Indian J Anim Health (2023), 62(2): 262-272
DOI: https://doi.org/10.36062/ijah.2023.13622

Research Article

Level and status of selected minerals in Surti buffalo milk and its alcohol stability

N. D. Chaudhary[1], S. C. Parmar[2*], A. K. Jain[2] and A. I. Shaikh[2]

[1]Gujarat Cooperative Milk Marketing Federation Ltd, Anand- 388 001, Gujarat, India; [2]Dairy Chemistry Department, SMC College of Dairy Science, Kamdhenu University, Anand- 388 110, Gujarat, India

Abstract

Pooled milk samples of Surti buffalo were collected weekly during August 2019 to March 2020 and analyzed for its selected minerals content as well as distribution pattern between soluble and colloidal phases, salt balance, Koestler number and alcohol stability. Fractionation of milk was carried out by rennet coagulation. Contents of Ca, Mg, P and citrate in the milk samples were 175.42, 19.06, 116.84 and 195.77 mg/100 mL, respectively, while those in soluble form were 38.19, 10.24, 39.63 and 164.93 mg/100 mL, respectively. Total and soluble chloride content in the Surti buffalo milk were 113 and 110 mg/100 mL, respectively. The Koestler's number for Surti buffalo milk was less than 3 (2.24±0.19), which corresponds to a milk of normal status. Calcium to phosphorus ratio (Ca:P) and salt balance (Calcium + Magnesium)/(Citrate + Phosphate) were found to be 1.51±0.16 and 0.62±0.03, respectively. Pooled milk samples of Surti buffalo showed negative results to the alcohol stability test for 66 to 72% v/v alcohol concentration, but not for 75% v/v alcohol concentration.

Keywords: Alcohol stability, Koestler number, Minerals, Salt balance, Surti buffalo milk

Highlights

- Calcium, magnesium, phosphorous and citrate content in Surti buffalo milk ranged from 151.73-189.65, 14.63-23.83, 94.34-130.23 and 168.76-221.85 mg/100 mL, respectively. The chloride content in Surti buffalo milk ranged from 97 -131 mg/100 mL.
- Of the total content, the soluble form of calcium, magnesium, phosphorus and citrate in milk were 21.81, 53.87, 33.96 and 84.32%, respectively. Almost all content of chloride in milk was present in soluble phase.
- The Koestler's number for Surti buffalo milk was less than 3 (2.24±0.19) which corresponds to a milk of normal status.
- Surti buffalo has shown stability for 66 to 72% v/v alcohol concentration.

INTRODUCTION

Historically, buffalo have been classified into swamp and river buffalo based on morphological, behavioral, and geographic criteria. Sometimes they are referred as separate subspecies: river buffalo as *Bubalus bubalis* and swamp buffalo as *Bubalus bubalis carabenesis* (Groeneveld *et al*., 2010). Conversely, river buffalo are mainly used for milk production (Han *et al*., 2007).

India has a rich repository of 13 recognized breeds of buffalo. Murrah, Nili-Ravi, Jaffarabadi, Surti and Mehsani are the best known dairy breeds of buffalo (Thiruvenkadan *et al*., 2013). Surti buffalo is also known as Deccani, Gujarati, Talabda, Charator and Nadiadi. Average lactation period for Surti buffalo is 292±8.0 to 311±7.0 days with an average daily milk yield of 4.90 to 5.30 kg and an average fat content of 7.85 to 8.10% (ICAR-CIRB, 2019).

The estimated production of milk worldwide is 827.88 million tonnes. In India, total milk production is 198.4 million tonnes with 406 g/day per capita availability (NDDB, 2022) with bulk of milk obtained from buffalo and cow. It is well known that the milk of these two species of animals varies considerably in composition.

Minerals are important for growth, development and regulation of various vital functions in an organism (Soetan *et al*., 2010). Almost all twenty minerals (sodium, potassium, chloride, calcium, phosphorus, magnesium, iron, zinc, copper, manganese, iodine, fluoride, selenium, cobalt, chromium, molybdenum, nickel, arsenic, silicon and boron) are considered important in the diet of humans are present in milk (Flynn, 1992). A functional way of assessing the sensitivity of milk to heat coagulation is the alcohol test. Milk powder manufacturers still use it widely for assessing raw milk freshness and detecting abnormal milk. This is very useful in knowing whether milk is capable of withstanding high heat treatment during milk processing, such as milk sterilization. Alcohol test is used to assess the sensitivity of milk to heat coagulation. Milk powder manufacturers still use it widely for checking raw milk freshness and detecting abnormal milk.

*Corresponding Author, E-mail: parmarsatish79@gmail.com

Literature published in recent years had little information on the minerals content and their distribution pattern between the soluble and colloidal phases in milk of indigenous breeds of buffalo. Over the years, rearing practices of milch animals in India have changed. Organized way of milk farming is on the rise with a significant change/improvement in feeding, healthcare facilities, breeding practices, etc. All these may have an effect on the composition of milk. Study on minerals content and their partitioning pattern in milk can provide important information to the dairy industry involved in condensing and drying of milk. Systematic study of such kind may generate useful information for academicians and researchers as well. Looking to above resume, this research work is planned to study milk of Surti buffalo for its important major minerals content, their distribution between the soluble and colloidal phases in milk and alcohol stability.

MATERIALS AND METHODS

Fresh pooled raw milk samples of Surti buffalo were collected from Reproductive Biology Research Unit (RBRU), Anand Agricultural University, Anand, Gujarat during the period from August 2019 to March 2020 in a clean and dry container. Analysis of these samples was carried out at the Dairy Chemistry Department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat. The samples of milk of Surti buffalo were collected every week throughout the whole lactation period. The animals were fed on dry fodder, green pasture and concentrates during the milking days.

All the chemicals used during the study were of 'Analytical grade' (AR). Glassware meant for analysis purpose were washed with dilute nitric acid followed by distilled water and dried in an oven.

The lactose content of Surti buffalo milk samples was determined by the Lane Eynon method (BIS, 1981).

Partitioning of minerals between soluble and colloidal phases: Partitioning of minerals in the milk samples of Surti buffalo was done at 37°C by rennet coagulation (de la Fuente *et al*., 1996) with suitable modifications, i.e., 1 L of milk was taken instead of 40 mL as mentioned in the reference. Milk (1 L) was warmed to 37°C in a water bath, and a rennet solution (Chr. Hansen Laboratorium, Copenhagen, Denmark) was added, i.e., 0.016 g rennet was dissolved in 10-15 mL of distilled water and added to 1 L of milk. After allowing a firm curd to form, the curd was sliced into 1-2 mm cubes. The whey was collected after filtration and used for analysis of selected minerals in diffusate.

Calcium and magnesium contents were determined simultaneously in milk by the complexometric method of Davies and White (1962) using disodium salt of ethylene diamine tetra acetic acid (EDTA).

Calcium in diffusate was determined by the method of Davies and White (1962) with suitable modifications. The quantity and volume of the reagents used in the procedure were reduced to half of its original values as a modification of the original method.

Phosphorus was determined by the colorimetric method of Fiske and Subba Row (1925), while the methods prescribed in BIS (1981) were followed to determine citrate content and alcohol stability of milk.

Statistical analysis: Descriptive statistics measures like mean, standard deviation, coefficient of variation (CV %), etc., were obtained using the statistical model of Steel and Torrie (1980) for analysis of data.

RESULTS

The results obtained for lactose content, selected minerals content and alcohol stability of Surti buffalo milk during the study are discussed below:

Lactose content

Lactose content in Surti buffalo milk ranged from 4.83-5.98% with an average of 5.12±0.09% (Fig. 1). The lactose content was lowest in the month of October (4.83%) and highest in the month of November (5.98%). It started declining then after and remained almost linear with slight variations from January to March.

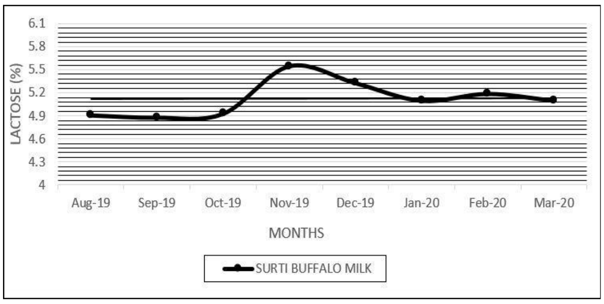

Fig. 1. Lactose content in Surti buffalo milk

Major salt constituents in Surti buffalo milk

In milk, minerals exist in two forms which are partly in soluble and partly in an insoluble/colloidal form associated with casein. Surti buffalo milk was analyzed for calcium, magnesium, phosphorous, chloride and citrate to get an idea about their level and partitioning status in milk.

Calcium: Calcium content in Surti buffalo milk ranged from 151.73-189.65 mg/100 mL with a mean value of 175.42±8.81 mg/100 mL (Fig. 2). Lowest calcium content in milk was observed in the month of February (151.73 mg/100 mL) and highest being in the month of October (189.65 mg/100 mL).

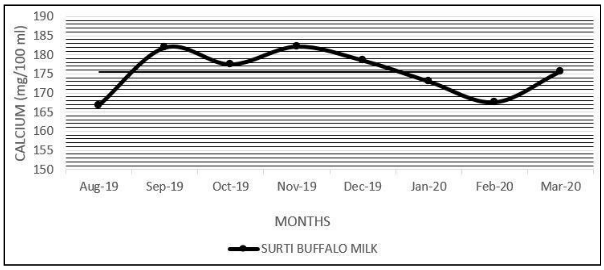

Fig. 2. Calcium content in Surti buffalo milk

Partitioning of calcium: The total concentration of calcium in Surti buffalo milk was 175.42±8.81mg/100 mL. Out of this, 38.19±1.71 mg/100mL (21.81±2.38%) was soluble calcium (Table 1).

Magnesium: The magnesium content in Surti buffalo milk ranged from 14.63-23.83 mg/100 mL with a mean value of 19.06±2.45 mg/100 mL (Fig. 3). The lowest magnesium content in milk was observed in the month of December (14.63) and the highest being in the month of January (23.83).

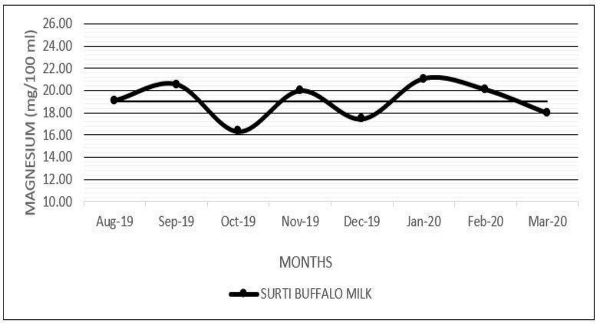

Fig. 3. Magnesium content in Surti buffalo milk

Partitioning of magnesium: The total concentration of magnesium in Surti buffalo milk was 19.06±2.45 mg/100 mL. Out of this, 10.24±0.61 mg/100 mL (53.87±2.18%) was soluble magnesium (Table 2).

Phosphorus: Phosphorus content in Surti buffalo milk ranged from 94.34-130.23 mg/100 mL with a mean value of 116.84±9.48 mg/100 mL (Fig. 4). Lowest phosphorus content in milk was observed in the month of November (94.34) while the highest being in the month of October (130.23) month. Later, an increase in the months of December, January and February was observed.

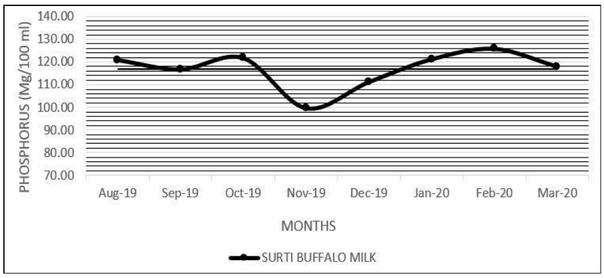

Fig. 4. Phosphorus content in Surti buffalo milk

Partitioning of phosphorus: The total concentration of phosphorus in Surti buffalo milk was 116.84±9.48 mg/100 mL. Out of this, 39.63±2.50 mg/100 mL (33.96±1.50%) was soluble phosphorus (Table 3).

Chloride: The chloride content in Surti buffalo milk ranged from 0.097%-0.131% with a mean value of 0.113% (Fig. 5). The lowest chloride content in milk was observed in September (0.097%) and the highest content was observed in October (0.131%) month. Much variation in chloride content was observed during November to March.

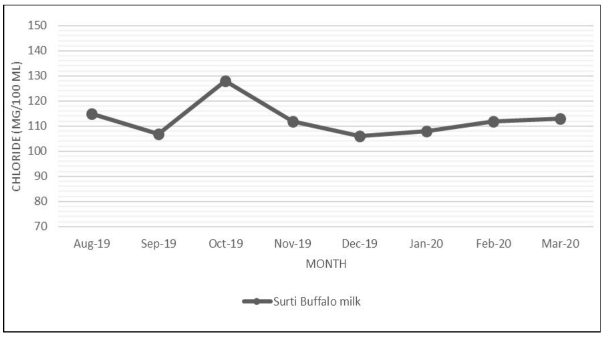

Fig. 5. Chloride content in Surti buffalo milk

Partitioning of chloride: Total concentration of chloride in Surti buffalo milk was 0.113%. Out of this, 0.11±0.65% was soluble chloride (Table 4).

Citrate: Citrate content of Surti buffalo milk was determined over the period of August 2019 to March 2020.

Citrate content in Surti buffalo milk ranged from 168.76-221.85 mg/100 mL with a mean value of 195.77±13.94 mg/100 mL (Fig. 6). The lowest citrate content in milk was observed in January (168.76mg/100 mL), and the highest content was observed in February (221.85 mg/100 mL) month.

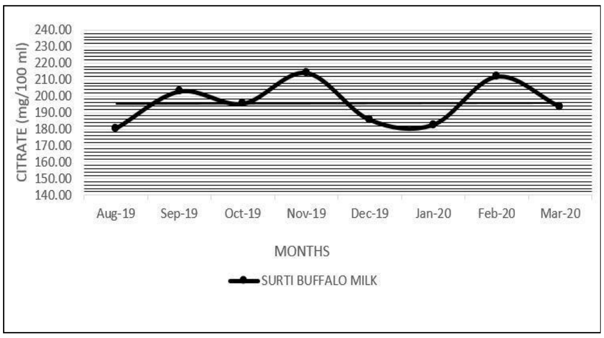

Fig. 6. Citrate content in Surti buffalo milk

Partitioning of citrate: The total concentration of citrate in Surti buffalo milk was 195.77±13.94 mg/100 mL. Out of this, 164.93±9.39 mg/100 mL (84.32±2.91%) was soluble citrate (Table 5).

Table 1. Partitioning of calcium between soluble and colloidal phases in milk

Months	Total Ca (mg/100 mL)	Soluble Ca (mg/100 mL)	Colloidal Ca* (mg/100 mL)	Soluble Ca (% of Total Ca)	Colloidal Ca (% of Total Ca)
August-19	166.85	38.48	128.37	23.06	76.94
September-19	181.86	36.56	145.30	20.10	79.90
October-19	177.51	31.28	146.23	17.62	82.38
November-19	182.15	41.03	141.12	22.53	77.47
December-19	178.55	37.39	141.16	20.94	79.06
January-20	173.09	35.54	137.55	20.53	79.47
February-20	167.67	43.29	124.38	25.82	74.18
March-20	175.70	41.93	133.77	23.86	76.14

* Colloidal calcium = Total calcium – Soluble calcium

Table 2. Partitioning of magnesium between soluble and colloidal phases in milk

Months	Total Mg (mg/100 mL)	Soluble Mg (mg/100 mL)	Colloidal Mg* (mg/100 mL)	Soluble Mg (% of Total Mg)	Colloidal Mg (% of Total Mg)
August-2019	19.09	10.13	8.96	53.06	46.94
September-2019	20.54	10.61	9.93	51.66	48.34
October-2019	16.33	9.44	6.89	57.81	42.19
November-2019	19.97	10.94	9.03	54.78	45.22
December-2019	17.45	9.87	7.58	56.56	43.44
January-2020	21.05	11.2	9.85	53.21	46.79
February-2020	20.07	10.29	9.78	51.27	48.73
March-2020	17.97	9.45	8.52	52.29	47.41

* Colloidal magnesium = Total magnesium – Soluble magnesium

Table 3. Partitioning of phosphorus between soluble and colloidal phases in milk

Months	Total P (mg/100 mL)	Soluble P (mg/100 mL)	Colloidal P* (mg/100 mL)	Soluble P (% of Total P)	Colloidal P (% of Total P)
August-2019	120.83	39.03	81.8	32.30	67.70
September-2019	116.68	39.4	77.28	33.77	66.23
October-2019	121.69	38.78	82.91	31.87	68.13
November-2019	99.6	35.69	63.91	35.83	64.17
December-2019	111.05	37.67	73.38	33.92	66.08
January-2020	121.13	39.7	81.43	32.77	67.23
February-2020	125.77	44.16	81.61	35.11	64.89
March-2020	117.98	42.62	75.36	36.12	63.88

* Colloidal phosphorus = Total phosphorus – Soluble phosphorus

Table 4. Partitioning of chloride (Cl) between soluble and colloidal phases in milk

Months	Total Cl (%)	Soluble Cl (%)	Soluble Cl (% of Total Cl)	Colloidal Cl (% of Total Cl)
August-2019	0.115	0.113	98.26	1.74
September-2019	0.107	0.104	97.20	2.80
October-2019	0.128	0.127	99.22	0.78
November-2019	0.112	0.11	98.21	1.79
December-2019	0.106	0.104	98.11	1.89
January-2020	0.108	0.105	97.22	2.78
February-2020	0.112	0.109	97.32	2.68
March-2020	0.113	0.111	98.23	1.77

Table 5. Partitioning of citrate between soluble and colloidal phases in milk

Months	Total citrate (mg/100 mL)	Soluble citrate (mg/100 mL)	Colloidal citrate* (mg/100 mL)	Soluble citrate(% of Total Citrate)	Colloidal citrate (% of Total Citrate)
August-2019	180.20	148.23	31.97	82.26	17.74
September-2019	202.84	173.94	28.90	85.75	14.25
October-2019	195.38	165.82	29.56	84.87	15.13
November-2019	214.27	180.07	34.20	84.04	15.96
December-2019	185.59	164.16	21.43	88.45	11.55
January-2020	182.46	154.52	27.94	84.69	15.31
February-2020	211.92	165.36	46.56	78.03	21.97
March-2020	193.55	167.31	26.24	86.44	13.56

* Colloidal citrate = Total citrate – Soluble citrate

Table 6. Koestler's number in Surti buffalo milk

Months	Lactose (%)	Chloride (%)	Koestler's number
August-2019	4.91	0.115	2.34
September-2019	4.87	0.107	2.20
October-2019	4.87	0.128	2.63
November-2019	5.29	0.112	2.12
December-2019	5.27	0.106	2.01
January-2020	5.06	0.108	2.13
February-2020	5.08	0.112	2.20
March-2020	5.02	0.113	2.25
Mean ± SD	**5.04±0.29**	**0.113±0.00**	**2.24±0.19**

Table 7. Major salt constituents in Surti buffalo milk

Months	Total Ca	Total Mg	Total Citrate	Total P	Ca : P	(Ca + Mg)/ (Cit. + PO_4)
August-2019	166.85	19.09	180.20	120.83	1.38	0.62
September-2019	181.86	20.54	202.84	116.68	1.56	0.63
October-2019	177.51	16.33	195.38	121.69	1.46	0.61
November-2019	182.15	19.97	214.27	99.60	1.83	0.64
December-2019	178.55	17.45	185.59	111.05	1.61	0.66
January-2020	173.09	21.05	182.46	121.13	1.43	0.64
February-2020	167.67	20.07	211.92	125.77	1.33	0.56
March-2020	175.70	17.97	193.55	117.98	1.49	0.62
Mean ± SD	**175.42±5.85**	**19.06±1.66**	**195.77±13.94**	**116.84±8.19**	**1.51±0.16**	**0.62±0.03**

Koestler's number

Koestler's number for Surti buffalo milk is 2.24±0.19 (Table 6) which is less than 3; therefore, the pooled Surti buffalo milk was found to be normal.

Salt balance in Surti buffalo milk

Pooled milk samples of Surti buffalo were analyzed for the total concentration of calcium, magnesium, phosphorus and citric acid for an understanding of the 'salts balance' of the milk. The ratios of calcium: phosphorus and (calcium + magnesium)/ (citrate + phosphate) were also calculated (Table 7).

The average calcium content was 175.42±5.85 mg/100 mL with a range of 151.73 to 189.65 mg/100 mL which is in agreement with the value normally

Table 8. Alcohol stability of Surti buffalo milk (No. of pooled sample = 32)

Months	Alcohol Stability			
	Ethanol Concentration (% v/v)			
	66	69	72	75
August-2019	-	-	-	+
September-2019	-	-	-	+
October-2019	-	-	-	+
November-2019	-	-	-	+
December-2019	-	-	-	+
January-2020	-	-	-	+
February-2020	-	-	-	+
March-2020	-	-	-	+

'+' and '-' signs indicate positive and negative alcohol tests, respectively under the conditions indicated in the table.

quoted for buffalo milk. The average magnesium content was 19.06±1.66 mg/100 mL with a range of 14.63 to 23.83 mg/100 mL. The average phosphorus content was 116.84±8.19 mg/100 mL with the values varying from 94.34 to 130.23 mg/100 mL. The average citric acid content was found to be 195.77±13.94 mg/100 mL with values ranging from 168.7 to 221.85 mg/100 mL. It is in general agreement with the average values reported for citrate content for pooled buffalo milk. The calcium: phosphorus ratio in Surti buffalo milk samples was 1.51±0.16. The salt balance, i.e., the ratio of (calcium + magnesium) to (citrate + phosphate) of Surti buffalo milk was found to be 0.62±0.03.

Alcohol stability

Ethyl alcohol of 66–75% (v/v) with an increment of 3% was used to measure the alcohol stability of Surti buffalo milk. Pooled milk samples of Surti buffalo showed negative result to alcohol stability test for 66 to 72% v/v alcohol concentration. All pooled milk samples showed positive result to alcohol test at 75% v/v alcohol concentration (Table 8).

DISCUSSION

Lactose content

A wide range was obtained for lactose content for Surti buffalo milk as against reported in the literature. However, it was well within the range for pooled buffalo milk. Similarly, average lactose content was slightly higher than those reported in the literature for Surti buffalo milk but in agreement with the average available for pooled buffalo milk.

Gajjar (2014) studied Surti buffalo milk for composition, physicochemical properties and nitrogen distribution and found the range for lactose content to be 4.38 to 4.87% with a mean value of 4.50%. Yoganandi *et al*. (2014) reported average lactose content in buffalo milk as 4.70±0.05%.

Tyagi *et al.* (2016) studied composition variability in milk samples of breeds of Surti and Mehsani buffalo collected at the 15th and 60th postpartum days. Average lactose in the milk of Surti buffalo at 15th and 60th day postpartum were 5.12±0.07 and 5.24±0.09%, respectively, whereas that of Mehsani buffalo, these values were 5.07±0.06 and 5.32±0.08%, respectively. Kapadiya *et al.* (2016) studied buffalo milk for gross composition, nitrogen distribution and selected minerals content and observed range for lactose to be 4.67 to 5.27% with a mean value of 4.86±0.24%. Pandya *et al.* (2017) studied variability in test day milk yield and milk composition at 15th and 60th day of lactation in Surti and Jaffarabadi buffaloes and found average lactose content in the milk of Surti buffalo at 15th and 60th day of lactation as 5.34±0.07 and 5.61±0.14%, respectively, whereas that of Jaffarabadi buffalo, these values were 6.21±0.12 and 5.83±0.07%, respectively. Sorathiya *et al.* (2018) studied the effect of non-genetic factors on milk compositions in Surti buffaloes, a total of 1515 milk samples were collected from 91 buffaloes during three year period and found average lactose content in milk was 5.01±0.01%.

Major salt constituents in Surti buffalo milk

In milk, minerals exist in two forms which are partly in soluble and partly in an insoluble/colloidal form associated with casein. Surti buffalo milk was analyzed for calcium, magnesium, phosphorous, chloride and citrate to get an idea about their level and partitioning status in milk.

Calcium: Range and average obtained for calcium content in Surti buffalo milk were in general agreement with data available for Surti buffalo milk in literature. It was well within the range available for pooled buffalo milk. Similarly, average calcium content was in general agreement with the data available for pooled buffalo milk.

Sindhu and Roy (1973) studied partitioning of buffalo milk minerals through dialysis and reported calcium as 176.3 mg/100 g. Garaniya *et al.* (2013) studied the nutrient profile of Jaffarabadi buffalo milk at different stages of lactation from 1 to 9 and found the range for calcium content in milk to be 1968.67 to 2083.07 mg/L. Kapadiya *et al.* (2016) studied buffalo milk for gross composition, nitrogen distribution and selected minerals content, and samples were collected from July to January 2014. The range observed for calcium content in buffalo milk was 167.98 to 185.66 mg/100 mL with a mean value of 178.59±6.79 mg/100 mL.

Partitioning of calcium: The result obtained in this study for the partitioning of calcium between soluble and colloidal phases in buffalo milk is in general agreement with that of data reported in the literature. Some variation in data may be due to the method used for the partitioning of minerals.

Kaur *et al.* (1983) studied the distribution of the major minerals between soluble and colloidal phases of buffalo milk as affected by pH and reported total Ca content as 175.78±2.64 mg/100 g, from which soluble Ca content was 40.28±1.70 mg/100 g which is around 22.92% of total Ca content in milk. It increased to 66.39 and 139.31 mg/100 g on lowering the pH of the milk to 5.97 and 5.02, respectively and corresponded to 37.77 and 79.25% of the total. On increasing the milk pH, soluble Ca showed uneven variations. Sindhu and Arora (2011) reported the concentration of salt constituents in the milk of buffalo and cow, and its dissolved phase. Calcium content in buffalo and cow milk was 182.6 and 114.2 mg/100 mL, respectively, while in the dissolved phase the values were 39.75 mg/100 mL (22%) and 39.2 mg/100 mL (34%), respectively. Singh *et al.* (2019) studied profiling and distribution of minerals content in Murrah buffalo milk from Livestock Research Center, ICAR-NDRI, Karnal during winter months and reported soluble calcium in milk as 44.91±4.91 mg/100 g (22%) of the total calcium 204.23±7.98 mg/100 g.

Magnesium: The range and average for magnesium content in Surti buffalo milk were slightly higher than the data available for Surti buffalo milk in literature. It also came within the range available for pooled buffalo milk. Similarly, average magnesium content was in general agreement with the data available in the literature for pooled buffalo milk.

Kapadiya *et al.* (2016) studied buffalo milk for gross composition, nitrogen distribution and selected minerals content and observed range for magnesium content in buffalo milk to be 14.59 to 21.40 mg/ 100 mL with a mean value of 18.29±2.52 mg/100 mL.

Partitioning of magnesium: Result obtained in the present study for the partitioning of magnesium in the soluble and colloidal phases is in general agreement with data reported in the literature for pooled buffalo milk.

Kaur *et al.* (1983) studied the distribution of the major minerals between soluble and colloidal phases of buffalo milk as affected by pH and reported total Mg content as 18.11±1.76 mg/100 g from which soluble Mg content was 8.95±0.73 mg/100 g which is around 49.42% of total Mg content in milk. It increased to 10.96 and 13.62 mg/100 g on lowering the pH of the milk to 5.97 and 5.02, respectively and corresponded to 60.52 and 75.21% of the total. On increasing the milk pH, soluble Mg constantly decreases. de la Fuente *et al.* (1996) studied the effect of ultracentrifugation, rennet coagulation, and dialysis on soluble phase separation in milk. They reported the total value of Mg as 11.9 mg/100 g. From the total, 48.7% soluble phase of Mg in rennet whey, 49.6% soluble phase in the supernatant of ultracentrifugation and 52.9% soluble fraction in diffusate of dialysis. Singh *et al.* (2019) studied profiling and distribution of minerals content in Murrah buffalo milk from Livestock Research Centre, ICAR-NDRI, Karnal during winter months and reported soluble Mg in buffalo milk was 11.33 ±0.67 mg/100 g (48%) of total Mg 23.53±1.33 mg/100 g.

Phosphorus: A wide range and higher value of average phosphorus content were observed than the reported literature for Surti buffalo milk. However, average phosphorus content was in accordance with the data available in the literature for pooled buffalo milk.

Kapadiya *et al.* (2016) studied buffalo milk for gross composition, nitrogen distribution and selected minerals content, and observed range for phosphorus content in buffalo milk to be 95.22 to 124.72 mg/100 mL with a mean value of 109.22±12.16 mg/100 mL.

Partitioning of phosphorus: The result obtained in the present study for the partitioning of phosphorus was in corroboration with the data reported in the literature for pooled buffalo milk.

Kaur *et al.* (1983) studied the distribution of the major minerals between soluble and colloidal phases of buffalo milk as affected by pH and reported total phosphorus content as 155.17±5.72 mg/100 g, from which soluble phosphorus content was 53±2.70 mg/ 100 g which is around 34.16% of total phosphorus content in milk. It increased to 70.49 and 107.24 mg/ 100 mL on lowering the pH of the milk to 5.97 and 5.02, respectively and corresponded to 45.43 and

69.11% of the total. On increasing the milk pH, soluble phosphorus showed small, irregular variations. de la Fuente *et al.* (1996) studied the effect of ultracentrifugation, rennet coagulation, and dialysis on soluble phase separation in milk and reported total value of phosphorus as 99.6 mg/100 g. From the total, 40.8% soluble phase of phosphorus in rennet whey. As well as 41.7% soluble phase in the supernatant of ultracentrifugation and 43.7% soluble phosphorus fraction in diffusate of dialysis. Sindhu and Arora (2011) reported phosphorus content in buffalo and cow milk as 82.39 and 84.8 mg/100 mL, respectively, while the values in the dissolved phase were 26.20 (31%) and 38.4 (45%), respectively. Singh *et al.* (2019) studied profiling and distribution of minerals content in Murrah buffalo milk during winter months, and reported soluble P was 38.37±1.8 mg/100 g (33%) of total P 117.45±5.26 mg/100 g.

Chloride: In the study, the chloride content of Surti buffalo milk was in general agreement with those reported in the literature for Surti buffalo milk, but the result was higher than reported values for pooled buffalo milk.

Sindhu and Roy (1973) studied partitioning of buffalo milk minerals through dialysis and reported chloride as 75.99 mg/100g. Gajjar (2014) studied Surti buffalo milk for composition, physicochemical properties and nitrogen distribution and found the range for chloride content to be 0.094 to 0.137% with a mean value of 0.110%. Yoganandi *et al.* (2014) studied evaluation and comparison of camel milk with cow milk and buffalo milk for gross chemical composition, 34 buffalo milk samples were collected from Gopalpura village, Anand district of Gujarat and reported average chloride content in buffalo milk was 0.12±0.00%. Kapadiya *et al.* (2016) studied buffalo milk for gross composition, nitrogen distribution and selected minerals content, and samples were collected from July to January 2014. The range observed for chloride content in buffalo milk was 0.10 to 0.12% with a mean value of 0.11±0.06%.

Partitioning of chloride: Results obtained in this study for the partitioning of chloride were in accordance with the data reported in the literature for pooled buffalo milk. The chloride content in Surti buffalo milk is almost completely in the soluble phase.

Sindhu and Roy (1982) studied partitioning of various mineral components and reported that chloride comes under completely soluble fraction. Kaur *et al.* (1983) studied the distribution of the major minerals between soluble and colloidal phases of buffalo milk as affected by pH and reported that chloride was completely soluble. Sindhu and Arora (2011) reported chloride content in buffalo and cow milk as 57.69 mg/100 mL and 106.3 mg/100 mL, respectively while the values in the dissolved phase were 57.03 mg/100 mL (99%) and 106.2 mg/100 mL (100%) respectively.

Citrate: Average citrate content found in Surti buffalo milk was in accordance with the data reported in the literature for buffalo milk.

Sindhu and Roy (1973) studied partitioning of buffalo milk minerals through dialysis and reported citrate content as 175.7 mg/100 g.

Partitioning of citrate: The results obtained in the study for the partitioning of citrate were in general agreement with data reported in the literature for pooled buffalo milk. Higher and lower values for soluble citrate were reported in the literature that might be due to the techniques used for the fractionation of milk.

Sindhu and Roy (1982) reported the total concentration of citrate in buffalo milk as 161.5 mg/100 g. Of which concentration in soluble citrate was 136.4 mg/100 g. Kaur *et al.* (1983) studied the distribution of the major minerals between soluble and colloidal phases of buffalo milk as affected by pH. They reported citrate content as 230.61 mg/100 mL, of which 199.92 mg/100 mL was present in soluble phase contributing almost 86.69% of the total citrate. de la Fuente *et al.* (1996) studied the effect of ultracentrifugation, rennet coagulation, and dialysis on soluble phase separation in milk and reported total value for citrate content was 176 mg/100 g, respectively. Out of that, 85.7% soluble phase of citrate in rennet whey. As well as 85.8% soluble phase in the supernatant of ultracentrifugation and 72.2% soluble citrate fraction in diffusate of dialysis. Sindhu and Arora (2011) reported citric acid content in buffalo and cow milk as 159.2 mg/100 mL and 166 mg/100 mL, respectively, while the values in the dissolved phase were 114.69 (84%) and 152 (96%), respectively.

Koestler's number

Milk has long been known to be in osmotic balance with blood, i.e., milk is isotonic to blood. NaCl concentration in milk increases during mastitis, which results in an increase in osmotic pressure. The rise is compensated by a decrease in the lactose content. Therefore, the chloride content of the milk is negatively correlated with lactose content. The inverse relationship between lactose and chloride concentration for abnormal milk is the basis of the Koestler's number. In the case of normal milk, the number is less than 3, whereas in abnormal milk, the number is considerably higher (i.e., 15).

Koestler's number for Surti buffalo milk is 2.24±0.19 (Table 6) which is less than 3, therefore, the pooled Surti buffalo milk was found to be normal.

Salt balance in Surti buffalo milk

In this study, the ratio of Ca and P is slightly lower than reported in the literature for buffalo milk, which may be due to a higher concentration of phosphorus in Surti buffalo milk. Low Ca:P ratio affects Ca metabolism and leads to bone loss and osteopenia, while high Ca:P ratio is good for bone health. Milk and milk products are a rich source of P and Ca as required for adequate Ca:P ratio and meet the recommended Ca:P ratio (Singh *et al*., 2019).

Tayal and Sindhu (1983) studied heat stability and salt balance of buffalo milk as affected by concentration and addition of casein in Murrah buffalo milk and reported Ca:P ratio as 1.60. Sindhu and Tayal (1983) studied the influence of homogenization on the heat stability and salt balance of buffalo milk and its 1:2 concentrate in Murrah buffalo milk and reported Ca:P ratio as 1.10 and 1.17 in unhomogenized milk and homogenized milk, respectively. Soliman (2005) studied chemical composition and mineral content of milk from various species such as human, cow, buffalo, camel and goat milk in Egypt and reported mean Ca:P ratio in pooled buffalo as 1.47±0.04. Khedkar *et al.* (2016) studied buffalo milk, and reported Ca:P ratio in buffalo milk was 1.8. Singh *et al.* (2019) studied profiling and distribution of minerals content in buffalo milk and reported Ca:P in Murrah buffalo milk as 1.74.

The salt balance [ratio of (calcium + magnesium) to (citrate + phosphate)] of Surti buffalo milk was slightly higher than the reported values. The variation in salt balance may be due to factors like temperature, breed, season, feed, nutritional profile, pH as well as fractionation method as reported by various researchers earlier. The ratio of soluble calcium + magnesium/ phosphorus + citrate plays an important role in the heat stability of buffalo milk.

Sharma *et al.* (1980) studied the composition of milk of different breeds of buffaloes and reported a salt balance in various breeds of buffaloes like Jafarabadi, Mehsani and Murrah buffalo were 0.401±0.074, 0.343±0.086 and 0.334±0.095, respectively. Sindhu and Tayal (1983) studied the salt balance of buffalo milk and reported the ratio of salt balance as 1.35.

The mineral composition of milk is not constant because it depends on the lactation phase, nutritional status of the animal, and environmental and genetic factors (Zamberlin *et al.*, 2012). The main component responsible for the variable salt balance ratio was usually soluble phosphorus (Donnelly and Horne, 1986).

Sharma *et al.* (1980) studied the composition of milk of different breeds of buffaloes and reported a salt balance in various breeds of buffaloes like Jafarabadi, Mehsani and Murrah buffalo and found seasonal and breed-wise variation in total and soluble minerals content and also upon the salt balance in the buffalo milk.

The reported data showed that the mineral contents of the various milk species and breeds varied considerably, and their content appeared to be affected by genetic, physical and environmental factors (Raynal-Ljutovac *et al.*, 2007). The concentration of major elements present in milk mainly depends on the species, individuality of animal, feed, lactation stage, and health condition of the udder (Cashman, 2006).

The milk salts have a crucially important impact on many properties of milk, including the formation and stability of the casein micelles, acid-base buffering and various colligative properties, as well as its key biological role (i.e., providing nutrition for the newborn). In addition, these salts have a powerful influence on protein stability during processing (e.g., rennet coagulation, heat and alcohol stability).

Alcohol stability

The alcohol test determines the susceptibility of milk to coagulation due to the development of acidity, disturbed salt balance or high albumin-globulin content. The milk giving a positive alcohol test would coagulate on concentrating in a similar manner as heat coagulation. That is why it is used to predict the heat stability of the milk. The alcohol stability of milk, which is the stability of milk proteins to precipitation by ethanol, is pH dependent; a typical ethanol stability pH-profile is sigmoidal, with stability increasing with pH (Horne and Parker, 1981). Donnelly and Horne (1986) studied the relationship between ethanol stability of bovine milk and natural variation in milk composition, and reported that ethanol stability at the natural pH of bulk milk from winter/spring and autumn calving animals was lowest in early and late lactation. The most important contributory factors appeared to be a high salt balance ratio in late lactation and a low natural milk pH in early lactation.

Sommer and Binney (1923) reported that a balance existed between calcium, magnesium, citrate and phosphate and this ratio in milk determines its alcohol stability. Coagulation by alcohol occurred as a result of an excess of calcium and magnesium over citrate and phosphate in the serum. Alcohol stability positive test in fresh milk depends mainly upon the relative amounts of these four salts present in it. Decreasing the salt balance ratio thus caused a shift in the ethanol stability/pH profile to acidic pH, whereas increasing the salt balance ratio shifted the profile to more alkaline values.

Davies and White (1958) reported the ethanol stability of milk at its natural pH to be significantly correlated with the level of free ionic calcium in the milk. Soluble ionic calcium is therefore the dominant factor in fixing the position of the profile along with pH-axis. Increasing the pH of milk thus will reduce the free calcium level and therefore increase in ethanol stability. In contrast, decreasing the pH frees ionic calcium and leads to a decrease in ethanol stability.

Puri *et al.* (1965) reported that buffalo milk was less stable than cow milk under the influence of alcohol. El-Salam and El-Shibiny (1966) studied the stability of buffalo milk to ethanol in relation to the stage of lactation, the chemical composition of milk, heat stability and rennet coagulation. It was found that total and soluble calcium contents of buffalo milk were inversely correlated to the stability of milk to ethanol.

Chavez *et al.* (2004) found a (positive) correlation between the concentrations of chloride, potassium, ionic Ca and ethanol stability. Moreover, an increase in Ca^{2+} concentration produced a decrease in alcohol stabilities. Sagar *et al.* (2016) studied the evaluation of camel milk for selected processing related parameters and comparisons with cow and buffalo milk. Eight samples of buffalo milk were collected from the local herd maintained in a village nearby Anand, Gujarat and analyzed for alcohol stability. Ethyl alcohol of 68% by volume and 75% by volume were used to measure the alcohol stability, and reported that the buffalo milk samples showed negative result to alcohol stability test i.e., there was no visible flakes/coagulation formation in buffalo milk samples.

The result obtained for alcohol stability (ethanol stability) of Surti buffalo milk was in accordance with those reported in the literature for bovine milk. As reported in the literature, the alcohol stability of milk is based on the pH profile of milk, lactation, feeding practices, temperature, breed, season as well as major mineral content in milk that is related to salt balance and concentration of the ionic form of salt.

The variation in lactose and different minerals of Surti buffalo milk might be due to factors like season, feed, stage of lactation and individuality of animal. The content of different minerals in milk as well as their partitioning between soluble and colloidal phases were in corroboration with other studies. However, method used for partitioning of milk can have its effect in variations in content and status of minerals in milk. The Koestler's number in the milk was 2.24±0.19, which is less than 3 and is falling in the normal range. The calcium: phosphorus ratio of Surti buffalo milk was 1.51±0.16, which is slightly lower than reported in the literature. The ratio of (calcium + magnesium) to (citrate + phosphate) was found to be 0.62±0.03. The salt balance of Surti buffalo milk was slightly higher than the reported values. Pooled milk samples of Surti buffalo showed negative result to alcohol stability test at 66 to 72% v/v alcohol concentration, which confirms suitability of the milk for products such as condensed, sterilized, evaporated milk, etc. which involves high processing temperatures. All pooled milk samples showed positive results to alcohol stability test at 75% by v/v alcohol concentration. The minerals content in milk, its salt balance and alcohol test are widely used to ascertain the freshness of milk meant for manufacture of concentrated and dried milks. The contents of minerals deviating from their usual range may be indicative of abnormality in milk (presence of colostrum) and/or bad animal health (mastitis). Knowledge on these aspects helps the milk processors to take precautionary measures so as to minimize losses during milk processing.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contributions: CND: Involved in investigation, data generation, preparing original draft; SCP, AKJ, AIS: Engaged in conceptualization, data curation, supervision and final editing.

ACKNOWLEDGEMENT

The authors thank Sheth MC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India for patronizing the research work.

REFERENCES

BIS, 1981. Handbook of Food Analysis. SP: 18 (Part XI–Dairy Products). Bureau of Indian Standards, Manak Bhavan, New Delhi, pp 1-43

Cashman KD, 2006. Milk minerals (including trace elements) and bone health. Int Dairy J, 16(11): 1389-1398, doi: 10.1016/j.idairyj.2006.06.017.

Chavez MS, Negri LM, Taverna MA and Cuatrin A, 2004. Bovine milk composition parameters affecting the ethanol stability. J Dairy Res, 71(2): 201-206, doi: 10.1017/s0022029904000172

Davies DT and White JCD, 1958. The relation between the chemical composition of milk and the stability of the caseinate complex. II. Coagulation by ethanol. J Dairy Res, 25(2): 256-266, doi: 10.1017/S0022029900009250

Davies DT and White JCD, 1962. The determination of calcium and magnesium in milk and milk diffusate. J Dairy Res, 29(3): 285-296, doi: 10.1017/S0022029900011109.

de la Fuente MA, Fontecha J and Juarez M, 1996. Partition of main and trace minerals in milk: effect of ultracentrifugation, rennet coagulation, and dialysis on soluble phase separation. J Agric Food Chem, 44(8): 1988-1992, doi: 10.1021/jf9506949

Donnelly WJ and Horne DS, 1986. Relationship between ethanol stability of bovine milk and natural variations in milk composition. J Dairy Res, 53(1): 23-33, doi: 10.1017/S0022029900024626

El-Salam MHA and El-Shibiny S, 1966. The chemical composition of buffalo milk. 1. General composition. Indian J Dairy Sci, 19(3): 151-154

Fiske CH and Subba Row Y, 1925. The colorimetric determination of phosphorus. J Biol Chem, 66(2): 375-400, doi: 10.1016/S0021-9258(18)84756-1

Flynn A, 1992. Minerals and trace elements in milk. Adv Food Nutr Res, 36: 209-252, doi: 10.1016/s1043-4526(08)60106-0

Gajjar SI, 2014. Evaluation of milk from Kankrej cow and Surti buffalo for composition, physico-chemical properties and nitrogen distribution (M. Tech. Thesis, SMC College of Dairy Science, Anand Agricultural University, Anand). Retrieved from https://krishikosh.egranth.ac.in/displaybitstream?handle=1/5810002725

Garaniya NH, Ramani HR and Golakiya BA, 2013. Nutrient profile of Jaffarabadi buffalo milk at different stages of lactation. Asian J Dairy Food Res, 32(2): 168-170

Groeneveld LF, Lenstra JA, Eding H, Toro MA, Scherf B *et al*., 2010. Genetic diversity in farm animals– A review. Anim Genet, 41(Suppl 1): 6-31, doi: 10.1111/j.1365-2052.2010.02038.x

Han BZ, Meng Y, Li M, Yang YX, Ren FZ *et al*., 2007. A survey on the microbiological and chemical composition of buffalo milk in China. Food Control, 18(6): 742-746, doi: 10.1016/j.foodcont.2006.03.011

Horne DS and Parker TG, 1981. Factors affecting the ethanol stability of bovine milk. I. Effect of serum phase components. J Dairy Res, 48(2): 273-284, doi: 10.1017/S0022029900021695

ICAR-CIRB, 2019. Buffalo pedia. Retrieved from https://buffalopedianew.cirb.res.in/surti/

Kapadiya DB, Prajapati DB, Jain AK, Mehta BM, Darji VB *et al*., 2016. Comparison of Surti goat milk with cow and buffalo milk for gross composition, nitrogen distribution, and selected minerals content. Vet World, 9(7): 710-716, doi: 10.14202/vetworld.2016.710-716

Kaur S, Sindhu JS and Roy NK, 1983. Distribution of the major minerals between soluble and colloidal phases of buffalo milk as affected by pH. J Food Process Preserv, 7(1): 9-18, doi: 10.1111/j.1745-4549.1983.tb00659

Khedkar CD, Kalyankar SD and Deosarkar SS, 2016. Buffalo milk. In Encyclopedia of food and health (vol. I, pp 522-528) (Eds.) B. Caballero, P. M. Finglas, & F. Toldra. Oxford: Acadamic Press in an imprint of Elsevier

NDDB, 2022. Milk Production in India-National Dairy Development Board. Retrieved from https://www.nddb.coop/information/stats/milkprodindia

Pandya GM, Ramani UV, Janmeda M, Tyagi KK, Kharadi VB *et al*., 2017. Variability in test day milk yield and milk composition at day 15 and 60 of lactation in Surti and Jaffarabadi buffaloes. Indian J Dairy Sci, 70(6): 763-766

Puri BR, Murari K and Toteja KK, 1965. Studies in stability of protein dispersion in milk. I. Ethanol Stability. Indian J Dairy Sci, 18: 112-114

Raynal-Ljutovac K, Park YW, Gaucheron F and Bouhallab S, 2007. Heat stability and enzymatic modifications of goat and sheep milk. Small Rumin Res, 68(1-2): 207-220, doi: 10.1016/j.smallrumres.2006.09.006.

Sagar SP, Mehta BM, Wadhwani KN, Darji VB and Aparnathi KD, 2016. Evaluation of camel milk for selected processing related parameters and comparisons with cow and buffalo milk. Int J Health Anim Sci Food Saf, 3: 27-37, doi: 10.13130/2283-3927/7446

Sharma UP, Rao SK and Zariwala IT, 1980. Composition of milk of different breeds of buffaloes. Indian J Dairy Sci, 33(1): 7-12

Sindhu JS and Arora S, 2011. Buffalo milk. In Encyclopedia of Dairy Sciences (2nd edn, pp 503-511) (Eds.) JW Fuquay, PF Fox and PLH McSweeney, London: Academic Press in an imprint of Elsevier

Sindhu JS and Roy NK, 1973. Partitioning of buffalo milk minerals. 1. Study through dialysis. Milchwissenschaft, 28(9): 573-575

Sindhu JS and Roy NK, 1982. Distribution of 45Ca between soluble and colloidal phases of buffaloes' milk as affected by processing. J Sci Food Agric, 33(3): 299-302, doi: 10.1002/jsfa.2740330317

Sindhu JS and Tayal M, 1983. Influence of homogenization on the heat stability and salt balance of buffalo milk and its 1: 2 concentrates. J Food Process Preserv, 7(4): 237-245, doi: 10.1111/j.1745-4549.1983.tb00825.x

Singh M, Sharma R, Ranvir S, Gandhi K and Mann B, 2019. Profiling and distribution of minerals content in cow, buffalo and goat milk. Indian J Dairy Sci, 72(5): 480-488, doi : 10.33785/IJDS.2019.v72i05.004.

Soetan KO, Olaiya CO and Oyewole OE, 2010. The importance of mineral elements for humans, domestic animals and plants- A review. Afr J Food Sci, 4(5): 200-222

Soliman GZ, 2005. Comparison of chemical and mineral content of milk from human, cow, buffalo, camel and goat in Egypt. Egypt J Hosp Med, 21(1): 116-130, doi: 10.21608/EJHM.2005.18054

Sommer HH and Binney TH 1923. A Study of the factors that influence the coagulation of milk in the alcohol test 1. J Dairy Sci, 6(3): 176-197, doi: 10.3168/jds.S0022-0302(23)94078-6.

Sorathiya LM, Tyagi KK, Patel MD and Raval AP, 2018. Effect of non-genetic factors on milk compositions in Surti buffaloes. Indian Vet J, 95(1): 43-45

Steel RGD and Torrie JH, 1980. Principles and Procedures of Statistics- A Biometrical Approach, 2nd edn, New York: McGraw Hill 137-167

Tayal M and Sindhu JS, 1983. Heat stability and salt balance of buffalo milk as affected by concentration and addition of casein. J Food Proc Preser, 7(3): 151-160, doi: 10.1111/j.1745-4549.1983.tb00673

Thiruvenkadan AK, Rajendran R and Muralidharan J, 2013. Buffalo genetic resources of India and their conservation. Buffalo Bull, 32(1): 227-235

Tyagi KK, Brahmkshtri BP, Ramani UV, Kharadi VB, Pandaya GM *et al*., 2016. Test day variability in yield and composition of Surti and Mehsani buffaloes milk at day 15 and 60 postpartum. Vet World, 9(6): 595-600, doi: 10.14202/vetworld.2016.595-600

Yoganandi J, Mehta BM, Wadhwani KN, Darji VB and Aparnathi KD, 2014. Evaluation and comparison of camel milk with cow milk and buffalo milk for gross composition. J Camel Pract Res, 21(2): 259-265, doi: 10.5958/2277-8934.2014.00046.0.

Zamberlin S, Antunac N, Havranek J and Samarzija D, 2012. Mineral elements in milk and dairy products. Mljekarstvo, 62(2): 111-125

Received- 30.09.2022, Accepted- 13.05.2023, Published- 05.07.2023 (Online), 01.12.2023 (Print)

Section Editor: Prof. M. K. Sanyal, Member, Editorial Board