DR-2130
[1-4]

RESEARCH ARTICLE Asian Journal of Dairy and Food Research

# Comparison of Total Antioxidant Capacity of Milk of Crossbred Holstein Friesian and Vechur Cattle during Different Lactation

V.R., Annie[1], K., Karthiayini[2], K.M., Lucy[1]

10.18805/ajdfr.DR-2130

## ABSTRACT

**Background:** Milk is a rich source of vitamins, minerals, fats and high quality proteins. Milk proteins such as casein, lactoferrin and amino acids in milk like tyrosine, cysteine, tryptophan, lysine, *etc.* have antioxidant properties. The objective of this study was to compare the antioxidant level of milk of crossbred Holstein Friesian (CBHF) and Vechur cattle during different stages of lactation.

**Methods:** Total antioxidant capacity (TAC) was estimated by using ferric reducing antioxidant assay. Milk samples from six animals each of CBHF and Vechur cattle were collected separately at early lactation, mid lactation and late lactation period.

**Results:** Milk samples from CBHF and Vechur cattle during the early stages of lactation had significantly higher total antioxidant capacity than milk samples from later stages of lactation. This could be because of the high fat content in the early phases. The total antioxidant content of Vechur milk was higher than that of CBHF milk throughout lactation. The milk of the Vechur cow contains higher beta casein A2 than A1. No significant difference in the TAC values noticed between milk of mid and late lactation in both CBHF and Vechur cattle. The high antioxidant capacity of milk from Vechur cattle suggests that it has a great therapeutic potential. Different animal milk and milk products with antioxidant defense systems can be considered to have health-beneficial effects against free radical damage.

**Key words:** Antioxidant capacity, CBHF, Ferric reducing antioxidant assay, Vechur cattle.

## INTRODUCTION

Milk is a rich source of vitamins, minerals, fats and high quality proteins. The composition of milk has been influenced by a number of genetic and environmental factors such as parity, breed, stage of lactation and climatic conditions (Bernard *et al.,* 2018). The most variable component in milk is fat, which is the primary determinant of its physical and organoleptic qualities (Magan *et al.,* 2021). Milk proteins such as casein, lactoferrin and amino acids in milk like tyrosine, cysteine, tryptophan, lysine, serine and leucine have antioxidant properties (Layman *et al.,* 2018). Antioxidant compounds have the ability to scavenge free radicals and help to relieve stress (Rio *et al.,* 2013). The antioxidants present in milk can be grouped into lipid-soluble and water-soluble antioxidants. Carotenoids, retinol and $\alpha$-tocopherol are lipid-soluble antioxidants, whereas ascorbic acid is water-soluble antioxidant (Khan *et al.,* 2019). The milk fat globule membrane contains a considerable amount of $\alpha$ -tocopherol, which is regarded as one of the milk's most potent lipid-soluble antioxidants (Bernard *et al.,* 2018). There are two primary groups of proteins in milk: caseins, which make up 80% of total proteins and come in a variety of forms and serum proteins, all of which contribute significantly to its antioxidant property (Davoodi *et al.,* 2016). Tyrosine and cysteine, Vitamins A and E, carotenoids and enzyme systems including superoxide dismutase, catalase and glutathione peroxidase are some of the sulfur-rich amino acids that contribute to milk and milk products' antioxidant potential (Gayathri and Renu, 2015). Equol, a polyphenolic metabolite of daidzein, is also present in milk in significant concentrations and research has shown that it has antioxidant properties (Mayo *et al.,* 2019). The antioxidant mechanisms of milk can prevent superoxide radicals (O2), hydroxyl radicals and peroxide radicals (Akhtar *et al.,* 2018).

Antioxidant properties of milk and its products may improve human health, immunity, nutritional value, prevent or treat health threats, aid in the treatment of diabetes, cancer and cholesterol-related issues and lessen allergic reactions (Manach *et al.,* 2017). A diet rich in antioxidants is crucial for managing the pro- and anti-oxidant balance. It is assumed that milk from the Vechur cattle is healthier than milk from crossbred bovines. In comparison to crossbreed

[1]Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Mannuthy-680 651, Thrissur, Kerala, India.
[2]Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy-680 651, Thrissur, Kerala, India.

**Corresponding Author:** V.R., Annie. Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. Email: annievraj@gmail.com

**How to cite this article:** Annie, V.R., Karthiayini K. and Lucy K.M. (2023). Comparison of Total Antioxidant Capacity of Milk of Crossbred Holstein Friesian and Vechur Cattle during Different Lactation. Asian Journal of Dairy and Food Research. DOI: 10.18805/ajdfr.DR-2130

**Submitted:** 20-06-2023 **Accepted:** 02-11-2023 **Online:** 27-11-2023

cattle, the Vechur cow has much more fat and solids not fat (SNF) content (Abraham and Gayathri, 2015). Different animal milk and milk products with antioxidant defense systems can be considered to have health-beneficial effects against free radical damage (Stobiecka *et al.,* 2022). Excessive formation of reactive oxygen species and oxygen-free radicals has been linked to oxidative stress, which may lead to chronic diseases such as cancer, cardiovascular diseases and ageing degenerative diseases (Pizzino *et al.,* 2017). The oxidation of membrane phospholipids, proteins and DNA as well as the modification of low-density lipoproteins, can all be exacerbated by oxidative stress (Lopez-Pedrera *et al.,* 2016). Free radicals, in particular reactive oxygen species (ROS) andreactive nitrogen species (RNS), which are produced during typical cellular and metabolic events, are crucial in the aetiology of chronic illnesses (Van-Raamsdonk *et al.,* 2017). When the natural defense mechanism of body fails to prevent the damage caused by reactive radicals, artificial and natural antioxidants are used to impede the negative consequences of oxidative stress. Artificial antioxidants have been found to be harmful, carcinogenic and hazardous (Uzombah, 2022). The dairy animals possess variable levels of antioxidant across different lactation period. Hence, the objective of the present study was to analyse the antioxidant properties of whole milk of CBHF and Vechur cattle and assess whether the Vechur milk is better in quality as far as antioxidant property is concerned.

## MATERIALS AND METHODS

The study was conducted in the College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala (Latitude: 10.536; Longitude: 76.265). From the Vechur cattle farm milk samples were collected for the present study. Total antioxidant capacity (TAC) of milk samples of CBHF and Vechur cattle were evaluated across early (5-15 days), mid (90-120 days) and late (>150 days) stages of lactation. A total of 36 milk samples from CBHF (n=6) and Vechur cows (n=6) were used for the study. All of the milk samples were obtained early in the morning and stored at 4°C till processing. The analysis was carried out on the same day of collection of samples.

### Determination of total antioxidant activity

The ferric reducing antioxidant power assay (FRAP) was used as a direct method for measuring the total antioxidant capacity. 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, which can be monitored by measuring the change in absorption at 593 nm. Working FRAP reagent was prepared by using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate and16 mL glacial acetic acid); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine), in 40 mM HCl; and 20 mM $FeCl_3$•$6H_2O$ in the ratio of 10:1:1. Milk samples (100 µl) at room temperature were mixed with 3ml of FRAP reagent and kept in dark for 10 minutes. The samples were then centrifuged at 8000 rpm for 5 minutes and supernatant solution was taken. The absorbance of the supernatant solution was measured using spectrophotometer at wavelength 593 nm. Aqueous solutions of $FeSO_4$•$7H_2O$ (100-3000 µM) were used as standard. The data is shown as FRAP values [µM $ml^{-1}$ Fe (II)].

### Statistical analysis

Data were expressed as means±standard error. For statistical comparisons between periods of lactation, the results were subjected to one-way ANOVA using Statistical Package for the Social Sciences (SPSS). Significant differences ($P$ <0.01) between CBHF and Vechur cattle at each stage of lactation were analyzed by Independent t-test (Gerald, 2018).

## RESULTS AND DISCUSSION

The present study shows that, milk samples from CBHF and Vechur cattle during the early stages of lactation had significantly higher total antioxidant capacity than milk samples from later stages of lactation (Table 1). This could be because of the high fat content in the early phases, which lead to higher readings. α-Tocopherol is largely present in milk fat globule membrane and it is considered as the most effective lipid-soluble antioxidant present in milk of dairy animals (Bernard *et al.,* 2018). Chen *et al.,* 2000) found that there was a link between milk fat content and antioxidant activity. In both CBHF and Vechur cattle, TAC values of early and mid-lactation (Fig 1) and early and late lactation (Fig 2) varied significantly. However, the TAC values of mid and late lactation milk in both CBHF and Vechur cattle, did not differ significantly (Fig 3).

The total antioxidant content of Vechur milk was higher than the total antioxidant capacity of CBHF milk throughout lactation. The TAC values of Vechur milk decreased as lactation progressed, but the net value remained significantly greater than the milk of CBHF. Vechur milk is distinguished from crossbred cow milk by its superior digestion, buffering capacity and therapeutic properties (Rajeev, 2007). Vechur

**Table 1:** Total anti-oxidant capacity of milk of CBHF cattle and vechur cattle.

| Period of lactation | CBHF | Vechur | t-value | p-value |
|---|---|---|---|---|
| Early lactation | 1589.43±159.81 | 1582.97±51.41 | 0.038[ns] | 0.971 |
| Mid lactation | 907.98±3 8.35 | 1073.78±39.91 | 2.95* | 0.013 |
| Late lactation | 980.67±52.41 | 1167.00±44.58 | 3.02* | 0.022 |

*Significant at 0.05 level; ns- Non-significant

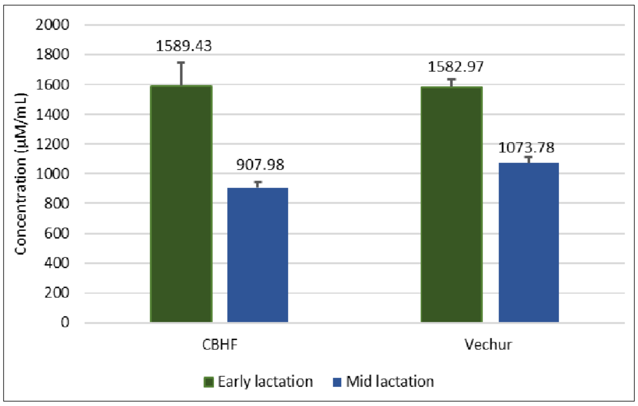


**Fig 1:** Total anti-oxidant capacity of milk at early and mid-lactation of CBHF cattle and Vechur cattle.

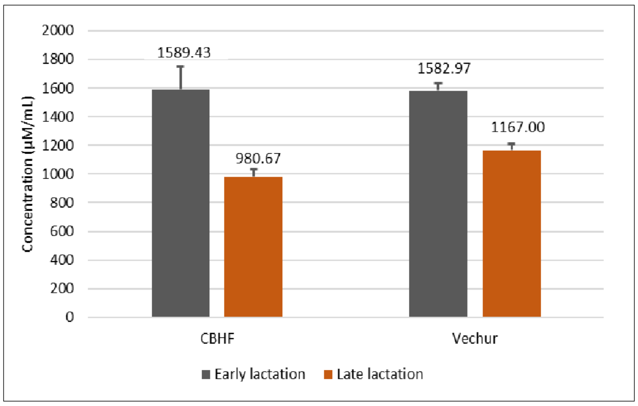


**Fig 2:** Total anti-oxidant capacity of milk at early and late lactation of CBHF cattle and Vechur cattle.

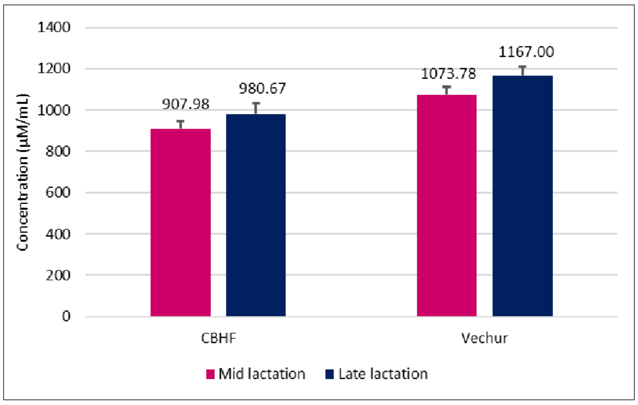


**Fig 3:** Total anti-oxidant capacity of milk at mid and late lactation of CBHF cattle and Vechur cattle.

milk had shown to have significant potential for decreasing blood cholesterol levels in numerous nutritional and medical studies (Chinnamma *et al.,* 2015). The milk of the Vechur cow contains higher beta casein A2 than A1. The levels of SNF, protein and lactose in Vechur cattle were stable throughout the seasons, suggesting the breed's adaptability and climatic resilience in terms of the primary milk ingredients (Anisha *et al.,* 2012). Along with the above mentioned previous studies, the higher TAC value of Vechur milk observed in the present study is indicative of its better medicinal value than the CBHF milk.

## CONCLUSION

Throughout lactation, the total antioxidant capacity of milk samples of CBHF and Vechur Cattle was examined in this study. Antioxidant levels were shown to fluctuate throughout different phases of lactation, with early lactation showed higher TAC values than later stages of lacation. In addition, when compared to CBHF milk, Vechur cattle milk had a higher total antioxidant capacity, indicating that Vechur cattle milk has a higher medicinal value.

## ACKNOWLEDGEMENT

The authors are grateful to Kerala Veterinary and Animal Sciences University for providing necessary facilities needed for carrying out the research.

**Conflict of interest:** None.

## REFERENCES

Abraham, B.L., Gayathri, S.L. (2015). Milk composition of crossbred and desi cattle maintained in the sub-tropical high ranges of Kerala. Indian Journal of Veterinary and Animal Sciences Research. 44(1): 53-55.

Akhtar, M.J., Mondor, M., Aider, M. (2018). Impact of the drying mode and ageing time on sugar profiles and antioxidant capacity of electro-activated sweet whey. International Dairy Journal. 80(1): 17-25.

Anisha, S., Bhasker, S., Mohankumar, C. (2012). Recombinant lactoferrin (Lf) of Vechur cow, the critical breed of Bos indicus and the Lf gene variants. Gene. doi: 10.5772/intechopen.103810.

Bernard, L., Bonnet, M., Delavaud, C., Delosiere, M., Ferlay, A., Fougere, H., Graulet, B. (2018). Milk fat globule in ruminant: Major and minor compounds, nutritional regulation and differences among species. European Journal of Lipid Science and Technology. 120(5): 1700039.

Chen, J., Lindmark-Mansson, H., Akesson, B. (2000). Optimisation of a coupled enzymatic assay of glutathione peroxidase activity in bovine milk and whey. International Dairy Journal. 10(1): 347-351.

Chinnamma, M., Shashidharan, A., Bhasker, S. (2015). Therapeutic Value of Milk of Vechur Cow, the Indigenous Breed of Kerala Listed as Critical by FAO. Biodiversity Conservation -Challenges for the Future. 103-111.

Davoodi, S.H., Shahbazi, R., Esmaeili, S., Sohrabvandi, S., Mortazavian, A., Jazayeri, S., Taslimi, A. (2016). Health-related aspects of milk proteins. Iranian Journal of Pharmaceutical Research. 15(3): 573-578.

Gayathri, B., Renu, A. (2015). Antioxidant activity and fatty acid profile of fermented milk prepared by *Pediococcus pentosaceus*. Journal of Food Science Technology. 51(12): 4138-4142.

Gerald, B. (2018). A brief review of independent, dependent and one sample t-test. International Journal of Applied Mathematics and Theoretical Physics. 4(2): 50-54.

Khan, I.T., Bule, M., Ullah, R., Nadeem, M., Asif, S., Niaz, K. (2019). The antioxidant components of milk and their role in processing, ripening and storage: Functional food. Veterinary World. 12(1): 12-15.

Layman, D.K., Lönnerdal, B., Fernstrom, J.D. (2018). Applications for á-lactalbumin in human nutrition. Nutrition Reviews. 76(6): 444-460.

Lopez-Pedrera, C., Barbarroja, N., Jimenez-Gomez, Y., Collantes-Estevez, E., Aguirre, M.A., Cuadrado, M.J. (2016). Oxidative stress in the pathogenesis of atherothrombosis associated with anti-phospholipid syndrome and systemic lupus erythematosus: New therapeutic approaches. Rheumatology. 55(12): 2096-2108.

Magan, J.B., Callaghan, T.F., Kelly, A.L., McCarthy, N.A. (2021). Compositional and functional properties of milk and dairy products derived from cows fed pasture or concentrate based diets. Comprehensive Reviews in Food Science and Food Safety. 20(3): 2769-2800.

Manach, C., Milenkovic, D., Van de Wiele, T., Rodriguez-Mateos, A., de Roos, B., Garcia-Conesa, M.T., Landberg, R., Gibney, E.R., Heinonen, M., Tomas-Barberan, F., Morand, C. (2017). Addressing the inter-individual variation in response to consumption of plant food bioactives: Towards a better understanding of their role in healthy aging and cardiometabolic risk reduction. Molecular Nutrition and Food Research. 61(9): 1600557.

Mayo, B., Vázquez, L., Florez, A.B. (2019). Equol: a bacterial metabolite from the daidzein isoflavone and its presumed beneficial health effects. Nutrients. 11(9): 2231-2235.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., Bitto, A. (2017). Oxidative stress: harms and benefits for human health. Oxidative Medicine and Cellular Longevity. 8(2): 1-13.

Rajeev, M. (2007). Molecular cloning and characterization of Alpha lactalbumin gene in Vechur cattle (Doctoral dissertation, Department of Animal Breeding and Genetics, College of Veterinary and Animal Sciences, Mannuthy).

Rio, D., Rodriguez-Mateos, A., Spencer, J.P.E., Tognolini, M., Borges, G., Crozier, A. (2013). Dietary (poly) phenolics in human health: structures, bioavailability and evidence of protective effects against chronic diseases. Antioxidants and Redox Signaling. 18(1): 1818-1892.

Stobiecka, M., Krol, J., Brodziak, A. (2022). Antioxidant activity of milk and dairy products. Animals. 12(3): 245-247.

Uzombah, T.A. (2022). The implications of replacing synthetic antioxidants with natural ones in the food systems. Natural Food Additives. DOI: 10.5772/intechopen.103810.

Van-Raamsdonk, J.M., Vega, I.E., Brundin, P. (2017). Oxidative stress in neurodegenerative disease: Causation or association? Oncotarget. 8(7): 10777-10778.