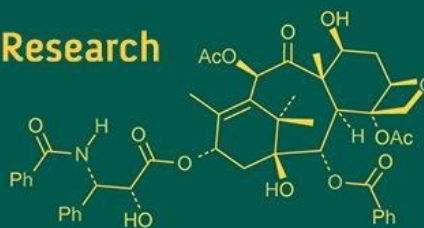


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Navin B

M.V.Sc. Scholar, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University (KVASU), Kerala, India

Karthiayini K

Professor and Head, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University (KVASU), Kerala, India

Haripriya K

M.V.Sc. Scholar, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University (KVASU), Kerala, India

Gleeja VL

Associate Professor and Head, Department of Statistics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University (KVASU), Kerala, India

Sajana OK

Teaching Assistant, Department of Statistics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University (KVASU), Kerala, India

Corresponding Author:**Navin B**

M.V.Sc. Scholar, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University (KVASU), Kerala, India

Seasonal influence on total antioxidant capacity of milk samples from different domestic species of Kerala

Navin B, Karthiayini K, Haripriya K, Gleeja VL and Sajana OK

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Abstract

Milk is rich in vitamins, minerals, fats and high-quality proteins. Milk proteins namely casein, lactoferrin and amino acids such as tyrosine, cysteine, tryptophan and lysine have antioxidant properties. The present study was conducted with the objective of comparing the seasonal influence on total antioxidant capacity of milk samples collected from different species present in various farms of Kerala Veterinary and Animal Sciences University, Mannuthy. The experiment was conducted in two seasons: summer (May) followed by monsoon (July). Pooled milk samples of Attapady and Malabari goats, Murrah buffaloes, crossbred (Holstein Friesian × Sahiwal) cows and native Vechur cows were collected during early morning milk collection for a period of 10 days in each season. Estimation of total antioxidant capacity was done on the same day of collection by ferric reducing antioxidant power (FRAP) assay. The results revealed that there was no seasonal effect on antioxidant capacity of milk of Vechur cows, buffaloes and crossbred cows. But milk of Attapady and Malabari goats shown highest antioxidant capacity in monsoon compared to summer. Analysing in different species, Vechur milk has the highest antioxidant capacity followed by buffalo milk and other species. Hence, it can be concluded from the study that besides the seasonal effects, Vechur and buffalo milks had highest antioxidant capacity followed by goat milk. Goat milk could be suggested instead of cow milk owing to its therapeutic significance.

Keywords: Total antioxidant capacity, ferric reducing antioxidant power, milk samples, domestic species, Vechur cows

1. Introduction

Milk contains high quality proteins, antioxidant-rich carotenoids and flavonoids, conjugated linoleic acid (CLA), vitamin C (ascorbic acid), vitamin E, phospholipids, coenzyme Q₁₀ and various physiologically functional components. Many genetic and environmental factors, including parity, breed, stages of lactation and climatic fluctuations have an impact on the composition of milk (Bernard *et al.*, 2018) [8]. Antioxidant capacity of milk and dairy products is mainly due to sulphur containing amino acids, vitamins, carotenoids, endogenous enzymes such as superoxide dismutase, catalase and glutathione peroxidase (Usta and Yilmaz, 2013) [21]. Amino acids in milk such as tyrosine, tryptophan, lysine, cysteine, leucine and serine have antioxidant properties (Rafiq *et al.*, 2016 [20]; Layman *et al.*, 2018 [17]). The major milk proteins such as casein and lactoferrin have antioxidant mechanisms among which caseins present in the form of macromolecular aggregates are considered as the significant scavengers of free radicals (Clausen *et al.*, 2009) [11] owing to their ability to inhibit thiobarbituric reactive substances (TBARS) and lipid peroxide formation. Antioxidants offer defense against free radicals, hence sufficient quantity of antioxidants is necessary to counterbalance the disruption caused by free radicals. Intake of antioxidants in the form of foods rich in antioxidants may protect the body from oxidative stress and cellular damage (Wang, 2006) [22]. Hence the consumer may be protected by drinking the milk that has a higher antioxidant capacity which may neutralise the free radicals. Decreased antioxidant defense mechanism in human body might result in oxidative stress leading to damage of DNA/RNA, proteins and lipid peroxidation of membranes and disruption of normal cellular metabolism. Recent trends in consumption of milk samples from various domestic species highlight their high antioxidant capacities and potential health benefits.

Buffalo milk, with its higher fat and tocopherol content, is being explored for consumption to enhance antioxidant intake. Goat milk's rich selenium and zinc content, along with bioactive peptides, makes it a focus for research on gut health and reducing oxidative stress. Meanwhile, previous reports shown that Vechur cow milk had the highest antioxidant capacity, attributed to its unique composition and high levels of bioactive peptides and antioxidants (Annie *et al.*, 2023) [5]. Therefore, Vechur milk and goat milk have better medicinal values and buffalo milk have higher antioxidant property when compared to *Bos taurus* (Annie *et al.*, 2019) [4]. However, studies were done to compare the antioxidant capacity of Vechur milk with goat milk. But no studies were done to compare the seasonal influence on total antioxidant capacity of different domestic species. Antioxidant status being an essential component of medicinal and therapeutic significance of milk, the present study was conducted to assess and compare the seasonal impacts on total antioxidant capacity (TAC) of milk from Attapady and Malabari goats, crossbred (Holstein Friesian × Sahiwal) cows, Murrah buffaloes and Vechur cattle of Kerala during months of May and July. The present study will also elucidate the antioxidant capacity of milk of these important breeds which are of medicinal and nutritional significance for human consumption during different environmentally challenged seasons.

2. Materials and methods

2.1 Experimental layout and Location of study

The experiment was conducted in two seasons: summer (May) followed by monsoon (July) in various farms of College of Veterinary and Animal Sciences, Mannuthy of Kerala Veterinary and Animal Sciences University. Pooled milk samples of 10 ml each were collected in a sterile container from Attapady and Malabari goats of University Goat farm, Mannuthy, crossbred cows of University Livestock Farm, Murrah buffaloes of buffalo farm and Vechur cows of Vechur cattle farm during early morning milk collection for 10 simultaneous days in each season. The samples were analysed for TAC on the same day of collection instantaneously for higher accuracy of antioxidant capacity of milk samples in the Central Instruments Laboratory (CIL), College of Veterinary and Animal Sciences, Mannuthy.

2.2 Estimation of total antioxidant capacity of milk

The ferric reducing antioxidant power (FRAP) assay by Benzie and Strain, 1996^[7] was used as a direct method for measuring the total antioxidant capacity of milk samples. In the FRAP assay, the ability of milk sample to reduce ferric ion is used as a criterion for the measurement of TAC. At low pH, ferric 2,4,6-tripyridyl-s-triazine [Fe (III)-TPTZ] complex gets reduced to ferrous 2,4,6-tripyridyl-s-triazine [Fe (II)-TPTZ] complex, which has an intense blue colour, can be monitored by measuring the change in absorption at 593 nm using a spectrophotometer. Working FRAP reagent was prepared by using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, plus 16 mL glacial acid); 10 mM TPTZ [2,4,6-tris (2-pyridyl)-s-triazine], in 40 mM HCl; and 20 mM FeCl₃•6H₂O in the ratio of 10:1:1. Milk sample (100 µL) at room temperature was mixed with 3 mL of FRAP reagent and kept in dark for 10 minutes. Then the sample was centrifuged at 10000 rpm for 5 minutes and supernatant solution was taken. The absorbance of the supernatant

solution was monitored at wavelength 593 nm with Perkin Elmer's LAMBDA UV-Vis spectrophotometer. Aqueous solutions of FeSO₄•7H₂O (100–3000 µM) was used as standard. The change in absorbance was translated into FRAP value (µM) by relating ΔA₅₉₃ of test sample with that of standard solution of known FRAP value using formula.

Calculation of FRAP value

$$\frac{0 - 5 \text{ min } \Delta A_{593} \text{ test sample}}{0 - 5 \text{ min } \Delta A_{593} \text{ standard}} \times \text{FRAP value of standard}$$

The data is shown as FRAP values (µM/mL Fe (II)).

2.3 Statistical analysis

The results were expressed as Mean ± Standard Error. Statistical analysis of the calculated data was carried out by univariate analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 24.0 software.

3. Results and Discussion

The present study shown that there was no significant difference found in the ferric reducing antioxidant power in the milk samples of Murrah buffaloes, crossbred cows and Vechur cattle between seasons (Table 1). Despite the seasonal comparison, Vechur cow milk shown the highest antioxidant capacity followed by buffalo milk (Fig. 1). The highest antioxidant capacity of Vechur milk was owing to its more fat and solid not fat (SNF) content (Abraham and Gayathri, 2015) [1]. Another reason might be due to α-Tocopherol, a compound present in milk fat globule membrane is the most effective lipid-soluble antioxidant present in milk of dairy animals (Bernard *et al.*, 2018) [8]. Chinnamma *et al.* (2015) [10] studied the structural and functional features of lactoferrin gene of Vechur cattle and the gene was expressed for the production of recombinant bovine lactoferrin protein which also contributes to the antioxidant capacity of Vechur cow milk by preventing the oxidation of fatty acids. Lactoferrin possess antimicrobial, antitumor and anti-inflammatory properties. According to Chen *et al.* (2000) [9], there was a relationship between milk fat content and antioxidant activity which might have contributed to the higher TAC of Vechur milk. Besides that, Vechur cow milk might have high buffering capacity, easily digestible and is therapeutically important value as it lowers blood cholesterol levels in humans (Chinnamma *et al.*, 2015) [10]. Also, the Vechur milk contains higher beta casein A2 than A1. According to Anisha *et al.* (2012) [3], SNF, protein and lactose levels in Vechur cows were stable throughout the seasons, elucidating the adaptability and climatic resilience of the breed in terms of primary milk ingredients which precisely validates the present findings. Buffalo milk due to its high fat content from the energy point of view is more valuable and has a higher antioxidant capacity compared to cow milk as it is rich in bioactive compounds promoting healthier diet (Khan *et al.*, 2017) [16]. But in Attapady and Malabari goats, the TAC values were significantly increased in the monsoon season when compared to summer season, indicating their highest antioxidant capacity in monsoon where the environmental challenges to the goats are lesser when compared to summer with highest temperature-humidity challenges. This finding is in accordance with Cloetens *et al.* (2013) [12] and

Alyaqoubi *et al.* (2014) [12] who reported that factors such as natural pasture and season influence the nutritional composition and antioxidant capacity of milk. Besides the seasonal comparison, species wise comparison in the study shown that goat milk has higher antioxidant capacity followed by Vechur and buffalo milk samples. Also, comparing the goat milk and crossbred cow milk, goat milk has significantly higher antioxidant capacity owing to its lipid content, higher specific gravity, viscosity and titrable acidity and lower refractive index and freezing point than cow milk (Haenlein and Wendorff, 2006; Park *et al.*, 2007, Barłowska *et al.*, 2011) [14, 19, 6].

Considering medicinal importance, goat milk supplies vitamin A, niacin, thiamine, riboflavin and pantothenic acid

for human infants (Haenlein, 2004) [13]. It also provides excess of Ca and P in relation to energy to human infant, which are easily absorbed by them (Jenness, 1980) [15]. It came into light that goat milk has been recommended as a substitute for patients allergic to cow milk. Reports shown that between 40-100% of patients allergic to cow milk proteins could tolerate goat milk (Park, 1994) [18]. Hence it was clear from the findings that milk of Vechur cows, buffaloes, Attapady and Malabari goats are highly recommended for human consumption when compared to milk of crossbred cows to improve their antioxidant capacity and prevent from ageing and lifestyle diseases.

Table 1: Seasonal comparison of ferric reducing antioxidant power ($\mu\text{M}/\text{mL}$ Fe (II)) values in milk samples of different domestic species (Mean $\pm$ SE, n=6)

Species/Seasons	Attapady goats	Malabari goats	Murrah buffaloes	Crossbred cows	Vechur cows
Summer (May)	1306.48 ^{aA} $\pm$ 31.04	1356.51 ^{aA} $\pm$ 31.04	1490.48 ^{bA} $\pm$ 31.04	1111.18 ^{cA} $\pm$ 31.04	1728.86 ^{dA} $\pm$ 31.04
Monsoon (July)	1405.68 ^{aB} $\pm$ 31.04	1444.40 ^{aB} $\pm$ 31.04	1534.67 ^{bA} $\pm$ 31.04	1188.42 ^{cA} $\pm$ 31.04	1740.23 ^{dA} $\pm$ 31.04
p-value	$p < 0.05$ ($p = 0.026$)	$p < 0.01$ ($p = 0.001$)	$p = 0.317^{\text{ns}}$	$p = 0.08^{\text{ns}}$	$p = 0.796^{\text{ns}}$

Means bearing different superscript within a row (a-c) and columns (A-B) differ significantly ($p < 0.05$), ns – non-significant at 0.05 level

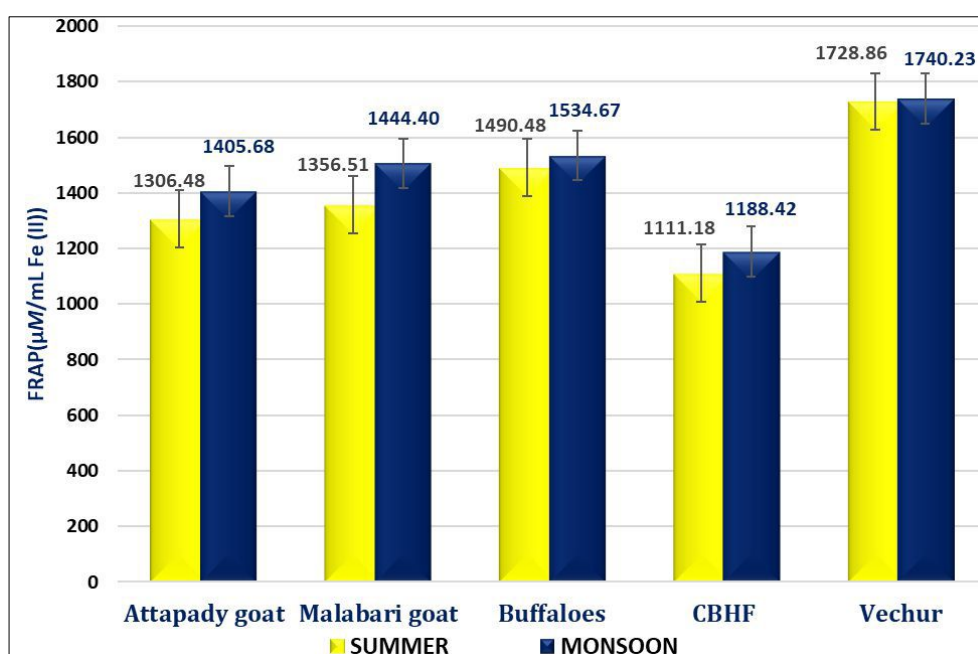


Fig 1: Ferric reducing antioxidant power ($\mu\text{M}/\text{mL}$ Fe (II)) in milk samples of different domestic species of Kerala during summer and monsoon seasons

4. Conclusion

The study highlighted the highest antioxidant capacity in milk of Vechur cows and buffaloes besides environmental factors in different seasons. Attapady and Malabari goat milk shown significantly higher antioxidant capacity in monsoon compared to summer elucidating the environmental challenges affecting the antioxidant capacity of milk of these goat breeds. Comparing the antioxidant capacity of crossbred cow milk with other species, it was evident that milk of Vechur cows, buffaloes, Attapady and Malabari goats are highly recommended for human consumption to enhance the antioxidant capacity for healthy human life style and well-being.

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6. Conflict of interest

The authors declare no conflict of interest.

7. References

- Abraham BL, Gayathri SL. Milk composition of crossbred and desi cattle maintained in the sub-tropical high ranges of Kerala. Indian J Vet Anim Sci Res. 2015 Jan;44(1):53-55.
- Alyaqoubi S, Abdullah A, Samudi M, Abdullah N, Addai ZR, Al-Ghazali M. Effect of different factors on

- goat milk antioxidant activity. *Int J Chem Tech Res.* 2014;6:3091-96.
3. Anisha S, Bhasker S, Mohankumar C. Recombinant lactoferrin (Lf) of Vechur cow, the critical breed of *Bos indicus* and the Lf gene variants. *Gene.* 2012. DOI: 10.5772/intechopen.103810.
 4. Annie VR, Karthiayini K, Sreekumar TR, Rajesh K, Lucy KM. Estimation of total antioxidant capacity of milk of Malabari goat and Vechur cattle during different lactation. *Pharma Innov J.* 2019;8:150-2.
 5. Annie VR, Karthiayini K, Lucy KM. Comparison of total antioxidant capacity of milk of crossbred Holstein Friesian and Vechur cattle during different lactation. *Asian J Dairy Food Res.* 2023. doi: 10.18805/ajdfr.DR-2130.
 6. Barłowska J, Szwajkowska M, Litwińczuk Z, Król J. Nutritional value and technological suitability of milk from various animal species used for dairy production. *Comprehensive reviews in food science and food safety.* 2011 Nov;10(6):291-302.
 7. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem.* 1996 Jul 15;239(1):70-6.
 8. Bernard L, Bonnet M, Delavaud C, Delosiere M, Ferlay A, Fougere H, *et al.* Milk fat globule in ruminants: major and minor compounds, nutritional regulation and differences among species. *Eur J Lipid Sci Technol.* 2018 May;120(5):1700039.
 9. Chen J, Lindmark-Månsson H, Åkesson B. Optimisation of a coupled enzymatic assay of glutathione peroxidase activity in bovine milk and whey. *Int Dairy J.* 2000 Jan 1;10(5-6):347-51.
 10. Chinnamma M, Shashidharan A, Bhasker S. Therapeutic value of milk of Vechur cow, the indigenous breed of Kerala listed as critical by FAO. In: *Biodiversity Conservation-Challenges for the Future.* 2015. p. 103-11.
 11. Clausen MR, Skibsted LH, Stagsted J. Characterization of major radical scavenger species in bovine milk through size exclusion chromatography and functional assays. *J Agric Food Chem.* 2009;57:2912-9.
 12. Cloetens L, Panee J. The antioxidant capacity of milk—the application of different methods *in vitro* and *in vivo*. *Cell Mol Biol.* 2013 Nov 3;59(1):43-57.
 13. Haenlein GF, Wendorff WL. Sheep milk. In: *Handbook of milk of non-bovine mammals.* 2006 Jan 13:137-94.
 14. Haenlein GF. Goat milk in human nutrition. *Small Rumin Res.* 2004 Feb 1;51(2):155-63.
 15. Jenssen H, Hancock RE. Antimicrobial properties of lactoferrin. *Biochimie.* 2009;91:19-29.
 16. Khan IT, Bule M, Ullah R, Nadeem M, Asif S, Niaz K. The antioxidant components of milk and their role in processing, ripening, and storage: functional food. *Vet World.* 2019 Jan;12(1):12.
 17. Layman DK, Lönnerdal B, Fernstrom JD. Applications for α -lactalbumin in human nutrition. *Nutr Rev.* 2018 Jun 1;76(6):444-60.
 18. Park YW, Juárez M, Ramos MG, Haenlein GF. Physico-chemical characteristics of goat and sheep milk. *Small Rumin Res.* 2007 Mar 1;68(1-2):88-113.
 19. Park YW. Hypo-allergenic and therapeutic significance of goat milk. *Small Rumin Res.* 1994 Aug 1;14(2):151-9.
 20. Rafiq S, Huma N, Pasha I, Sameen A, Mukhtar O, Khan MI. Chemical composition, nitrogen fractions and amino acids profile of milk from different animal species. *Asian-Australas J Anim Sci.* 2016;29:1022-8.
 21. Usta B, Yilmaz-Ersan L. Antioxidant enzymes of milk and their biological effects. *J Agric Faculty Uludag Univ.* 2013;2:123-30.
 22. Wang Y, Yu R, Chou C. Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. *Food Microbiol.* 2006;23:128-35.