International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(7): 760-763
www.biochemjournal.com
Received: 10-05-2025
Accepted: 14-06-2025

Rachel Jemimah E
Assistant Professor, Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Kattupakkam, Tamil Nadu, India

S Jaishankar
Professor and Head, Department of Livestock Production Management, Veterinary College and Research Institute, Salem, Tamil Nadu, India

D Balasubramanyam
Professor and Head, Department of Animal Genetics and Breeding, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, Tamil Nadu, India

P Tensingh Gnanaraj
Professor and Head, Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Kattupakkam, Tamil Nadu, India

Corresponding Author:
Rachel Jemimah E
Assistant Professor, Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Kattupakkam, Tamil Nadu, India

Influence of non-genetic factors on milk composition traits in Murrah buffaloes under a tropical savanna climate

Rachel Jemimah E, S Jaishankar, D Balasubramanyam and P Tensingh Gnanaraj

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i7Sj.4982

Abstract
The study was conducted to evaluate the influence of non-genetic factors namely lactation order, lactation stage, level of milk yield, and season on the milk composition traits of Murrah buffaloes under a tropical savanna climate. A total of 798 milk samples were collected from Murrah buffaloes reared at the Post Graduate Research Institute in Animal Sciences, Kattupakkam, Tamil Nadu, between April 2019 and August 2021. The samples were analyzed using an automatic milk analyzer to determine fat, solids-not-fat (SNF), total solids (TS), protein, lactose, and salt concentrations. The data were categorized and analyzed using an appropriate statistical model. The level of milk yield, lactation order, and lactation stage significantly influenced ($p<0.01$) all milk constituents except salt. Buffaloes in their fourth lactation and at the third lactation stage exhibited higher levels of milk constituents. Milk yield levels of 0-3 litres were associated with the highest concentrations of fat, SNF, TS, and protein. Seasonally, milk collected during the monsoon had significantly higher fat and protein content, followed by summer. Positive correlations were observed among fat, SNF, TS, protein, and lactose, as well as between lactation order/stage and most milk constituents. A negative correlation was noted between season and fat, SNF, TS, and protein. These findings suggest that milk composition in Murrah buffaloes is notably influenced by physiological and environmental factors, with implications for improving milk quality and nutritional value through management strategies tailored to these non-genetic influences.

Keywords: Murrah, milk composition, lactation order, lactation stage, season

Introduction
India is regarded as a home of the world's best buffalo germplasm. Buffaloes, with a population of 109.85 million, contribute around 20.45% to the total livestock population in India (Livestock Census, 2019) [7] and account for about 49.2% of total milk production in the country (BAHFS, 2017) [1]. Murrah is one of the superior breeds of Indian buffaloes. The home tract of Murrah buffaloes is Haryana, but graded Murrah buffaloes are found throughout the country due to their higher milk production potential, wide adaptability to different environmental conditions, and efficient feed conversion.
Although Murrah is considered the best and highest milk-producing buffalo breed, its milk yield, composition, and reproductive efficiency vary under different management and environmental conditions (Bishnoi and Singh, 2009) [3]. Compared to cow milk, buffalo milk is richer in almost all the major milk nutrients (El-Salam and El-Shibiny, 2011) [5]. Milk fat represents the major constituent of buffalo milk, followed by lactose (Boro *et al.*, 2018) [4]. Murrah is the best performing breed in terms of fat, total protein, and casein contents (Yadav *et al.*, 2013) [14].
Milk composition is not constant in dairy buffaloes and is influenced by several factors including breed and species differences, parity or lactation order, age and size of the animal, dietary composition, season, pregnancy, sire, service period, BCS, DP, lameness, heat stress, behaviour or milking temperament, udder health, locality, and stage of lactation (Boro *et al.*, 2018) [4]. Determining the genetic and non-genetic estimates and correlations of these parameters might help in selecting appropriate methods of selection, predicting direct and correlated responses, estimating genetic gains, and identifying suitable breeding

systems for future improvement (Verma *et al.*, 2017) [13]. Developing breeding programs aimed at altering milk composition requires an understanding of the relative influence of both genetic and environmental factors (Boro *et al.*, 2018) [4]. Furthermore, understanding the behaviour of milk components at various stages of lactation will help in producing value-added milk and milk products that promote health (Boro *et al.*, 2018) [4].

With this background, the current work was carried out to understand the variations in milk composition across different stages and orders of lactation, and seasons, in Murrah buffaloes under a tropical savanna climate.

Materials and Methods

Fat, Solids Not Fat (SNF), Total Solids (TS), protein, lactose, and salt concentrations of 798 milk samples were analyzed using an automatic milk analyzer between April 2019 and August 2021 at the Post Graduate Research Institute in Animal Sciences, Kattupakkam and Tamil Nadu. To study the effect of various non-genetic factors on milk composition in Murrah buffaloes, the data were categorized based on lactation order (1st, 2nd, 3rd, 4th), lactation stages (Early: 5-90 days; Mid: 91-180 days; Late: 181-305 days), level of milk yield (1st : 0-3 litres; 2nd : 3-6 litres; 3rd: 6-9 litres; 4th: > 9 litres) and season (Summer: March-June; Monsoon: July to October; Winter: November to February). A one-way ANOVA was initially performed to evaluate the individual effects of season, lactation order, and lactation stage on milk composition. Subsequently, a multivariate general linear model (GLM) was employed using IBM SPSS Statistics v.25 to analyze the combined effect of fixed factors on milk composition traits. Non-significant interactions (p>0.05) were excluded from the model, and the data were re-analyzed with only significant main effects and their interactions.

The model fitted was

Yijk=μ+LOi+LSj+Sk+LOi*Sk+LOi*LSj+Sk*LSj+LOi*LSj*Sk+eijk

Where,

- Yijk = Milk composition of Murrah buffaloes during different lactation order, lactation stage and season.
- μ = Mean.
- LOi = Effect of ith order of lactation.
- LSj = Effect of jth stage of lactation.
- Sk = Effect of kth season.
- LOi*Sk = Effect of interaction of ith order and kth season.
- LOi*LSj = Effect of interaction of ith order and jth stage of lactation.
- Sk*LSj = effect of interaction of kth season and jth stage of lactation.
- LOi*LSj*Sk = Effect of interaction of ith order of lactation, jth stage of lactation and kth season.
- eijk = Random error.

Additionally, Pearson's correlation analysis was conducted to assess the relationships among milk composition traits and influencing factors. Statistical differences were considered significant at p<0.05, and Turkey's post hoc test was used to separate the means.

Results and Discussion

Milk composition is the outcome of various biological processes, which are influenced by environmental conditions, feed intake, and the physiological status of animals (Neville *et al.*, 2001) [8].

Lactation Order

The changes in milk composition across different lactation orders in Murrah buffaloes are presented in Table 1. Lactation order had a significant effect on all milk components except salt. A higher concentration (p<0.01) of all milk constituents was observed during the 4th lactation, followed by the 1st lactation. The overall trend of milk component concentrations across lactation orders was: 4th > 1st > 2nd > 3rd. Similar results were reported by Sundaram and Hariharan, (2013) [12], who observed significant effects (p<0.01) of lactation number on fat%, protein%, total solids%, and casein% in Murrah buffaloes. Yadav *et al.*, (2013) [14] also noted increased fat and protein concentrations with advancing parity up to the fifth lactation.

- **Fat:** The highest fat percentage (p<0.01) was recorded in the 4th lactation, followed by the 1st. This is consistent with Yadav *et al.*, (2013) [14]. However, Pawar *et al.*, (2012) [10] and Verma *et al.*, (2017) [13] reported no consistent trend in fat percentage across parities in Murrah buffaloes.
- **SNF and TS:** Significantly higher (p<0.01) SNF and TS percentages were observed in the 4th lactation, followed by the 1st. Sundaram and Hariharan, (2013) [12] observed higher TS in the 1st lactation. Verma *et al.*, (2017) [13] reported that SNF was unaffected by parity, contrasting with the present findings.
- **Protein:** The highest protein percentage (p<0.01) was recorded during the 4th lactation. No significant differences were observed between the 1st, 2nd, and 3rd lactations. In contrast, Sundaram and Hariharan, (2013) [12] reported peak protein concentration in the 3rd parity, and Yadav *et al.*, (2013) [14] found no variation in protein up to the 6th parity.
- **Lactose:** A significantly higher (p<0.01) lactose percentage was noted in the 4th lactation, followed by the 1st. Yadav *et al.*, (2013) [14], however, reported increased lactose in the 2nd and 5th parities, attributing it to water content variations.
- **Salt:** Salt concentration remained consistent across all lactation orders, similar to findings in Marathwadi buffaloes (Kekan *et al.*, 2021) [6].

Lactation Stage

Milk composition across different lactation stages in Murrah buffaloes is presented in Table 2. All components, except salt, were significantly affected (p<0.01) by lactation stage. Similar findings were reported by Bhonsle *et al.*, (2003) [2], Yadav *et al.*, (2013) [14], and Siddiqui *et al.*, (2021) [11]. Late lactation showed significantly higher concentrations of fat, SNF, TS, protein, and lactose, followed by mid-lactation. The lowest concentrations were observed in early lactation.

Increases in fat, protein, and lactose with advancing lactation stage were also reported by Yadav *et al.*, (2013) [14] and Siddiqui *et al.*, (2021) [11]. However, Yadav *et al.*, (2013) [14] noted a decrease in protein, and Siddiqui *et al.*, (2021) [11] reported a decline in lactose at later stages.

Bhonsle *et al.*, (2003) [2] found constant SNF levels across stages but observed a rising trend in TS, aligning with the present results.

Level of Milk Yield

Changes in milk composition across different milk yield levels are presented in Table 3. A decrease in milk composition (p<0.01) was observed with increasing milk yield. Higher concentrations of fat, SNF, TS, protein, and lactose (except salt) were noted in animals yielding up to 3 litres, followed by the 3-6 liter group. Milk composition decreased with every 3-litre increase in yield up to 6 litres, then stabilized. This may be due to the dilution effect, where constituent concentration per unit volume decreases as yield increases (Siddiqui *et al.*, 2021) [11].

Season

Seasonal variations in milk composition are presented in Table 4. Season had a significant effect on fat (p<0.05), protein, and salt (p<0.01) content. Fat and protein concentrations were highest during the monsoon, followed by summer, and lowest in winter. SNF, TS, and lactose were not significantly influenced by season. (Yadav *et al.*, (2013) [14] reported reduced milk fat during the monsoon and increased protein and lactose during winter, which contrasts with our results. Conversely, Pawar *et al.*, (2012) [10] found higher fat concentrations in winter. Seasonal reductions in milk constituents may be due to increased yield diluting solids in winter, and the reverse effect during hotter months (Yadav *et al.*, 2013) [14]. However, Verma *et al.*, (2017) [13] reported that fat and SNF were unaffected by season in Murrah buffaloes.

Interactions

Multivariate analysis revealed that milk composition is significantly influenced by the interactions between lactation order, stage, and season. Fat, TS, protein, and salt were significantly (p<0.01) affected by double interactions including LO × Season, LO × LS, and Season × LS. Lactose was significantly influenced by the LO × Season interaction. Salt showed significance under the LO × LS × Season triple interaction. The model explained 54% of the variation in fat, 45% in TS, and 48% in protein due to these factors and their interactions. Contrary to these findings, Yadav *et al.*, (2013) [14] observed no significant interactions among parity, lactation stage, and season in Murrah buffaloes.

Correlation

Correlations among non-genetic factors and milk constituents are shown in Table 5. Milk fat, SNF, TS, protein, and lactose were positively correlated (p<0.01), indicating that an increase in fat was associated with increases in the other components. Lactation order and lactation stage were also positively correlated (p<0.01) with fat, SNF, TS, protein, and lactose, meaning that these constituents increased with advancing order and stage.

Season, however, showed a negative correlation (p<0.01) with fat, SNF, TS, and protein, indicating that as seasons progressed from summer to winter, these component concentrations declined. Bhonsle *et al.*, (2003) [2] also reported positive correlations among fat, TS, and protein, but noted a negative correlation between fat and SNF, which contradicts our findings. Pandey, (1981) [9] similarly observed positive and significant correlations between various milk constituents, including protein and lactose, in buffaloes.

Table 1: Changes in milk composition over different order of lactation in Murrah buffaloes

SI. No.	Lactation order	N	Mean ±S.E.					
			Fat	SNF	TS	Protein	Lactose	Salt
			(%)					
1.	1st	190	7.24^b±0.09	9.21^b±0.05	16.42^b±0.14	3.49^a±0.02	4.94^b±0.03	0.69±0.16
2.	2nd	145	6.75^a±0.13	9.02^{ab}±0.08	15.67^a±0.20	3.40^a±0.03	4.87^{ab}±0.04	0.69±0.24
3.	3rd	355	6.59^a±0.13	8.80^a±0.08	15.39^a±0.20	3.31^a±0.03	4.74^a±0.04	1.16±0.24
4.	4th	108	8.51^c±0.13	9.75^c±0.08	18.10^c±0.21	3.94^b±0.04	5.23^c±0.04	0.69±0.25
	F-Value		30.43**	20.75**	24.20**	52.43**	24.00**	4.29^{NS}
7.	Overall mean	798	7.16±0.06	9.14±0.04	16.24±0.09	3.50±0.02	4.92±0.02	0.94±0.11

^{NS} Non-significant; ** Significant at p<0.01; Means bearing different superscript in the same column differ significantly.

Table 2: Changes in milk composition over different stage of lactation in Murrah buffaloes

SI. No.	Lactation stage	N	Mean ±S.E.					
			Fat	SNF	TS	Protein	Lactose	Salt
			(%)					
1.	Early (Day 5 to 90)	319	5.58^a±0.10	8.67^a±0.06	14.23^a±0.16	3.15^a±0.03	4.71^a±0.03	1.59±0.19
2.	Mid (Day 191 to 180)	283	6.99^b±0.09	9.04^b±0.06	15.96^b±0.15	3.43^b±0.03	4.82^b±0.03	0.68±0.18
3.	Late (Day 181 to 305)	196	8.77^c±0.11	9.68^c±0.06	18.36^c±0.17	3.89^c±0.03	5.21^c±0.03	0.67±0.20
	F-Value		80.12**	24.00**	55.43**	47.80**	18.35**	1.63^{NS}

^{NS} Non-significant; ** Significant at p<0.01
Means bearing different superscript in the same column differ significantly.

Table 3: Changes in milk composition over different level of milk yield in Murrah buffaloes

SI. No.	Level of milk yield (litres)	N	Mean ±S.E.					
			Fat	SNF	TS	Protein	Lactose	Salt
			(%)					
1.	1st (0-3)	71	8.46^c±0.17	9.72^c±0.09	18.18^c±0.24	3.81^c±0.05	5.19^b±0.05	0.68±0.01
2.	2nd (3-6)	376	7.45^b±0.10	9.24^b±0.05	16.59^b±0.15	3.57^b±0.03	4.98^b±0.03	0.68±0.01
3.	3rd (6-9)	338	6.05^a±0.08	8.79^a±0.04	14.84^a±0.11	3.24^a±0.02	4.73^a±0.02	0.90±0.21
4.	4th (> 9)	16	6.11^a±0.34	8.65^a±0.17	14.76^a±0.50	3.22^a±0.09	4.66^a±0.09	0.67±0.01
		F value	62.43**	31.32**	50.45**	50.37**	30.30**	0.52^{NS}

^{NS} Non-significant; *Significant at p<0.05; ** Significant at p<0.01; Means bearing different superscript in the same column differ significantly.

Table 4: Changes in milk composition over different season in Murrah buffaloes

SI. No.	Season	N	Mean ±S.E.					
			Fat	SNF	TS	Protein	Lactose	Salt
			(%)					
1.	Summer (March to June	356	7.04^b±0.09	9.17±0.05	16.46±0.14	3.44^b±0.02	4.92±0.03	1.32^b±0.17
2.	Monsoon (July to October)	178	7.74^c±0.12	9.31±0.07	16.97±0.19	3.75^c±0.03	5.04±0.04	0.67^a±0.23
3.	Winter (November to February)	264	6.37^a±0.10	8.95±0.06	15.32±0.16	3.25^a±0.03	4.80±0.03	0.70^a±0.19
		F value	3.91*	0.56 ^{NS}	1.89 ^{NS}	5.60**	1.99 ^{NS}	8.37**

^{NS} Non-significant; *Significant at p<0.05; ** Significant at p<0.01; Means bearing different superscript in the same column differ significantly.

Table 5: Correlation between non-genetic factors and milk composition in Murrah buffaloes (n=798)

SI. No.	Factors	SNF	TS	Protein	Lactose	Salt	Lactation order	Lactation stage	Season
1.	Fat	0.794^{**}	0.948^{**}	0.916^{**}	0.653^{**}	-0.052^{NS}	0.161^{**}	0.641^{**}	-0.219^{**}
2.	SNF		0.870^{**}	0.893^{**}	0.840^{**}	-0.021^{NS}	0.101^{**}	0.412^{**}	-0.095^{**}
3.	TS			0.922^{**}	0.722^{**}	-0.042^{NS}	0.147^{**}	0.579^{**}	-0.168^{**}
4.	Protein				0.788^{**}	-0.036^{NS}	0.188^{**}	0.561^{**}	-0.147^{**}
5.	Lactose					-0.010^{NS}	0.102^{**}	0.371^{**}	-0.068^{NS}
6.	Salt						0.031^{NS}	-0.044^{NS}	-0.031^{NS}

^{NS} Non-significant; *-Significant p<0.05; ** Significant p<0.01

Conclusion

The present study highlights the significant influence of non-genetic factors such as lactation order, lactation stage, level of milk yield, and season on the milk composition traits of Murrah buffaloes under tropical savanna climate. Among these, lactation order and stage showed a consistent positive impact on milk constituents like fat, SNF, TS, protein, and lactose, with the fourth order and late lactation stage yielding the highest concentrations. Conversely, increased milk yield levels were associated with a dilution effect, leading to reduced concentration of most milk components. Seasonal variation also influenced certain milk constituents, with monsoon season showing higher fat and protein concentrations, while winter recorded lower values. Significant interactions among these factors were observed, particularly affecting fat, TS, protein, and salt content, emphasizing the complex interplay between physiological and environmental influences on milk composition. The positive correlations among milk constituents and with lactation parameters further reinforce the interconnected nature of these traits. These findings can serve as a basis for nutritional and management interventions aimed at optimizing milk quality in Murrah buffaloes under tropical conditions.

Acknowledgement

The Authors express their sincere gratitude to the facilities provided by the Vice-Chancellor, TANUVAS.

References

1. Basic Animal Husbandry and Fisheries Statistics (BAHFS). Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Government of India; 2017 (cited 2024 Jun 6). Available from: https://dahd.nic.in/sites/default/files/Basic%20Animal%0Husbandry%20and%20Fisheries%20Statistics%202017.pdf
2. Bhonsle D, Chourasia SK, Singh M, Jain RK. Factors influencing major milk constituents in Murrah buffaloes. Indian J Anim Sci. 2003;73(1):107-109.
3. Bishnoi DK, Singh R. Comparative feeding practices of milking animals in Haryana. Indian J Anim Nutr. 2009;26(3):243-246.
4. Boro P, Debnath J, Das TK, Naha BC, Debbarma N, Debbarma P, *et al*. Milk composition and factors affecting it in dairy buffaloes: A review. J Entomol Zool Stud. 2018;6(3):340-3.
5. El-Salam MHA, El-Shibiny S. A comprehensive review on the composition and properties of buffalo milk. Dairy Sci Technol. 2011;91(6):663-699.
6. Kekan PM, Wankar K, Daware SB, Amrutkar SA. Effect of seasons × meteorological variables on normal milk constituents in Marathwadi buffaloes. Int J Curr Microbiol Appl Sci. 2021;10(2):388-392.
7. Livestock Census. Key Results & Annexure. Department of Animal Husbandry and Dairying, Government of India; 2019 (cited 2024 Jun 6). Available from: https://www.dahd.nic.in/sites/default/files/Key%20Results%2BAnnexure%2018.10.2019.pdf
8. Neville MC, Morton J, Umemura S. Lactogenesis: the transition from pregnancy to lactation. Pediatr Clin North Am. 2001;48(1):35-52.
9. Pandey HS. Genetic studies on the milk constituents of buffaloes and their association with related traits (M.V.Sc. thesis). Izatnagar: Indian Veterinary Research Institute; 1981.
10. Pawar HN, Ravikumar GVPPS, Narang R. Effect of year, season and parity on milk production traits in Murrah buffaloes. J Buffalo Sci. 2012;1(1):122-5.
11. Siddiqui ASS, Kodape AH, Ingole SD, Kharwadkar MD. Effects of different stages of lactation on milk components of Murrah buffalo. Pharm Innov J. 2021;SP-10(2):91-96.
12. Sundaram M, Hariharan G. Preliminary study on evaluation of effect of lactation number on milk yield and milk composition in Murrah (*Bubalus bubalis*) buffaloes. Res J Anim Vet Fish Sci. 2013;1(7):21-23.
13. Verma MK, Sachdeva GK, Yadav AK, Gautam S, Ali MM, Kumar S. Effect of genetic and non-genetic factors on milk yield and milk constituents in Murrah buffalo. Indian J Anim Res. 2017;51(2):387-390.
14. Yadav SP, Sikka P, Kumar D, Sarkar S, Pandey AK, Yadav PS, *et al*. Variation in milk constituents during different parity and seasons in Murrah buffaloes. Indian J Anim Sci. 2013;83(7):747-751.