bitan Mondal[5](✉)

Received: 20 April 2023 / Accepted: 19 September May 2023 / Published online: 23 June 2024

Abstract: A study on assessing the profitability and economic viability of sixty commercial dairy farms was carried out in the Trans-Gangetic Plains of India. The study's focus region, the Trans-Gangetic Plains, was found pivotal due to its significant dairy orientation and robust dairy development programs. The selected farms were classified into small, medium and large commercial dairy farms based on herd size category. The total cost per farm per year was worked out to be ₹90.66 lakh, ₹ 154.96 lakh and ₹254.62 lakh in small, medium and large commercial dairy farms, respectively. The net returns per farm per year were ₹ 16.10 lakh, ₹52.79 lakh and ₹95.39 lakh in small, medium and large commercial dairy farms, respectively. The net income per income per milch animal per day was ₹ 66.76, ₹130.00 and ₹ 139.13 in ascending order of herd size category. The cost of milk production per litre was estimated as ₹31.50, ₹29.10 and ₹26.97 in small, medium and large commercial dairy farms, respectively. The return per litre was ₹6.10, ₹10.57 and ₹ 11.03 in same order of farm category. Financial assessments of dairy enterprises in this region showed that as farms grow larger, they tend to have a more substantial proportion of long-term debts. The efficiency and profitability ratios indicated that all farm sizes could recover their capital invested at least once in a year. However, medium-sized farms demonstrated a better margin of safety and efficiency in herd management than smaller or larger. The findings suggest the potential for enhancing profitability through value-added milk products and the importance of managing costs for the sustained economic viability of dairy farms. Policy formulation should emphasize capacity building for small farms, promote value addition, and ensure efficient management practices across all farm sizes.

[1*5]Assistant Professor, Department of Agricultural Economics, Institute of Agriculture, Visva-Bharati, West Bengal

[2&3]Principal Scientist, DES&M Division, ICAR-NDRI, Karnal

[4]Assistant Professor, Department of Extension, CSSS PG College Chaudhary Charan Singh University, Meerut, Uttar Pradesh.

Bitan Mondal(✉)
Email: bitan.mondal@visva-bharati.ac.in

Introduction

In the contemporary global milieu, dairy farming is undergoing significant transformations fuelled by shifting economic, demographic, and technological dynamics (Gayathri et al. 2023). This paper aims to evaluate the profitability and economic viability of commercial dairy farms, particularly in the Trans-Gangetic Plains of India, a region pivotal to the nation's dairy sector. Global milk production forecasted to an impressive 935.9 million tonnes in 2022, reflecting a growth of 0.05% (FAO, 2020). The dairy farming landscape, however, is characterized by remarkable diversity due to myriad geographical variations, making generalized assessments challenging.

A closer scrutiny of data reveals that the global number of dairy farms, which stood at approximately 119.60 million in 2016, is on a decline at a rate of 1.00% (Hemme, 2017). This decline, juxtaposed with the International Farm Comparison Network's (IFCN) classification of dairy farms into household, family, and business categories, presents an intriguing trend. Notably, business farms, which house more than 100 cows, are witnessing an annual growth rate of 1.70% from 2010 to 2016, while household farms are diminishing at 0.50% annually (Hemme, 2017).

India, often referred to as 'the oyster of the global dairy industry', leads in milk production with a staggering 221.1 million tonnes in 2021-22 (BAHS, 2022). This dominance is further bolstered by its possession of over 61% of the world's dairy farms (Hemme, 2017). Yet, the average farm size in India remains modest at 1.80 cows per farm in 2016, paling in comparison to dairy-developed nations. As India's dairy sector transitions towards commercialization, there is an imperative to understand the economic implications of this shift, especially in the context of the Trans-Gangetic Plains. This changing economic scenario shows various open challenges and various opportunities to increase milk production through breeding, feeding and scientific management of dairy farms. The success of any enterprise depends upon its management. It is the costs and returns which should be managed properly. The production of milk at the lowest cost, as well as its marketing, is also important on the farm. The determination of economic and cash costs are the key indicators for sustainable dairy farming (Vanchalker, 2005). These costs play a significant role in determining commercial dairy farms' economic viability. Any enterprise's profitability can be increased by either increasing milk production or decreasing production costs. The dairy farm deals with higher daily expenses, so these expenses must be met with the profit which is earned on the farm. Hence, present study was undertaken to find out the profitability and economic viability of various types of farms.

Study Area and Data Collection

The present study was undertaken in Trans-Gangetic Plains of India with an objective of comprehensively analysing the economic viability and profitability of commercial dairy farms. A sample of sixty commercial dairy farms comprising twenty-five farms from Punjab and Haryana, each and ten from two districts of Rajasthan *i.e.*, Ganganagar and Hanumangarh. In the selected sample, commercial dairy farms possessed minimum 50 in-milch animals. The study conducted by the Kumar in 2009 found that the optimum herd size in dairy farms was 54 animals in Haryana. So, our sample farms satisfy the optimum size criterion. This region was chosen as the focal area due to its distinctive prominence in commercial dairy orientation and the widespread existence of dairy development programs. Statistical data further accentuated the region's significance, with states such as Punjab, Rajasthan and Haryana showcasing commendable per capita milk availabilities during the fiscal year 2021-22, with figures standing at 1271g/day, 1150g/day, and 1051g/day, respectively (BAH&S, 2022).

In the research endeavor, a structured personal interview approach was employed to gather comprehensive data from commercial dairy farmers, focusing on predetermined survey schedules. This data encompassed a range of dimensions, including livestock counts, labour inputs, feed and fodder consumption, as well as miscellaneous expenditures like vaccination, artificial insemination and insurance premiums etc. The incurred costs associated with these facets were meticulously computed for commercial dairy enterprises, thereby facilitating the assessment of profitability.

Analytical Framework

Various components of costs and returns were described below:

Fixed Cost

In our research study, the concept of fixed costs encompassed depreciation on assets and the interest associated with fixed capital. To compute fixed costs, we employed standard procedure of the Capital Recovery Cost (CRC) method given by Sirohi et al. (2015).

The useful life of assets was assumed to be 50 years for *pucca* cattle shed, 25 years for tractor, milking machine, bulk milk cooler, and Total Mixed Ration (TMR) wagon, 15 years for fodder harvester, 10 years for *katcha* shed and power operated chaff cutter, and 6 years for manual chaff cutter. The useful life of milch animals also vary with the type of animal (local, crossbred or buffalo). Based on the advice of subject matter specialists, useful productive life of milch animals, defined in terms of age (years) and order of lactation viz. average number of calvings per animal was assumed as 10 years (6 calvings) for local cow and buffalo, and 8 years (5 calvings) for crossbred cow.

Variable Costs

This cost is subject to modification in the short term and pertains to expenses associated with variable factors of production. The primary components of variable costs encompass expenses related to feed and fodder, labour, and various miscellaneous expenditures. These costs are calculated by adopting Sirohi et al. (2015).

In case of Labour cost 1 day of woman labour = 0.67 man day (3 women = 2 men) by considering 8 working hours a day. The cost of family labour was determined on the basis of existing wage rate of permanent farm labour. Gross cost, Net cost, Gross Return, Net return and Cost of milk production were calculated using standard methodology (Sirohi et al. 2015) and presented in the table given in result section.

We also analysed the financial ratios of different farms to know the liquidity, solvency, efficiency and profitability of the farms. We have analysed the farms' ability to meet current to long term financial obligations in liquidity ratio. Standard values of different ratios were presented in Table 1. Efficiency ratios, profitability ratios, Break- even level of herd size, Margin of safety were calculated.

Results and Discussion

The financial assessment of commercial dairy enterprises offers insights into the cost-benefit dynamics, thereby elucidating the profitability of dairy farms. This foundational understanding aids in devising strategies to enhance return on investment while optimizing operational costs (Bhowmik and Sirohi 2008). The

Table 1: Standard values for the ratios

Ratios	Standard values
Current ratio	>2
Debt –equity ratio	<0.5
Net capital ratio	>2
Fixed ratio	<0.5
Gross ratio	<1
Capital turnover ratio	>5

subsequent sections elucidate the findings from this research study.

Categorization of Commercial Dairy Farms

Based on the herd size, commercial dairy enterprises were systematically classified using the cumulative square root frequency method. Sequentially, the number of Standard Animal Units (SAU) across varying dairy farms was observed to be 67-124, 125-203, and 204-382, corresponding to the ascending order of herd categories.

As evident from the Table 2, approximately 53.33% of the sampled dairy enterprises were categorized as small, succeeded by medium-sized farms (30.00%) and subsequently by large dairy enterprises (10.00%). The average per cent of milch animals was highest in medium dairy farms *i.e.,* 73.62 per cent followed by large commercial dairy farms (72.54%) and small dairy farms (68.93%).

The average SAU was registered as 96 SAU, 151 SAU, and 259 SAU for small, medium, and large dairy farms, respectively.

Distribution of commercial dairy farms

The encompassed commercial dairy farms manifested a diversified distribution comprising crossbred farms, buffalo-centric farms, mixed farms, and those hosting indigenous breeds. Remarkably, 48.33 per cent of the farms were dominated by crossbred species, shadowed by indigenous farms (21.67 per cent), mixed breed farms (16.67 per cent), and buffalo-centric farms (13.33 per cent) within the sampled cohort. In small commercial dairy farms, crossbred farms accounted for about 50 per cent, followed by indigenous farms (21.88 %), mixed farms (15.63%) and buffalo farms (12.50%). In contrast, medium-sized dairy enterprises reported 44.44% of farms as crossbred, succeeded by an equal distribution of mixed and indigenous farms (22.22% each) and a smaller fraction of buffalo farms (11.11%).

The crossbred dairy farms contribute about 50 per cent followed by indigenous and buffalo farms (20%) each and mixed farms (10.00 per cent) in large commercial dairy farms. The table 3 showed that there was dominance of crossbred dairy farms followed by indigenous dairy farms in the study area. This observation underscores the emphasis of dairy farms on commercial production, as evidenced by the predominance of crossbred dairy animals on these farms. Notably, the average milk yield in crossbred animals surpasses that of their indigenous counterparts, a finding corroborated by the Basic Animal Husbandry Survey (BAHS, 2022).

Cost and Return from Commercial Dairy Farms

The Table 4 outlines the cost and return from the commercial dairy farms. The fixed costs contributed about 14.83 per cent in large commercial dairy farms followed by small commercial dairy farms (12.92%) and medium commercial dairy farms (12.64%). This may be due to higher level of automation in large commercial dairy farms as compared to other type of farms. The total feed cost was found to be highest in small commercial dairy farms,

Table 2: Categorization of sampled commercial dairy farms

Particulars	Category of Herd Size			
	Small	Medium	Large	Overall
	(67-124)	(125-203)	(204-382)	(67-382)
Dairy farms	32	18	10	60
	(53.33)	(30.00)	(16.67)	(100)
Average SAU /farm	96	151	259	136
Average milch SAU/farm	66	111	188	100

Note: Figures in parentheses specify percentage of row total

Table 3: Distribution of commercial dairy farms according to animal type

Commercial dairy farms	Animal type				
	CB farm	Buffalo farm	Mixed farm	Indigenous Farm	Total
Small	16	4	5	7	32
	(50.00)	(12.50)	(15.63)	(21.88)	(100.00)
Medium	8	2	4	4	18
	(44.44)	(11.11)	(22.22)	(22.22)	(100.00)
Large	5	2	1	2	10
	(50.00	(20.00)	(10.00)	(20.00)	(100.00)
Overall	29	8	10	13	60
	(48.33)	(13.33)	(16.67)	(21.67)	(100.00)

Note: Figures in parentheses indicate the percentage of the row total which accounted for 71.07 per cent, followed by medium (70.91 per cent) and large commercial dairy farms (70.69 per cent). The percentage of in-milk animals was found to be highest in small diary dairy farms as compared to other farms. This reason may be attributed to more feed cost in case of small commercial dairy farms as compared to medium and large farms. The percentage of concentrate cost was highest in small farms (62.82%) followed by large (62.28%) and medium dairy farms (57.49%). Of all the feed types, including green fodder, dry fodder, concentrates, and total supplements, concentrates accounted for the greatest portion of the overall costs which is consistent with past studies conducted by Vishnoi (2014), Kumar, (2015), Lal, (2016), Acharya, (2020) and Naresha, (2022). Labour cost was found to be lowest in large commercial dairy farms. The dependency on labour is less as large farms are capital-intensive. The total cost was around ₹ 9066.445 thousand, ₹ 15495.96 thousand and ₹ 25461.95 thousand in small, medium and large commercial dairy farms, respectively. The return from sale of milk was contributed highest in small dairy farms, followed by medium and large commercial dairy farms and percentage was around 91.12 per cent, 88.40 per cent and 84.00 per cent in ascending order of category of farms. Small commercial dairy farms have less mechanization or processing facilities and are mainly focused on selling raw milk rather than processing it into different milk products. A small chunk of milk is processed and sold in small commercial dairy farms.

The return from sale of processed food products was highest in large (10.28 %), followed by medium (6.20%) and small dairy farms (3.64%) respectively. The percentage of milk used for processing of milk products was highest in large commercial dairy farms *i.e.* 8.55 per cent followed by medium commercial dairy farms (4.87 per cent) and small commercial dairy farms (3.11 per cent). Small dairy farms' capacity building towards processing milk into different milk products is crucial for enhancing profitability as value added products fetch higher margin.

The gross return obtained from the different commercial dairy farms was around ₹ 10676.27 thousand, ₹ 20763.81thousand and ₹ 3500.40thousand per farm per year in small, medium and large dairy farms, respectively. The net return/day/farm was ₹ 4.410 thousand, ₹ 14.432 thousand and ₹ 26.135 thousand in small, medium and large commercial dairy farms, respectively. The net income per milch animal per day was highest in large commercial

Table 4: Cost and Returns from Different Commercial Dairy Farms (in 000 ₹/year/farm)

Particulars	Commercial dairy farms			Overall
	Small	Medium	Large	
Fixed cost	1171.831 (12.92)	1958.949 (12.64)	3775.969 (14.83)	1719.278 (13.84)
Total Feed Cost	6443.563 (71.07)	10987.990 (70.91)	18000.619 (70.69)	8642.401 (69.58)
Total Labour cost	1118.750 (12.34)	1955.333 (12.62)	2412.040 (9.47)	1061.940 (10.16)
Total Miscellaneous Cost	239.501 (2.64)	373.209 (2.41)	628.128 (2.47)	272.051 (4.12)
Total Variable Cost	7801.815 (86.05)	13316.532 (85.94)	21040.787 (82.64)	10416.392 (83.86)
Total Production Cost	8973.645 (98.98)	15275.481 (98.58)	24816.756 (97.47)	12135.670 (97.70)
Total Processing Cost	92.800 (1.02)	220.480 (1.42)	645.200 (2.53)	285.352 (2.30)
Total Cost	9066.445 (100.00)	15495.961 (100.00)	25461.956 (00.00)	12421.022 (100.00)
Sale of Milk	9729.170 (91.12)	18355.888 (88.40)	29402.006 (84.00)	12083.054 (79.65)
Sale of Products	387.000 (3.64)	1286.500 (6.20)	3597.333 (10.28)	1650.380 (10.88)
Sale of Dung	60.517 (0.57)	89.758 (0.43)	158.161 (0.45)	85.564 (0.56)
Sale of Animals	499.581 (4.67)	1031.667 (4.97)	1843.900 (5.27)	1350.933 (8.90)
Gross Return	10676.268 (100.00)	20763.813 (100.00)	35001.401 (100.00)	15169.931 (100.00)
Net Return/Year	1609.823	5267.852	9539.445	2748.909
Net Return/Day	4.410	14.432	26.135	7.531
Net Income/Milch/Day	0.06676	0.130	0.139	0.075

dairy farms, followed by medium and small commercial dairy farms in the study area. The reason may be attributed to the fact that with higher processing of raw milk output. The margin and, subsequently the profit from the dairy business is enhanced. Processing and value addition of raw milk plays a crucial role here.

Cost of Milk Production from Commercial Dairy Farms

The cost of milk production is helpful to know whether farmer is able to cover per litre of cost of production. The cost of milk production is shown in Table 5. This helps in making various decisions related to profit generation as well as expansion of dairy farms. The fixed cost accounted for about 15.22 per cent, 13.06 per cent and 12.82 per cent in large, small and medium commercial dairy farms, respectively in the total cost. A higher level of automation can be attributed to higher fixed costs in large commercial dairy farms. The total variable cost accounted for about 85.83 per cent in all types of commercial dairy farms. The returns from sale of milk were around ₹ 10041.250 thousand, ₹ 19296.50 thousand and ₹ 32150.66 thousand per annum in small, medium and large commercial dairy farms, respectively. The per litre average selling price of milk was ₹37.60, ₹ 40.50 and ₹ 38.00 in small, medium and large commercial dairy farms, respectively.

The per litre cost of milk production was highest in small commercial dairy farms i.e. ₹ 31.50 followed by medium ₹ 29.10 and large commercial dairy farms (₹ 26.97/litre). The return from milk production was maximum in large commercial dairy farms, i.e. ₹ 11.03/litre, followed by medium ₹ 10.57 and small commercial dairy farms (₹ 6.10/litre), which is mainly due to the existence of economies of scale.

Economic viability of commercial dairy farms

Economic viability delineates the financial performance of a farm which was worked out by using various financial ratios such as liquidity ratio, solvency ratio, efficiency ratio and profitability ratios and break-even analysis.

Financial ratios

Financial ratios are important parameters for measuring commercial dairy farms' economic viability. Four types of ratios: liquidity ratio, solvency ratio, efficiency ratio and profitability ratio were presented in Table 6.

Liquidity ratios

A farm's aptitude to honor its short-term financial commitments can be gauged by its liquidity ratios. The 'current ratio' is a prime metric indicative of a farm's liquidity stance. For instance, small, medium, and large dairy enterprises recorded current ratios of 3.28, 2.43, and 2.11, respectively. This suggests that small dairy farms have existing assets thrice that of their current liabilities, positioning them favourably. However, larger farms seem less equipped to address their immediate liabilities than their smaller and medium-sized counterparts. Notably, all the farms surpassed the benchmark value (>2), which signifies their adeptness in meeting short-term financial demands via extant assets.

Solvency ratio

The solvency ratio measures the dairy farm's ability to meet its long-term debt obligations. Solvency ratios offer insights into a dairy farm's prowess to meet its prolonged financial commitments. Two specific metrics, the debt-equity ratio and net capital ratio, clarify these ventures' long-term solvency.

Table5: Cost and Return of Milk Production from Different Commercial Dairy Farms

Particulars	Herd Size Category			Overall
	Small	Medium	Large	
Total Fixed cost (thousand ₹/annum)	1171.831 (13.06)	1958.949 (12.82)	3775.969 (15.22)	1719.278 (14.17)
Total Variable Cost (thousand ₹ /annum)	7801.815 (86.94)	13316.532 (87.18)	21040.787 (87.78)	10416.392 (85.83)
Total Cost (thousand ₹ /annum)	8973.645 (100.00)	15275.481 (100.00)	24816.756 (100.00)	12135.670 (100.00)
Sale of Dung (thousand ₹/annum)	60.517	89.758	158.161	85.564
Sale of Animals (thousand ₹/annum)	499.581	1031.667	1843.900	1350.933
Net Cost (thousand ₹/annum)	8413.548	14154.056	22814.695	10699.173
Sale of Milk (thousand ₹/annum)	10041.250	19296.501	32150.660	12972.254
Net Return (thousand ₹/annum)	1627.703	5142.445	9335.965	2273.081
Milk Production (thousand litres/annum)	267.055	486.457	846.070	346.750
Cost (₹/litre)	31.50	29.10	26.97	30.86
Sale Price of Milk (₹/litre)	37.60	40.50	38.00	37.41
Return (₹/litre)	6.10	10.57	11.03	6.56

Debt-equity ratio

This ratio delineates the relationship between a farm's total liabilities and its equity. These ratios for small, medium, and large dairy enterprises stood at 0.38, 0.39, and 0.43 respectively. Notably, as the herd size augmented, there was a commensurate rise in the ratio. This indicates that larger farms have a more substantial proportion of long-term debts than their smaller counterparts. These findings echo the research conclusions of Sharma (2013).

Net capital ratio

This ratio represents the financial stability of a dairy farm; the net capital ratios for small, medium, and large farms were 3.55, 3.65, and 3.34, respectively. These figures, which surpass the benchmark value of 2, indicate the security of the lending agency's funds. It implies that these dairy farms are poised to honour their long-term financial obligations on time.

Efficiency ratio

The efficiency ratio measures dairy farm's ability to use their assets efficiently and effectively to generate returns. Efficiency is defined as the returns obtained by investing one rupee in the dairy farm. Fixed ratio and gross ratio were used to measure the efficiency of dairy farm. These ratios show the proportion of fixed and total costs in the total/gross returns. The optimum value of this ratio should be 0.50. Lower the value of these ratios shows higher efficiency.

Fixed ratio

Our observations revealed fixed ratios of 0.11, 0.09, and 0.12 for small, medium, and large farms, respectively.

This metric highlights the proportion of fixed costs invested to achieve a rupee of total return. The fixed ratio was 0.11, 0.09 and 0.12 in small, medium and large commercial dairy farms. Intriguingly, only 9-12% of fixed costs were needed to realize a rupee of total return across these farms. The ratio was three times less than the standard value, i.e., 0.50, on all types of commercial dairy farms.

Gross ratio

Gross ratio measures the amount of total expenses or cost incurred to gain one rupee of total profit. The ratio was 0.89, 0.77 and 0.77 in ascending order of commercial dairy farms. The gross ratio was found to be highest in small dairy farms, whereas in medium and large commercial dairy, it was found to be the same. In small commercial dairy farms, a total ₹ 89 were invested to earn a total return of ₹100, which led to a net profit of ₹ 11. The gross cost was highest in small dairy farms compared to medium and large dairy farms, with high cost of maintaining crossbred animals as in small dairy farms were dominated by crossbred animals. All the ratios were less than one, meaning all the farms invested less to generate net income.

Table 6: Different financial ratios of dairy farms

Particulars		Small	Medium	Large
a)	Liquidity ratio			
	Current ratio	3.28	2.43	2.11
b)	Solvency ratio			
	Debt-equity ratio	0.38	0.39	0.43
	Net capital ratio	3.55	3.65	3.34
c)	Efficiency ratio			
	Fixed ratio	0.11	0.09	0.12
	Gross ratio	0.89	0.77	0.77
d)	Profitability ratio			
	Capital-turnover ratio	1.12	1.30	1.30

Particulars	Commercial dairy farm category			Overall
	Small	Medium	Large	
Average milch animal per farm	66	111	188	100
Total fixed cost per farm (₹)	1171831	1958949	3775969	1719278
Total variable cost per animal (₹)	118102	119742	111943	104803
Total cost per animal	135841	137357	132032	122102
Gross return per animal	161615	182699	181702	153907
Break-even herd size (number)	27	31	54	35
Break-even to average herd (%)	40.77	27.98	28.80	34.66
Margin of safety (%)	59.23	72.02	71.20	65.34

Profitability ratio

This ratio is used to find out the farm's ability to gain return to its total initial investment. The ratio was found to be 1.12, 1.30 and 1.30 on small, medium and large dairy farms, respectively, indicating total cost was recovered only once in a year. There is a huge gap in the actual and standard value *i.e.* 5, but dairy farms may still improve their situation as farms are already recovering once a year. This indicates that total capital invested was recovered only once in a year. This ratio was less than the standard value, *i.e.,* 5, but dairy farms could still recover its capital invested once a year.

Break-even herd size analysis of commercial dairy farms

Break-even analysis is essential for finding the no profit and loss situation. In the present study, the break-even level of herd size calculated the minimum level required to cover its total costs. The break-even herd size in different commercial dairy farms was 27, 31 and 54 milch animals on small, medium and large commercial dairy farms, respectively. The actual herd sizes of different commercial dairy farms were found to be higher than that of break-even herd size. The actual in milch herd sizes were 66, 111 and 188 milch animals in ascending order of herd size categories. The break-even to average herd percentage was 41, 23 and 29 in small, medium and large commercial dairy farms, respectively. This indicates that medium dairy farms kept more animals than small and large dairy farms. The margin of safety was found to be lowest in small dairy farms, followed by large and medium dairy farms.

The lowest return per milch animal in small dairy farms was the main reason for less margin of safety in these farms. The margin of safety was 59.23, 72.02 and 71.20 per cent in ascending order of herd size category, respectively. The margin of safety can be improved by proper management of small dairy farms.

Conclusion

The commercial dairy farming landscape in the Trans-Gangetic Plains of India presents a dynamic and evolving sector, with an intricate interplay of economic, demographic, and technological factors. The economic analysis reveals that larger dairy farms, despite their higher initial investments, tend to have lower costs of milk production, thus benefiting from economies of scale. However, small farms, although producing milk at a slightly higher cost, still have a significant role in the dairy landscape due to their sheer number and contribution to total milk output. The solvency and liquidity ratios of the farms, irrespective of size, suggest overall financial health, but there is room for improvement, especially in terms of efficiency and profitability. The break-even analysis further elucidates that while most farms operate above their break-even point, there is a significant potential to enhance profitability.

Policy Formulation

Encouraging value addition, such as processing raw milk into various dairy products, can serve as a lucrative avenue for enhancing profitability. Small dairy farms, in particular, can benefit immensely from such ventures, given the higher margins associated with value-added products. Dairy farmers should be equipped with modern dairy farming practices, business management skills, and market access strategies. This can be achieved through targeted training programs, workshops, and exposure visits. The introduction of technology, be it in the form of automation, dairy farm management software, or advanced milking machinery, can significantly enhance the efficiency and productivity of dairy farms.

References

Acharya KK, Malhotra R, (2020) Economic analysis of milk production in peri-urban dairy farms of Odisha. Indian J Dairy Sci. 73(2):155-159

BAH&S (2022) Basic Animal Husbandry Statistics-2022. Government of India Ministry of Fisheries, Animal Husbandry & Dairying Department of Animal Husbandry and Dairying Krishi Bhawan, New Delhi

Bhowmik P, Sirohi S, (2008) Cost of milk production and returns from dairying in South Tripura. Indian J Dairy Sci 61: 303-307

FAO (2020) OECD-FAO Agricultural Outlook 2019-2028, OECD Publishing, Paris/Food and Agriculture Organization of the United Nations, Rome. https://doi.org/10.1787/agr_outlook-2020-en

Gayathri SL, Bhakat M, Mohanty TK (2023) An outlook on commercial dairy farming in India: A review. Indian J Anim Prod Mgt 37(1): 45-56

Hemme, T (ed.) (2017) IFCN Dairy Report 2017, IFCN, Kiel, Germany.

Kumari B, Malhotra R, Chauhan AK, (2016) Impact of women dairy co-operatives on economics of milk production in Begusarai district of Bihar. Indian J Dairy Sci 69: 487:491

Lal P, Chandel BS (2016) Economics of milk production and cost elasticity analysis in Sirsa district of Haryana. J Econ Affairs 61: 405-411

Narehsa N, Dixit AK, Singh A, Meena BS (2023) Economic analysis of milk production in Southern and North coastal region of Andhra Pradesh. J Dairy Sci 76: 163-168

Sirohi S, Bardhan D, Chand P (2015) Costs and returns in milk production: Developing standardized methodology and estimates for various production systems. Project Report submitted to Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Govt. of India, New Delhi.

Vancalker KJ, Berentsen PBM, Giesen GWJ, Huirne RBM (2005) Identifying and ranking attributes that determine sustainability in Dutch dairy farming. Agric Human Values 22:53-63

Indian J Dairy Sci 77(3): 282-290
https://doi.org/10.33785/IJDS.2024.v77i03.013

RESEARCH ARTICLE

Comparative analysis of management practices of calves of dairy animals under organic and non-organic farming system

Saikat Maji[1], B S Meena[2], Neela Madhav Patnaik[3] and Priyajoy Kar[4]

Received: 13 February 2023 / Accepted: 14 September 2023 / Published online: 23 June 2024

Abstract: Dairy farming provides livelihood and nutritional security to the vast majority of families throughout rural India. In this livelihood system dairy calves play a very important role as most of the farming families carefully raise the young calves to become efficient producers in terms of milk or as a source of drought power. For organic farmers, the necessity of proper calf management assumes more importance due to restrictions on diet choice and also due to more stress on welfare-based animal rearing. In this context, the present study was undertaken in Uttarakhand, one of the leading states in organic farming, as a part of a larger study on dairy-based organic farming. A number of organic and non-organic farmers compulsorily have either cattle, buffalo, or goats as dairy animals or any combination of these enterprises, selected as per the sampling plan presented in the main text, and were surveyed, Calf management practices were studied from the context of recommended practices of organic farmers. It was found that variation existed between organic and non-organic farmers in plain and hilly regions and also for different species of dairy animals. Keeping the calf separate from the respective mother was the most common practice at least for a few days after birth. Mutilation practices like castration were higher in hilly regions irrespective of the farming system especially for cattle than in buffalo. Disbudding was also practiced by many organic farmers in violation of organic standards. Under miscellaneous management practices, colostrum feeding after birth was found to be followed by all organic farmers in plain and hilly regions. Also, the paper shed some light on varied probability (increasing or decreasing) of practicing/ compliance to various calf management practices if farmers moved from non-organic to organic farming. The calf mortality pattern between organic and non-organic management was not found to be significantly different.

Keywords: Calf mortality; Housing Management; Mutilation practices; Non-organic farmers Odds ratio; Organic farmers

Introduction

Calves play an important role in the development of the dairy sector, as the future of the dairy herd solely depends upon the successful raising of young calves thus transforming them into a future efficient producer. With many constraints in terms of shortage of fodder, lack of high-yielding breeds, inability to give quality feed, *etc.,* affecting the ability of dairy producers, timely management and healthcare like preventive measures, de-worming, and timely treatment right from the calf stage is a prerequisite to ensure proper health of animals that promote their productivity despite of various shortfalls (Singh *et al.*2007; Ahirwar et al. 2011; DAHD 2018). Thus, understanding the livestock management practices adopted by the farmers is necessary to identify the strength and weaknesses of the rearing systems and to formulate suitable intervention policies (Gupta *et al.* 2008). Among these management practices calf management practices is one of the most important one to be followed diligently. Poor management practices lead to economic losses to the farmers in terms of higher calf mortality, poor growth rate, delayed maturity, and poor productivity. Further, not feeding colostrum to newborn calves reduces the immunity of calves and makes them susceptible to diseases (Maousami *et al.* 2013; Sabapara et al. 2015) which increases the cost of rearing and farmers to face economic loss by calf mortality. Ensuring proper management of calf assumes even more significance under an organic farming regime not only for transforming a calf into a sustainable producer or efficient worker but also for the utmost importance placed on the welfare of animals under organic dairy farming principles. Especially, the scale and intensity of livestock farming have increased significantly over the past few decades.

[1], I.Ag.Sc., Banaras Hindu University, Varanasi (UP), 221005

[2] Dairy Extension Division, ICAR-NDRI, Karnal, Haryana,132001

[3] ICAR-MGIFRI, Piprakothi, Bihar, 845429

[4] ICAR-NRC Pig, Garbhanga F.V., Assam, 781015

Saikat Maji(✉)
Email: saikatm@bhu.ac.in

Despite having many positive aspects, this intensive dairy farming has also negatively affected the factors like sustainability including animal welfare (Cardoso et al. 2019; Henchion et al. 2022). On the other hand, consumers are now becoming increasingly sensitive towards aspects like how their food is produced, cruelty towards animals, and psychological well-being (like freedom of grazing, etc.) of farm animals. Thus, the interest in organic crop and livestock farming remerges in recent times across the world along with India, given the adverse environmental, social, and economic impacts of so-called modern agriculture. But unlike organic crop farming the concept and practice of organic dairy farming is relatively new (Oruganti, 2011). Organic Dairy farming means rearing cattle on organic feed (*i.e.,* feed crops cultivated without the use of fertilizers or pesticides), having access to pasture or outside, along with the restricted usage of antibiotics and hormones. It deliberately avoids the use of synthetic inputs such as drugs, feed additives, and genetically engineered breeding inputs. The welfare of animals is also of prime importance in the organic dairy farming system (Chander et al. 2013; Wolde and Tamir, 2016).

Uttarakhand, which is one of the leading organic states in India, has a predominance of mostly traditional integrated crop-livestock farming (Subrahmanyeswari and Chander, 2008). A vast number of farmers practicing organic farming and the state have a well-developed structure for promoting organic farming as well as for marketing of organic products. With dairy farming acting as one of the most important means of providing livelihood and nutritional security to the vast majority of rural masses (Srivastava, 2011) studying the calf-rearing practices followed by organic farmers was of utmost importance not only to ensure increased productivity but also to ensure the integrity of organic system. The calf-rearing practices followed by non-organic farmers and calf mortality patterns were also studied not only to have a comparative view of calving and calf-rearing practices of organic and non-organic farms but also for gaining knowledge of existing calf management practices prevalent in the study region which in turn will help in better policy intervention for the development of the overall dairy sector of the state.

Research Methodology

Multistage random sampling was followed in the selection of ultimate sampling units. The field survey was conducted across four districts of the Uttarakhand namely; Haridwar, Udham Singh Nagar (as a plain region) and Tehri and Pithoragarh (as a hilly region) selected purposively based on the number of organic farmers and area under organic cultivation (as per data available in Uttarakhand Organic Certification Board (UOCB) Website, 2018). Two blocks from each district, thus, a total of eight blocks were selected randomly for surveying the farmers. Farmers having rice-wheat-dairy animals (Cattle buffalo or goat along with at least a pair of drought animal) as a component of the farming system was selected for final data collection which include both registered organic farmers (registered with UOCB as per guideline of APEDA) and non-organic farmers. The selection method differed between farm types though the basic criteria for entry into the list of farmers to be surveyed were indifferent. The organic farms had to be certified for at least three years. Non-organic farmers were selected in the vicinity of organic farms to minimize bias in the farming system based on geographic location advantages. The presence of cattle, buffalo or goat, or any combination of them was also the essential criteria for selection for both groups of farmers across two regions (i.e., plain and hilly). The selected farmers were classified based on the type of dairy animal reared. A farmer has multiple types of dairy animals included in multiple categories thus not mutually exclusive in the sample. The sample distribution is presented in Table 1. The interview schedule was developed based on calf-rearing practices recommended under the organic farming regime in national standards with an appropriate mix of local traditional welfare-based practices. Previous studies and expert opinion were considered for the construction of the interview schedule item which was part of a larger study on dairy-based organic farming. Frequency data was further subjected to a chi-square test to find out whether calf-rearing practices were significantly different for cattle, buffalo, and goats between organic and non-organic farmers in the plain and hilly regions of the state separately. Before applying chi-square, the sample data has been adjusted for differential sample size between comparing sample groups.

$$\chi^2 = \sum_i (O_i - E_i)^2 / E_i$$

where,

÷ 2 = Chi-Square value

Oi = Observed frequency

Ei = Expected frequency

The odds ratio was calculated to find out the probability of compliance with each practice as the farming system changed from non-organic to organic system (organic farmers/non-organic farmers).

$$Probablity\ (p) = \frac{Odds\ ratio}{(1 + Odds\ Ratio)}$$

If the odds ratio is less than one there will be decreasing the chance of following a particular practice as farmers moved from non-organic to organic management and if the odds ratio is more than one probability to follow particular practice increases as farmers moved from non-organic to organic management.

Results and Discussion

Management practices adopted for calves of cattle, buffalo, and goats have been presented in sections *viz.* housing arrangement for calves, mutilation practices, and miscellaneous management practices for caring for newborns which are often applied when animals are in their early ages for large animals especially. Mortality patterns among calves and their reasons were also presented to enquire into the common belief of high calf mortality under the organic system as compared to the non-organic system among the researchers (ICROFS, 2016; Wilhelm et al. 2017)

Housing Arrangement Practices

The majority of organic farmers in plain regions used to keep their newborn cattle and buffalo with their dam separately (53.13 % and 73.81 %, respectively) for some period after birth (Table 2). In the case of non-organic farmers in plain regions, the practice of keeping newborns with their mothers separately was also followed by the majority of farmers (65.51 and 76.47 %, respectively) similar to those with organic farming systems but with a higher number of cases. In the case of hilly regions majority of organic reported keeping their cattle and buffalo calves with the dam separately (59.65 % and 66.67, respectively). But in hilly regions, though the majority of non-organic farmers reported keeping their newborn cattle and buffalo calve with their dam separately (55 and 60 %, respectively) at early stages but the proportion of farmers was lower than those of their organic counterparts. The tendency to keep newborns with a dam was higher in the case of buffalo than cattle in both plain and hilly regions. The higher mortality rate of buffalo calf and less milk production in case of separation of dam and calf in buffalo was reported as the reasons. A comparatively higher number of organic farmers in Hill also reported keeping their calf in a common shed (21.05 % and 22.22 % of cattle and buffalo farmers, respectively) but not immediately after birth. The lack of space for separate arrangements in hilly patches was the main reason for this. Some comparatively wealthy farmers in plain and hilly regions also had the facility to keep newborns in separate pens but often inside the same shed in the case of hilly regions. The number of non-organic farmers with the facility of separate pens was comparatively higher in both plain and hilly regions than their organic counterparts in respective regions. In the case of goats also the majority of organic farmers in plain and hilly areas (75 and 77.78 %, respectively) keep their newborns with other calves. In the hilly region, 22.22 percent of organic farmers and 16.67 percent of non-organic farmers reported to keep the goat kids in the common animal shed which was not found to be practiced by any organic farmers in plain regions. Possession of a higher number of goats compared to large animals and the birth of often up to 4 kids at one delivery in the case of goats was the natural reason to place goat kids with other kids.

The chi-square analysis as presented in Table 3 did not find any significant difference in any of the housing management practices between organic and non-organic farmers in both plain and hill ecosystems except in the case of keeping calves in a common shed between organic and non-organic farmers in plain region. None of the non-organic farmers reported keeping their cattle in the common shed as compared to 6.25 percent of organic farmers in this case. For keeping the newborn calf separately with the dam which is the recommended practice for an organic system it was found that as farmers moved from non-organic to organic management the probability to keep the calf separately with the dam decreased by 37.34 percent and 45.86 percent, respectively for cattle and buffalo in the plain region. Contrary to this in the case of the hilly region as farmers moved from non-organic to organic management the probability to keep the calf separately with the dam increased by 54.73 percent and 42.86 percent, respectively for cattle and buffalo rearers.

Mutilation Practices

The proliferation of modern dairy farming for exclusively business purposes has resulted in the use of animal husbandry practices that are painful, like castration and debudding, removing calves from their dams, etc. which interfere with 'the natural behavior of animals (Regan et al. 2022). Such mutilation practices are largely prohibited under the organic regime except for medical necessity. But despite of that some of the practices were found to be applied to dairy animals, especially at the calf stage by organic farmers as well as non-organic farmers in the same region (Table 2). Castration of male calves was done by 53.12 percent of cattle owners and 33.33 percent of buffalo rearers, having an organic farming system especially when the calves were intended to be used as drought animals in the future. Cases of castration of male calves were found to be higher in the case of cattle as well as buffalo in plain regions (53.12 % and 33.33 %, respectively) under the management of organic farmers than their non-organic counterparts. Less dependence as well as no compulsion for non-organic farmers to use animal drought power may be the reason non-organic farmers are less prone to have the male calf for future use as a bullock. Whereas, in hilly regions, the percentage of farmers who followed castration was almost similar between organic and non-organic farmers (57.89 % and 58.33 %

Table 1: Sample distribution under the study

Sl. No.	Type of farming system	No of selected farmers based on the type of animal reared by them*					
		Cattle rearer		Buffalo rearer		Goat rearer	
		Plain	Hill	Plain	Hill	Plain	Hill
1.	Organic system	32	57	42	9	4	9
2.	Non-organic system	29	60	34	15	0	12

*Not mutually exclusive

for cattle, 22.22 % and 20.00 % for buffalo under organic and non-organic systems, respectively). The castration of male calves was higher in hilly regions and especially higher for male calf of cattle where bullocks are the main source of drought power often irrespective of the farming system. A higher rate of castration was reported by Subrahmanyeswari and Chander, 2008 where 69.44 percent of organic farmers in Uttarakhand hills were found to be applying castration to their draft animals. Castration of male goats at an early age is done by all organic farmers in plain and 44.44 percent of organic farmers in the hilly region for getting better physical growth to be sold as slaughtered animals in the future. Docking or cropping was not common in this region and was neither reported by any organic as well as non-organic farmers in both plain and hilly regions. Among the organic farmers, 71.87 percent of cattle rearers in the plain region and 19.29 percent in the hilly region subjected their cattle to dehorning/disbudding practices which are often used for both male and female animals and containing the aggressiveness was reported as the main reason. 25.00 percent of organic farmers in plain and 33.33 percent in hilly regions reported disbudding of their goat calf.

The chi-square analysis as presented in Table 3 indicates a significant difference in castration and dehorning/disbudding practices between organic and non-organic farmers in the plain region. As farmers moved from non-organic to organic farming systems the probability to use castration and dehorning/ disbudding increased by 81.25 and 88.92 percent. It is against the regulation and spirit of organic farming and thus needs to be controlled if the government decided to promote specialized organic dairy farming in the state in the line of organic farming. Contrary to this, in the hilly region there was a significant difference in the use of dehorning/disbudding practice in cattle but the probability to use dehorning/disbudding decreased by 20.38 percent as farmers moved from non-organic to organic farming system which is welcoming sign. The use of castration and dehorning/disbudding practices in the case of small dairy animals (goats) was also significantly different between organic and non-organic farmers in the hilly regions where, as farmers moved from non-organic to organic system the probability of subjecting goats to castration and dehorning/disbudding practices decreased by 6.80 percent and 4.30 percent, respectively. For buffalo, there were no significant differences between organic and non-organic farmers in the use of these mutilation practices in both plain and hilly regions.

Miscellaneous Management Practices

In the case of other important management practices ranging from cleaning of calf immediately after birth to the vaccination of the calf have been documented in Table 4. It was found that in the case of cattle all farmers in plain region (both organic and non-organic farmers) practice cleaning of calf immediately after birth with old clothes and asses or husk to keep it worm. It was also followed by the vast majority of both organic and non-organic farmers in the plain region for buffalo (92.85 % and 82.35 %, respectively) and goat (75.00 %). Whereas, in Hill, a relatively lower number of organic farmers deliberately clean newborn calves (57.89, 33.33, and 22 %, respectively for cattle, buffalo, and goat) leaving the calf to be cleaned by licking of their concerned mother. In the case of non-organic farmers in the hilly region also the cleaning of a newborn using cloths or ash was practiced by a smaller number of farmers (55.00 %, 26.67 %, and 25.00 % of cattle, buffalo, and goat owners, respectively) compared to plain farmers and also their organic counterparts in the same region. The result was in agreement with the findings of Meena et al. 2008; who reported only 29 percent of cases in the Kumaon hills of Uttarakhand where such cleaning is done. But, Tiwari et al. 2018, found the complete opposite result of this when they reported only 8.00 percent of farmers used to clean their calves using old rags immediately after birth in the Tarai region (plain) of Uttarakhand.

Cutting the naval cord and applying of any type of disinfectant was practiced by only 34.37 percent of organic farmers in plain regions for cattle and half of the organic farmers for buffalo. Whereas, proper cutting and disinfection of naval cord using iodine solution or other antibiotic solution was followed by 65.61 percent cases for cattle and 88.23 percent cases for buffalo by non-organic farmers in plain regions which was higher than their organic counterparts in the same region. But these differences are somewhat attributed to better socio-economic conditions of non-organic farmers who got selected in the sample. Similarly, Cutting the naval cord and applying any type of disinfectant was lower in the case of organic farmers in the hilly region (29.82 % and 33.33 % for cattle and buffalo, respectively) than their non-organic counterparts in the hill (41.67 % and 46.67 % for cattle and buffalo, respectively). From an overall sense, the use of cutting and disinfection of naval cords was lower in the case of hill farmers than the farmers in plain regions. Such practice in the case of goats was not common either in plain or hilly areas of the state.

Colostrum feeding immediately after birth was universal for all farmers in both regions for all species of dairy animals. But continuing feeding of colostrum in sufficient quantity (up to 4 days after birth) was not followed by all farmers in hilly regions for all species. 87.77 percent of organic farmers and 68.33 percent of non-organic farmers in Hill continue to feed colostrum to cattle up to four days after birth. Meena et al. 2007 reported that 89.00 percent of hill farmers used to continuously feed colostrum for up to 7 days whereas others ceased to do so after the first day. In the case of goats, 25.00 percent of organic farmers in plain and 66.67 percent of non-organic farmers in hill keep feeding the newborn goat with colostrum up to four days after birth.

Weaning is not common in India under the traditional mixed crop-dairy farming system. In the study area, weaning was not practiced

Table 2: Housing arrangement and Mutilation practices for calves of various dairy animals followed by farmers across regions and farming systems {n= number of farmers having a particular type of dairy animal}

Sl. No.	Management Practices	Cattle				Buffalo				Goat			
		Plain		Hill		Plain		Hill		Plain		Hill	
		Org n=32	Non-Org n=29	Org n= 57	Non-Org n= 60	Org n= 42	Non-Org n= 34	Org n= 9	Non-Org n= 15	Org n= 4	Non-Org n= 0	Org n= 9	Non-Org n= 12
Housing Arrangement Practices													
1.	Separate pen	9.37	13.80	5.27	10.00	4.77	5.88	0.00	6.67	0.00	-	0.00	0.00
2.	With other calves	31.25	20.69	14.03	18.33	11.90	11.77	11.11	20.00	75.00	-	77.78	83.33
3.	With the dam separately	53.13	65.51	59.65	55.00	73.81	76.47	66.67	60.00	25.00	-	0.00	0.00
4.	In common shed	6.25	0.00	21.05	16.67	9.52	5.88	22.22	13.33	0.00	-	22.22	16.67
Mutilation practices													
1.	Castration	53.12	20.68	57.89	58.33	33.33	17.70	22.22	20.00	100	-	44.44	91.67
2.	Docking	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00
3.	Cropping	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00
4.	Dehorning/ Disbudding	71.87	24.13	19.29	48.33	0.00	0.00	0.00	0.00	25.00	-	33.33	91.67

Table 3: Significance of differences in Housing arrangement pattern and Mutilation practices for calves and probability of compliance across regions and farming systems

Practices	Org_Plain vs. Non-org_Plain				Org_Hill vs Non-org_Hill			
	χ^2	Effect Size (Phi)	Odds Ratio	p (%)	χ^2	Effect Size (Phi)	Odds Ratio	p (%)
			Cattle					
Separate pen	.292	-.069	.609	37.89	.924	-.089	0.5	33.33
With other calves	.877	.120	1.746	63.58	.397	-.058	.727	42.09
With the dam separately	.966	-.126	.596	37.34	.258	.047	1.209	54.73
In common shed	53.50*	-.936	-	-	.368	.056	1.333	57.13
Castration	6.814**	.334	4.334	81.25	.002	-.004	.982	49.54
Dehorning/Disbudding	13.87**	.477	8.032	88.92	10.95**	-.306	.256	20.38
			Buffalo					
Separate pen	.047	-.025	0.8	44.44	.626	-.162	-	-
With other calves	.000	.002	1.01	50.24	.320	-.115	0.5	33.33
With the dam separately	.071	-.031	.867	45.86	.107	.067	1.333	42.86
In common shed	.343	.067	1.684	62.74	.320	.115	1.857	64.99
Castration	2.384	.177	2.333	69.70	.017	.026	1.143	53.34
			Goat					
With other calves	-	-	-	-	-.103	.070	.700	41.17
In common shed	-	-	-	-	.103	.070	1.429	58.83
Castration	-	-	-	-	5.619*	-.517	.073	6.80
Dehorning/Disbudding	-	-	-	-	7.875**	-.612	.045	4.30

(-) On at least one case, the value of the weight variable was zero, negative, missing or not computed due lack of comparison
(*) Significant at 1 % level, (**) Significant at 5 % level, (#) Significant at 10 % level (p) Probability

Table 4: Miscellaneous management practices in case of calves of various dairy animals followed by farmers across regions and farming systems {n= number of farmers having particular type of dairy animal}

Sl. No.	Practices	Cattle				Buffalo				Goat			
		Plain		Hill		Plain		Hill		Plain		Hill	
		Org n=32	Non-Org n=29	Org n= 57	Non-Org n= 60	Org n= 42	Non-Org n= 34	Org n= 9	Non-Org n= 15	Org n= 4	Non-Org n= 0	Org n= 9	Non-Org n= 12
1.	Cleaning of new born	100	100	57.89	55.00	92.85	82.35	33.33	26.67	75.00	-	22.22	25.00
2.	Cutting and disinfection of naval cord	34.37	65.51	29.82	41.67	50.00	88.23	33.33	46.67	0.00	-	0.00	0.00
3.	Colostrum feeding (Within 1 hr of birth)	100	100	100	100	100	100	100	100	100	-	100	100
4.	Colostrum feeding (Up to 4 days)	100	100	87.77	68.33	100	100	100	100	25.00	-	100	66.67
5.	Weaning	21.87	48.27	10.52	18.33	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00
6.	De-worming	75.00	72.41	43.85	28.33	83.33	88.23	22.22	40.00	0.00	-	0.00	8.33
7.	Vaccination	84.33	93.10	40.35	26.67	73.80	73.52	11.11	40.00	0.00	-	0.00	0.00

Table 5: Significance of difference in miscellaneous management practices for calves and probability of compliance across regions and farming systems

Practices	Org_Plain vs. Non-org_Plain				Org_Hill vs Non-org_Hill			
	χ^2	Effect Size (Phi)	Odds Ratio	p (%)	χ^2	Effect Size (Phi)	Odds Ratio	p (%)
			Cattle					
Cleaning of new born	-	-	-	-	.100	.029	1.125	52.94
Cutting and disinfection of naval cord	5.903*	.311	.276	21.63	3.825#	.187	.459	31.45
Colostrums feeding (up to 4 days)	-	-	-	-	6.356*	.233	3.310	76.79
Weaning	4.697*	.277	.300	23.07	1.435	.111	.524	34.38
De-worming	.053	.029	1.143	53.33	3.062#	.162	1.976	66.39
Vaccination	1.141	.137	.400	28.57	2.463	.145	1.860	65.03
			Buffalo					
Cleaning of new born	1.986	.162	2.786	73.58	.121	.071	1.375	57.89
Cutting and disinfection of naval cord	12.444*	.405	.133	11.73	.411	.131	.571	36.34
De-worming	.365	.069	.667	40.01	.800	.183	.429	30.02
Vaccination	.001	.003	1.015	50.37	2.272	.308	.188	15.82
			Goat					
Cleaning of new born	-	-	-	-	.022	.032	.857	46.15
Colostrums feeding (up to 4 days)	-	-	-	-	3.706#	.420	1.50	60.00
De-worming	-	-	-	-	.787	.194	1.09	52.15

(-) On at least one case, the value of the weight variable was zero, negative, missing or not computed due lack of comparison

(*) Significant at 1 % level, (**) Significant at 5 % level, (#) Significant at 10 % level (p) Probablity

Table 6: Mortality pattern of calves in case of various dairy animals across regions and farming systems {n= number of farmers having particular type of dairy animal}

Sl. No.	Practices	Cattle				Buffalo				Goat			
		Plain		Hill		Plain		Hill		Plain		Hill	
		Org n=32	Non-Org n=29	Org n= 57	Non-Org n= 60	Org n= 42	Non-Org n= 34	Org n= 9	Non-Org n= 15	Org n= 4	Non-Org n= 0	Org n= 9	Non-Org n= 12
Experienced Calf Death in last 10 Years		43.75	27.59	56.14	58.33	45.23	55.89	66.67	73.33	50.00	-	77.78	75.00
1.	Diseases	57.15	37.50	59.37	48.57	36.85	31.57	50.00	45.45	100	-	57.15	55.55
2.	Accidents	0.00	0.00	12.50	5.71	10.52	0.00	0.00	0.00	0.00	-	14.28	11.11
3.	Snake bites	14.28	12.50	9.37	5.71	15.78	10.52	0.00	0.00	0.00	-	0.00	11.11
4.	Wild animal injury	0.00	0.00	0.00	2.87	0.00	5.27	0.00	0.00	0.00	-	28.57	22.23
5.	Abortion and Breeding time complications	28.57	50.00	18.75	37.14	36.84	52.63	50.00	54.55	0.00	-	0.00	0.00

for buffalo and goats across farming systems and regions. But, the practice of weaning is reported to be used by a few farmers in the plain region for cattle calves. Despite being organic farmers 21.87 percent of cattle owners in plain and 10.52 percent in hilly regions reported weaning of calves which are prohibited under the organic doctrine. The practice of weaning was comparatively higher under non-organic management in the respective region. Subrahmanyeswari and Chander, 2008 reported a sufficiently high number of organic farmers used to feed their newborn calves with colostrum (86.00 %) but not by cent percent farmers found this research but the percentage of organic farmers who practiced weaning was almost similar (18.80 % in hilly regions).

De-worming through tablets was practiced by a vast number of organic farmers in the plain region (75.00 % and 83.33 % of cattle and buffalo owners). The trend was found to be almost similar in the case of their non-organic counterparts in plain regions in terms of the percentage of farmers. Deworming was not common in the case of goats across the regions and farming systems. Tiwari et al. 2018, reported deworming practices used by 75 percent of farmers in the Tarai region of Uttarakhand for large dairy animals. A similar trend of deworming practice was also reported by Pawar et al. 2006; Prajapati et al. 2015.

Vaccination, mostly for Foot and Mouth Disease (FMD) and Hemorrhagic Septicemia (HS) was continually done by 84.32 percent of cattle and 73.80 percent of buffalo owners in plain regions having organic systems. Vaccination was higher in the case of non-organic counterparts in plain regions for cattle (93.10 %). Vaccination was comparatively lower in hilly regions. When compared across farming systems in hilly regions, vaccination was done by a higher percentage of organic farmers in the case of cattle (40.35 %). The lack of access to animal health facilities fueled by difficult terrain and lack of awareness was the main reason behind the lower use of different animal health instruments by farmers in hilly regions. Also, less production potential of animals used mainly for home consumption often subconsciously de-motivate some farmers to use these preventive health measures as felt by the researcher during interaction in the process of data collection. But the percentage of farmers who use vaccination was found to be increased from the level reported by Subrahmanyeswari and Chander, 2008, where they found only 7.22 percent of hill organic farmers using vaccination in their dairy calves.

Differences in the use of some of the miscellaneous management practices (as presented in Table 5) *i.e.,* for "Cutting and disinfection of naval cord" and "Weaning" was found to be significant in the plain region for cattle, as farmers moved from non-organic to organic farming there will be 21.63 percent and 23.07 percent of less probability to use above two management practices, respectively. Whereas, in hilly regions, organic and non-organic farmers were significantly different in the case of the use of Colostrum feeding (up to 4 days after birth) along with "Cutting and disinfection of naval cord" and "Deworming". Also, as farmers moved from non-organic to organic systems there will be 31.45 percent less probability to use cutting and disinfection of naval cord after birth. But in contrary to plain region there will be 76.79 percent and 66.39 percent increased probability to use Colostrum feeding (up to 4 days after birth) and Deworming, respectively as farmers moved from non-organic to an organic system of farming in hilly regions. Thus, it represents a more suitable ecosystem to adopt organic dairy farming in cattle in hilly regions. The use of different management practices in the miscellaneous category was not significantly different between organic and non-organic farmers for buffalo across regions except for the use of cutting and disinfection of naval cords in plain regions. The value of the odds ratio indicates as farmers moved to the organic system there will be an 11.73 percent less probability to use the practice for buffalo rearing. In the case of the goat significant difference in rearing practices was only found in the case of colostrum feeding (up to four days after birth) between organic and non-organic farmers in hilly regions with a 60 percent increase in probability to comply as farmers move to organic farming.

Mortality Pattern among Dairy Calves

The incidence of calf mortality was experienced by all farmers across the region and farming system in the last 10 years from the time of data collection (Table 6). For cattle buffalo and goat calf mortality was experienced by 43.75, 45.23, and 50 per cent of organic farmers in the plain region. For the non-organic counterpart calf mortality was reported by 27.59 and 55.89 per cent of farmers in the plain region for cattle and buffalo, respectively. In the hilly region, calf mortality was reported by 56.14, 66.67, and 77.78 percent of organic farmers for cattle, buffalo, and goat, respectively, and by 58.33, 73.33 and 75.00 per cent of non-organic farmers for cattle buffalo and goat, respectively. Calf mortality was comparatively lower in the case of the plain region than in the hilly region for all species of dairy animals and particularly higher for buffalo and goats in the hilly region. The trend is not surprising as calf mortality in India was mainly attributed to diseases like diarrhea, pneumonia, and septicemia, the primary cause of which is unhygienic shed conditions. Other faulty managemental practices like delayed and inadequate first colostrum feeding, not deworming of the pregnant dam, parasitic infestations (both internal and external), and meteorological factors of harsh weather conditions. (Yadav et al. 2019; Patbandha et al. 2023). The incidence of these faulty practices is more in hilly regions as discussed in the previous section too including harsher weather which led to higher calf mortality.

When comparing between organic and non-organic farmers in each region and animal species separately no significant difference in distribution was found through chi-square statistics. Available research data indicates no conclusive evidence about higher or lower mortality rates under organic systems. Studies by Alvasen et al. 2012; Van Wagenberg et al. 2017 reported lower mortality in organically managed herds. Whereas, Kijlstra and Eijck, 2006; Akerfeldt et al. 2020 reported a higher mortality rate among newborns especially before weaning. Table 6 also indicates higher calf mortality due to disease factors in the case of organic farmers in the plain region and due to abortion or breeding-related factors in the case of non-organic farmers in the same region for both cattle and buffalo. But in the case of the hilly region, the dominant factor was diseases in the case of cattle and goats under both organic and non-organic systems whereas abortion or breeding-related factors in the case of buffalo. In organic animal production systems, the animals are particularly at risk due to outdoor rearing and the ban on prophylactic medication (Hermansen, 2003; Maji et al. 2017). Mortality due to wild animal injury was found to be the second dominant factor behind calf mortality for small animals (goats) in hilly regions for both organic and non-organic farming systems. But the significance of these unique observations in terms of the distribution of mortality across mortality factors could not be established when compared between organic and non-organic counterparts in respective regions for each animal species separately.

Thus, the practice followed by organic and non-organic farmers varied between regions as well as different species of dairy animals. Variations existed in mortality patterns also some of which could be attributed to health-related practices retrospectively. For some practices, organic farmers' approaches were found to be more in line with the doctrine of animal welfare leaving rest parameters where practices followed by non-organic farmers were more geared towards animal welfare often unknowingly. But, an increase in the probability of following some recommended practices as extracted from odds ratio calculation as farmers moved towards an organic system is of quite importance for the future proliferation of organic dairy farming in the state or similar conditions in other states.

Conclusions

From the result presented in the above section, it may be concluded that some of the practices followed by organic and non-organic farmers varied not only between plain and hilly regions but also among cattle, buffalo, and goats. But this variation could not be solely attributed to the type of farming system they followed but to the geographic location and socio-economic status of the farmers. Though for some parameters organic farmers' approaches to calf management were found to be more in line with the doctrine of animal welfare, but sanction violation in terms of commonly used practices was also noticed. Thus, promoting organic dairy farming among organic farmers and also among non-organic farmers required intensive awareness and knowledge creation campaigns supported by mass media to make the farmers aware about do and don'ts of organic farming. Only after these primary stimuli, further material support could be initiated to convert the farmers into successful organic dairy farmers throughout the state.

References

Ahirwar RR, Nanavati S, Nayak NK (2011) Feeding and breeding management of buffaloes under rural and urban areas of Indore district of M.P. Indian J Field Vet 6: 29-30

Åkerfeldt MP, Gunnarsson S, Bernes G, Blanco-Penedo I (2020) Health and welfare in organic livestock production systems-a systematic mapping of current knowledge. Organic Agriculture 1-28

Alvåsen K, Mörk MJ, Sandgren CH, Thomsen PT, Emanuelson U (2012) Herd-level risk factors associated with cow mortality in Swedish dairy herds. Journal of Dairy Science 95(8): 4352-4362

Cardoso CS, von Keyserlingk MG, Hötzel MJ (2019) Views of dairy farmers, agricultural advisors, and lay citizens on the ideal dairy farm. J Dairy Sci 102: 1811-1821

Chander M, Subrahmanyeswari B, Mukherjee R (2013) Organic animal husbandry. In. Handbook of Animal Husbandry, pp 365-382. New Delhi, Directorate of Knowledge management in Agriculture, ICAR, 1549 p

DAHD (2018) 19th Livestock Census Annual Report 2017-18, Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Govt. of India.

Gupta DC, Suresh A, Mann JS (2008) Management practices and productivity status of cattle and buffaloes in Rajasthan. Indian J Anim Sci 78(7): 769-774

Henchion MM, Regan Á, Beecher M, Macken-Walsh Á (2022) Developing 'Smart' Dairy Farming Responsive to Farmers and Consumer-Citizens: A Review. Animals 12(3): 360

Hermansen JE (2003) Organic livestock production systems and appropriate development in relation to public expectations. Livestock Production Science 80(1-2): 3-15

ICROFS (2016) Mortality among organic dairy calves to be reduced. Retrieved from https://icrofs.dk/en/aktuelt/nyheder/viewnews/artikel/mortality-among-organic-dairy-calves-to-be-reduced/ on 18.06.21

Kijlstra A, Eijck IAJM (2006) Animal health in organic livestock production systems: a review. NJAS-Wageningen Journal of Life Sciences 54(1): 77-94

Maji S, Meena BS, Paul P, Rudroju V (2017) Prospect of organic dairy farming in India: A review. Asian Journal of Dairy & Food Research 36(1): 1-8

Maousami SBP, Kumar R, Kumar V, Dohare A (2013) Analysis of buffalo calf management practices followed by buffalo owners. *J Anim Sci Adv* **3**(3): 129-133

Meena HR, Ram H, Singh SK, Mahapatra RK, Sahoo A, Rasool TJ (2007) Animal husbandry practices at high altitudes (> 6000 feet) in Kumaon region of Uttarakhand, India. Livestock Research for Rural Development 19(11): 7

Oruganti M (2011) Organic Dairy Farming- A new Trend in Dairy Sector. Veterinary World 4: 128-130

Patbandha TK, Garg DD, Maharana BR, Chavda MR, Pathak R, Gamit VV (2017) Factors Associated with Calf Mortality under Field Condition in Saurashtra Region of Gujarat, India. International Journal of Current Microbiology and Applied Science 6(7): 4184-4192

Pawar BK, Nalawade TH Jagtap DZ (2006) Adoption of bovine heeding practices and constraints faced by tribal farmers of Pune district. *J* Maha Agric Univ 31: 329-330

Prajapati VS, Singh RR, Kharadi VB, Chaudhary SS (2015) Status of breeding and health care management practices of dairy bovines in the rural and urban areas of South Gujarat of India. J Anim Sci Advances **5:** 1514-1521

Regan Á, Kenny U, Macken-Walsh Á, (2022). What does the public want to know about farming? Findings from a farmer-initiated public consultation exercise in Ireland. Sustainability 14(9): 5391

Sabapara GP, Fulsoundar AB, Kharadi VB (2015) Milking and Health Care Management Practices Followed by Dairy Animal Owners in Rural Areas of Surat District. Sch J Agric Vet Sci 2: 112-117

Singh M, Chauhan A, Chand S, Garg MK (2007) Studies on housing and health care management practices followed by the dairy owners. Indian J Anim Res 41: 79-86

Srivastava AK (2011) Vision 2030. National Dairy Research Institute (Deemed University), Karnal, Haryana, India, pp vii

Subrahmanyeswari B, Chander M (2008) Animal husbandry practices of organic farmers: an appraisal. Veterinary World 1(10): 303-305

Tiwari H, Kumar S, Singh DV, Rath R, Tyagi K (2018) Studies on existing milking and health care practices adopted by dairy farmers in Tarai region of Uttarakhand, India. Indian Journal of Animal Research 52(3): 454-458

Van Wagenberg CPA, De Haas Y, Hogeveen H, Van Krimpen MM, Meuwissen MPM, Van Middelaar CE, Rodenburg TB (2017) Animal Board Invited Review: Comparing conventional and organic livestock production systems on different aspects of sustainability. Animal 11(10): 1839-1851

Wilhelm AL, Maquivar MG, Bas S, Brick TA, Weiss WP, Bothe H, Schuenemann GM (2017) Effect of serum calcium status at calving on survival, health, and performance of postpartum Holstein cows and calves under certified organic management. Journal of dairy science 100(4): 3059-3067

Wolde DT, Tamir B (2016) Organic Livestock Farming and the Scenario in the Developing Countries: Opportunities and Challenges. *Global Veterinaria* 16: 399-412

Yadav S, Boora A, Singh, KP, Singh I (2019) A study of pattern in calf mortality at an organized Murrah buffalo herd in India: A 24-year review. Buffalo Bulletin 38(2): 237-248

NAAS Score: 5.24 (January 2024)

Regd. No. 15665/68

Covered by Clarivate Analytics Services: Emerging Sources Citation Index

https://mjl.clarivate.com/search-results

INDIAN JOURNAL OF DAIRY SCIENCE JULY-AUGUST VOL. 77, NO. 4, 2024

Contents

ISSN 0019-5146 (Print)
ISSN 2454-2172 (Online)

RESEARCH ARTICLES