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Sialic Acid Content in Colostrum of Two Cross Breed Dairy Goat: Effect of Breed and Lactation

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ABSTRACT

The present study aims to investigate the sialic acid content in colostrum of two crossbred goats, Sannen × Beetal and Alpine × Beetal, and to compare the effect of milking time on the sialic acid content between the breeds. Colostrum samples were collected from all the animals, at an interval of 12 hours, beginning at kidding till 72 hours thereafter. The sialic acid concentrations were estimated from the processed colostrum samples by fluorimetric method. The sialic acid content in colostrum of Alpine × Beetle and Sannen × Beetle goats ranged from 0.19 to 1.03 and 0.17 to 0.98 mg/ml, respectively. Sialic acid concentrations of colostrum in both the breeds were higher at 0 hour of milking and a gradual decrease in their levels was observed till 72 hours of milking. The mean ± S.E. sialic acid concentrations of Alpine × Beetal colostrum at 12, 24, 36, 48, 60 and 72 hours were significantly ($P < 0.001$) higher than their respective values for Sannen × Beetle colostrum. This study indicated that the breed and milking time have significant effect on sialic acid concentration of goat's colostrum.

Keywords: Alpine × Beetal, Sannen × Beetle, Goat, Sialic acid, Colostrum

Colostrum is the first milk produced by female mammals immediately after parturition (Uruakpa *et al.* 2002). Colostrum contains many kinds of biofunctional constituents, including growth factors, anti-pathogenic compounds and immune-enhancing components like immunoglobulins as well as nutrients such as casein, lactose, fats, vitamins, and minerals. The colostrum or the first secretion is characterized by high concentrations of total protein, total solids, fat, and ash during the first three days following parturition when compared with milk (Kumar *et al.* 2014). Sialic acids are important constituents of mammalian milk (Meyrand *et al.* 2013). Structurally, it is composed of nine carbon acidic sugars that includes N-acetylneuraminic acid, N-glycolylneuraminic acid, and 2-Quito-3-deoxy-D-glycero- D galactonononic acid (Fukushima *et al.* 2014). Sialic acid present in colostrum and milk is regarded as an important biological component that plays a role in the development of the brain and in increasing immunity, in infants (Varki, 2008; Ednie *et al.* 2015). Studies have indicated that the sialic acid profile of goat colostrum and milk is similar to the human milk (Urashima *et al.* 2001). Claps *et al.* (2014) observed that the sialiloligo saccharides content of goat's milk was significantly higher than the milk of other ruminants. Several studies have reported the composition, structure

and some bioactivities of sialic acid in human milk, but little information is available about sialic acid in milk or colostrum of domestic mammals. Several factors may influence the total sialic acid concentration in milk, including species, feed management and climatic conditions (Urashima *et al.* 2001). To our knowledge, no investigation has been made on the effect of goat breed and the different stages of lactation on total sialic content in goat colostrum. Therefore, a study was designed to investigate the sialic acid content in colostrum of two crossbred goats, Sannen × Beetal and Alpine × Beetal and to compare the effect of milking time on the sialic acid content between the breeds. Significant ($P < 0.001$) effects of breed and sampling time on sialic acid concentrations were observed.

MATERIALS AND METHODS

Collection of goat colostrum samples

The study was conducted on fifteen Sannen Beetal and fifteen Alpine × Beetal crossbred dairy goats nearing parturition at National Dairy Research Institute, Karnal, India. All the goats were healthy and were provided with similar housing system and diet regime. Colostrum samples were collected from all the animals, at an interval of 12 hours, beginning at kidding till 72 hours thereafter. After collection, the samples were immediately transported to the laboratory and stored at -20°C in aliquots till analysis.

Determination of sialic acid in goat colostrum (0-72 hours)

A simple and rapid fluorimetric method developed for detection and quantification of sialic acids by Matsuno and Suzuki (2008) was used to quantify the sialic acid content of colostrum in the present study.

Sample preparation

Colostrum samples of both the breeds were skimmed (4000 g for 30 min at 4°C), and then they were diluted 15 times with distilled water. Thereafter, the sialic acid content of these processed samples was estimated by the below mentioned protocol.

Procedure

Six hundred microlitre of processed samples were taken in a 10 ml polypropylene test tube, and 60 µl of freshly prepared sodium periodate (10mM) was added to it. The test tube was placed in an ice- bath for 45 min and then 300 µl of sodium phosphate was added to terminate the reaction. Thereafter, 1.5 ml of ammonium acetate and 1.2 ml of acetoacetanilide solutions were added and the test tube was incubated at 25°C for 10 min. The Fluorescence intensity of the content was measured in a fluorimeter (carry Eclipse, Varian) using excitation and emission wavelength at 372 nm and 471 nm, respectively.

Standard curve of sialic acid

Appropriate volumes of aqueous stock solution (0 16mM) of neuraminic acid (NeuNAc) were diluted with distilled water to obtain a series of standard solutions of sialic acid (8 3 to 66 6 nM) in 600 µl. These standard solutions were treated as described above. The Fluorescence intensity obtained was plotted against concentration of NeuNAc expressed as sialic acid.

Statistical analysis

Effects of breed, sampling time, and their interaction on sialic acid concentrations of colostrum was determined by two way ANOVA. In order to determine the statistical difference between the sialic acid concentrations of colostrum collected at different intervals of time in both the breeds, one way ANOVA was used. The sialic acid concentration of Alpine Beetal colostrum sample collected at a particular time interval was compared with their respective concentration of Sannen Beetal by student's t–test. Graph Pad prism (Sigma Stat for Windows; Version 2.0; Jadel Corporation, San Rafael, CA, USA) software was used for statistical analysis.

RESULTS AND DISCUSSION

The sialic acid content in colostrum of Alpine Beetle ranged from 0.19 to 1.03 mg/ml whereas it's content in colostrum of Sannen Beetle ranged from 0.17 to 0.98 mg/ml. There were significant ($P<0.001$) effects of breed and sampling time on sialic acid concentrations, whereas breed by sampling time interaction was non-significant. The changes in the sialic acid content of colostrum with sampling time in Alpine Beetle and Sannen Beetle breeds are shown in Table 1.

Sialic acid concentrations of colostrum in both the breeds were higher at 0 hour of milking and a gradual decrease in their levels was observed till 72 hours of milking. The mean ± S.E. sialic acid concentrations of Alpine Beetle and Sannen Beetle colostrum at 0 hour of milking were 1.03 ± 0.04 and 0.98 ± 0.03 mg/ml, respectively and at 72 hours of milking were 0.19 ± 0.02 and 0.17 ± 0.02 mg/ml, respectively. Similar decreases in sialic acid concentration with milking time, was observed in bovines (Puente *et al.*, 1994; Nakamura *et al.,* 2003). Martin *et al.* (2001) also reported a similar pattern of decrease in the sialic acid content in bovine colostrum.

Table 1. Changes in sialic acid concentration (mg/ml) in goat colostrum of Alpine × Beetle and Sannen × Beetle during postpartum (0-72 h) periods

Breeds	Sampling time (hours)						
	0	12	24	36	48	60	72
AB	1.03 ± 0.14^{a}	0.85 ± 0.01^{ak}	0.63 ± 0.01^{bako}	0.58 ± 0.02^{cgoq}	0.38 ± 0.04^{dhl}	0.27 ± 0.02^{eimpq}	0.19 ± 0.02^{fjnq}
SB	0.98 ± 0.05^{a}	0.67 ± 0.01^{bh}	0.48 ± 0.06^{ci}	0.32 ± 0.04^{di}	0.25 ± 0.06^{ejk}	0.20 ± 0.02^{fjkl}	0.17 ± 0.03^{gl}

AB - Alpine × Beetle

SB -Sannen × Beetle

Mean without a common letter differ significantly by one way ANOVA

Variation in the sialic acid levels between colostrum and milk samples of bovines, goats, and sheep was observed by Useh *et al.* (2008). They observed the sialic acid concentrations to be higher in colostrum of all the three species when compared with the milk samples.

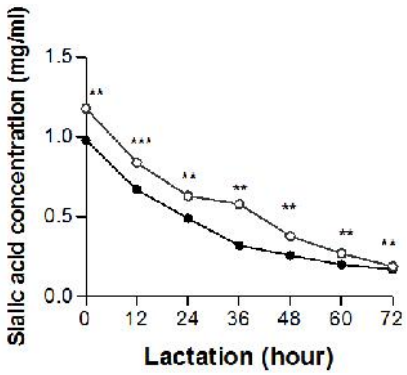

Fig. 1: Comparison of sialic acid concentration of goat colostrums between Alpine × Beetal ( )and Sannen x Beetal ( ) with statistical significance shown as * (P<0.05), ** (P<0.01), ***(P<0.001).

The mean ± S.E. sialic acid concentrations of Alpine Beetal colostrum at 12, 24, 36, 48, and 60 hours were significantly (P<0.001) higher than their respective values of Sannen Beetle colostrum (Fig. 1). Nakamura *et al*. (2003) suggested that the sialic acid content of colostrum is highly influenced by the period of collection and the breed of animal under investigation.

CONCLUSION

In the present study, significant effects of breed and sampling time on sialic acid concentration were observed. Sialic acid content of goat milk is a potential source of bioactive compounds which has beneficial effects on human and animal health. Among the breeds investigated, Alpine × Beetal showed higher concentrations of sialic acid than the Sannen × Beetal crossbred goats. The variation between the colostrum samples collected during different periods of milking from the two breeds of goats might be useful in the quality improvement of colostrum derived products *i.e.* colostrum powder.

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