

Antioxidant Activity of Milk from Vechur and Kasargod Dwarf Cattle by DPPH Method

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Abstract

The present study was conducted to evaluate the antioxidant activity of milk from Vechur and Kasargod Dwarf cattle indigenous to the state of Kerala, India. Vechur cattle is registered as an indigenous breed as per National Bureau of Animal Genetics Resources whereas Kasargod Dwarf is labelled as 'non-descript'. The antioxidant capacity of the milk samples was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method. The study revealed that the average antioxidant potential of Vechur and Kasargod Dwarf milk were 44.11 ± 8.60 and $48.75 \pm 7.65\%$ RSA respectively. The activity ranged between 33.35 to 63.70% for Vechur cow milk and 32.13 to 57.31% for Kasargod dwarf milk. The results of the study suggest that the enhanced radical scavenging activity of milk from native cattle varieties may be due to the variation in compositional parameters.

Keywords: Vechur • Kasargod dwarf • Antioxidant activity • RSA • DPPH • Lactoferrin

Introduction

India has the world's most diverse livestock resources, but most of our cattle are unidentified and grouped under the heading "non-descript." National Bureau of Animal Genetic Resources (NBAGR), Karnal is the nodal organization for registering newly identified cattle breeds and 50 cattle are registered as indigenous cattle breeds till date. However, in South India, there are several unique dwarf cow breeds which were mostly ignored in official bovine breed assessments. The Vechur and Kasargod Dwarf are small-sized cows native to the state of Kerala. Vechur is registered as an indigenous breed as per NBAGR, whereas Kasargod Dwarf is under the label 'non-descript'. These cattle are well known for their low feed to milk ratio and resistance to hot and humid climates. Studies have explored the increased antioxidant potential of Lactoferrin isolated from the milk of Vechur Anisha S, et al. [1] and colostrum of Kasargod Dwarf cattle Aswathy A, et al. [2].

Oxidative stress was characterized as the lack of balance between the formation of reactive oxygen/nitrogen species (ROS/RNS) and the organism's ability to counteract their effects by the antioxidative system Persson T, et al. [3]. The onset of most age/diet-related chronic illnesses is associated with increased oxidative stress Benzie IFF [4]; Ames BN, et al. [5]. Hence dietary antioxidants have a major role in fighting against cell damages due to oxidative stress.

Milk and Milk products have long traditions in human nutrition. Bovine milk is a source of lipids, proteins, carbohydrates, vitamins, minerals, and the nutrients required for growth and development. It contains immunoglobulins,

hormones, growth factors, nucleotides, peptides, polyamines, enzymes, and other bioactive peptides. Major antioxidants in milk can be categorized as lipid-soluble and water-soluble. Carotenoids, retinol, and tocopherol are lipid-soluble antioxidants, while ascorbic acid is a water-soluble antioxidant Cacho NT and Lawrence RM, [6]. Lactoferrin, an iron-binding glycoprotein, present in milk is associated with various physiological and protective actions, some of the most reviewed are antioxidant, antibacterial and anti-inflammatory activities. Its role in various biological functions is now recognized by the scientific community. The antioxidant potential of milk from Indigenous cattle breeds during different stages of lactation is demonstrated in various studies. However, literature on the antioxidant activity of milks from Vechur and Kasargod Dwarf breeds is least available. In the present study the antioxidant capacity of these milks were determined using DPPH assay and was represented as percentage radical scavenging activity (% RSA).

Materials and Methods

Collection of milk

Pooled fresh milk samples from Vechur and Kasargod Dwarf breeds of cattle were collected from Livestock farm, Kerala Veterinary and Animal Sciences University, Mannuthy. Raw milk samples were collected at the time of milking in clean and sterile containers. The samples were immediately transported to the laboratory and stored at 4 °C until analysis. The radical scavenging activity was analysed on a monthly basis for a total period of one year.

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is one of the common tests done to determine the antioxidant potential of biological compounds. The free radical scavenging activity of milk samples was measured by the DPPH method with a slight modification Guravaiah M, et al. [7]. One hundred μ L of milk sample was added to 2 mL of DPPH in ethanol solution (100 μ M) in a test tube. After incubation at 37°C for 30 min, 1 mL of chloroform was added and centrifuged at 3000 g for 5 min. The absorbance of clear solution was determined at 517 nm using spectrophotometer. An ethanolic solution of DPPH (100 μ M) was used as control and the percentage of DPPH radical scavenging activity was calculated according to the following equation:

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Radical Scavenging activity (%) = $\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$

Control absorbance

Statistical analysis

The monthly analysis of milk samples was done in three trials and the results were presented as mean \pm SD. The data were analysed for significant differences among the different months of the study by one-way ANOVA test using the Statistical Packages for Social Sciences (SPSS) version 23.

Result and Discussion

In the present study the free radical scavenging activity of the milks from Vechur and Kasargod Dwarf cattles were determined during December, 2020 to November, 2021. The results obtained are presented in (Figure 1) (Table 1).

Figures are Mean \pm Standard deviation of triplicate analyses. Means with different superscripts within a column differ significantly ($p < 0.05$).

Among the two different Indigeneous cattle milks, Kasargod dwarf milk exhibited higher mean percentage RSA (48.75 ± 7.65) than Vechur milk ($44.11 \pm 8.60\%$). Moreover, the results revealed that no significant difference ($p < 0.05$) in the scavenging activity of milk from Vechur and Kasargod Dwarf cattles among different months of the period of study. The results obtained are comparable with that observed for milk of Sahiwal cattle (*Bos indicus*) in the study conducted by Mann S, et al. [8], where the free radical scavenging activity of milk from Sahiwal cows (Indian native cattle), Karan Fries cows (Cross-bred), Holstein Friesian cows (exotic cattle) and Murrah buffaloes (Riverine buffaloes) were assessed during four different lactation stages. The study revealed that the percentage scavenging activity of Sahiwal cow milk in the early, peak, mid and late lactation were 55.66 ± 0.15 , 53.54 ± 0.86 , 52.70 ± 1.2 and 53.94 ± 1.04 percent scavenging activity, respectively. Vechur milk was reported to have higher total antioxidant capacity than Malabari goat milk throughout the lactation Annie VR, et al. [9]. Khan IT, et al. [10] compared the antioxidant activity of heat treated cow and buffalo milk and noticed a scavenging activity of $24.3 \pm 0.49\%$ for raw cow milk. However, it should be noted, that the results may be difficult to compare due to the use of different antioxidant activity assays and different values and units, within the same method.

Lactoferrin (Lf) is known to play a key function in antioxidant capacity by inhibiting fatty acid oxidation. Single nucleotide polymorphisms detected in the Lf gene revealed the presence of a higher amount of arginine, which illustrates the enhanced bioactivity of Lactoferrin in Vechur milk Shashidharan A, et al. [11]. The structural and functional features of the Lactoferrin gene from Vechur cow were elaborated by Shashidharan A, et al. [11]. The gene expressed for production of recombinant bovine lactoferrin was found to exhibit bacteriocidal properties. Studies have revealed that Lactoferrin isolated from colostrum of Kasargod Dwarf cattle possesses more effective antioxidant potential when compared to commercially available bovine lactoferrin Aswathy A, et al. [2].

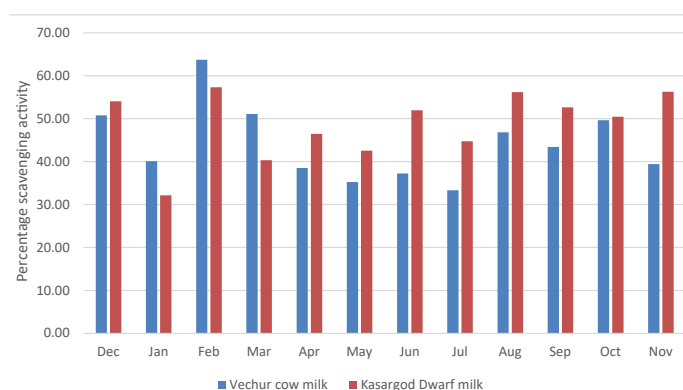


Figure 1. Percentage radical scavenging activity of Vechur and Kasargod Dwarf milk from december 2020 to november 2021.

Table 1. Radical scavenging activity of Vechur and Kasargod Dwarf milk from december-2020 to november-2021.

Month	Radical scavenging activity (%RSA)	
	Vechur	Kasargod Dwarf
December 2020	50.75 \pm 1.63 ^f	54.03 \pm 2.59 ^{efg}
January 2021	40.11 \pm 2.98 ^{cd}	32.13 \pm 1.53 ^a
February 2021	63.70 \pm 3.53 ^e	57.31 \pm 1.88 ^{ef}
March 2021	51.08 \pm 0.81 ^f	40.34 \pm 0.93 ^b
April 2021	38.51 \pm 2.36 ^{bc}	46.45 \pm 3.07 ^{cd}
May 2021	35.23 \pm 1.42 ^{ab}	42.56 \pm 0.97 ^{bc}
June 2021	37.25 \pm 1.37 ^{abc}	51.95 \pm 1.79 ^{ef}
July 2021	33.35 \pm 1.32 ^a	44.74 \pm 2.97 ^{bc}
August 2021	46.84 \pm 2.91 ^{ef}	56.18 \pm 2.91 ^{fg}
September 2021	43.41 \pm 1.57 ^{de}	52.62 \pm 1.33 ^{efg}
October 2021	49.65 \pm 1.11 ^f	50.47 \pm 1.34 ^{de}
November 2021	39.41 \pm 1.21 ^{bcd}	56.25 \pm 1.73 ^{fg}
Mean \pm SD	44.11 \pm 8.60	48.75 \pm 7.65

Figures are Mean \pm Standard deviation of triplicate analyses. Means with different superscripts within a column differ significantly ($p < 0.05$).

Conclusion

The antioxidant capacity of milk samples from Vechur and Kasargod Dwarf cattle was assessed during December 2020 - November 2021. Kasargod Dwarf milk showed a higher antioxidant capacity when compared to Vechur milk. The antioxidant potential of milk has both technological and nutritional significance. The results are suggestive of higher therapeutic potential of milk from native cattle varieties. Further studies are needed to isolate the antioxidant components in Vechur and Kasargod Dwarf milk and utilize its therapeutic potential in pharmaceutical formulations.

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