



# Eletrophoretic profile of serum proteins of goat kids fed with bovine colostrum *in natura* and lyophilized

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## ARTICLE INFO

### Article history:

Received 1 October 2012

Received in revised form 22 February 2013

Accepted 25 February 2013

Available online 19 March 2013

### Keywords:

Passive immunity

Immunoglobulins

Small ruminant

Artificial feeding

Antibodies

## ABSTRACT

Bovine colostrum *in natura* or lyophilized was evaluated as an alternative source of passive immunity for goat kids. Twenty-five female newborn goat kids were randomly allotted into five treatments: goat colostrum A (GCA) or bovine colostrum A (BCA) with 45–55 mg/mL of IgG, lyophilized bovine colostrum (LBC) with 45–55 mg/mL of IgG, goat colostrum B (GCB) or bovine colostrum B (BCB) with 15–25 mg/mL. The animals received 5% of body weight of colostrum at 0, 12 and 24 h after birth, and then, cow milk twice a day and concentrate *ad libitum* until 60 days of age. Blood samples were collected at 0 to 60 days of age to determine total serum protein (TP) and serum protein fractions by electrophoresis. In the last experimental days, 50 and 60, the TP concentration for all groups were higher than at 0 h ( $P < 0.05$ ) and differences in values after colostrum ingestion were not observed ( $P < 0.05$ ). The LBC group ( $2.39 \pm 0.09$  g/dL) showed lower ( $P < 0.05$ ) albumin concentration than GCA, GCB, BCA and BCB,  $3.20 \pm 0.09$ ,  $2.93 \pm 0.09$ ,  $3.25 \pm 0.09$  and  $3.07 \pm 0.10$  g/dL, respectively ( $P < 0.05$ ), and from birth to five days of life, albumin values were lower ( $P < 0.05$ ) than at 40 to 60 days of life. At birth, the globulin concentration,  $1.87 \pm 0.05$  g/dL, was lower ( $P < 0.05$ ) than at 10 to 60 days of life ( $P < 0.05$ ). The LBC group ( $1.25 \pm 0.04$ ) showed lower ( $P < 0.05$ ) albumin/globulin ratio than GCA, GCB, BCA and BCB ( $1.36 \pm 0.04$ ,  $1.38 \pm 0.04$ ,  $1.48 \pm 0.04$  and  $1.44 \pm 0.05$ , respectively) and, at birth ( $1.52 \pm 0.05$ ), the ratio was higher ( $P < 0.05$ ) than 0.5 and 1 day of life. The LBC group ( $0.67 \pm 0.04$  g/dL) showed higher ( $P < 0.05$ ) gamma globulin concentration than GCB and BCB ( $0.48 \pm 0.04$  and  $0.50 \pm 0.04$  g/dL, respectively), and did not differ ( $P > 0.05$ ) from GCA and BCA ( $0.62 \pm 0.04$  and  $0.50 \pm 0.04$  g/dL, respectively). The lowest ( $P < 0.05$ ) gamma globulin concentration was observed at birth,  $0.24 \pm 0.04$  g/dL. The results indicate that bovine colostrum can be used as an alternative source of initial protection for newborn goat kids.

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## 1. Introduction

The ruminants' placenta consists of five membranes between fetal and maternal circulation, thereby preventing the transference of immunoglobulins to the fetus during gestational period. Thus, colostrum consumption

immediately after birth is critical for newborns survival since this lacteal secretion constitutes the only source of immunoglobulins and, consequently, passive protection (Campbell et al., 1977; O'Brein and Sherman, 1993; Castro et al., 2005; Castro-Alonso et al., 2008).

Contraindications to goat colostrum and milk consumption are related to the transmission of infectious diseases to offspring, the most important being the Caprine Arthritis Encephalitis Virus (CAEV) (Guerrault, 1990). Since mammary secretions of infected mothers are a major source of virus contamination, alternatives to reduce or eliminate

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contact of newborns with their mothers have been developed (Argüello et al., 2003; Castro et al., 2005). Among the alternatives, the supply of lyophilized goat colostrum and bovine colostrum is considered a promising alternative (Castro et al., 2005; Lima et al., 2009). In commercial operations, a bovine colostrum bank is an important management tool, ensuring the supply of adequate amounts of immunoglobulins to newborn small ruminants (Lima et al., 2009; Moretti et al., 2010). The homology between cattle, goats and sheep immunoglobulins ensures a biological activity of these macromolecules in the different species (Curtain and Fudenberg, 1973).

This study investigated the fluctuation of serum proteins during the process of passive immunity acquisition in newborn goat kids fed with bovine colostrum *in natura* and lyophilized.

## 2. Materials and methods

### 2.1. Animals, feeding and experimental procedures

The experiment was conducted at the Intensive System of Sheep and Goats Production (ESALQ – University of São Paulo – Piracicaba city – São Paulo state – Brazil). The experiment was comprised of 25 Saanen × Boer female goat kids. The animals were maintained and treated in adherence to accepted standards for humane treatment of animals (authorized by ESALQ/USP ethics committee).

Bovine and goat first milking colostrum from Holstein cows and Saanen × Boer goats were collected before the experiment. The colostrums were homogenized to produce two bovine pools and one goat pool, respectively. The pools were stored at  $-20^{\circ}\text{C}$  and samples were evaluated for determination of IgG content by radial immunodiffusion (Mancini et al., 1965; Besser et al., 1985). One frozen pool of bovine colostrum was lyophilized (Modulyo, EC Apparatus INC.) and the resulting powder was homogenized and stored in a tightly sealed container at  $-20^{\circ}\text{C}$ .

At feeding, the frozen goat and bovine pools were thawed in warm water (up to  $50^{\circ}\text{C}$ ) and diluted with whole milk until reaching a concentration of 45–55 mg/mL of IgG, constituting colostrum A, or 15–25 mg/mL of IgG, constituting colostrum B. The bovine colostrum powder, however, was resuspended in water until it reached the original colostrum chemical composition taken in the lyophilization process and, subsequently, diluted with whole milk until a concentration of 45–55 mg/mL of IgG was reached.

Aliquots of colostrum meals were used to determine their chemical composition using standard AOAC (2000) procedures (Table 1).

The newborn goat kids were separated from their mothers immediately after birth, without maternal colostrum intake. At 0, 12 and 24 h of life, the newborns received 5% of body weight of goat colostrum A (GCA), bovine colostrum A (BCA) or lyophilized bovine colostrum (LBC), goat colostrum B (GCB) or bovine colostrum B (BCB). After the first three meals, the newborns were fed with cow milk twice a day (400 mL/feed) up to 60 days of life (weaning). From the first day of life, the animals were fed with concentrate *ad libitum*, formulated to meet the goats' needs from birth to weaning (Table 2).

Blood samples were collected from the jugular vein at 0, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 days of life, centrifuged and the resulting serum stored at  $-20^{\circ}\text{C}$ .

**Table 1**

Chemical composition of colostrum meals fed to newborn goat kids.

|                           | Goat colostrum I appropriated | Goat colostrum low | Bovine colostrum appropriated | Bovine colostrum low | Lyophilized bovine colostrum |
|---------------------------|-------------------------------|--------------------|-------------------------------|----------------------|------------------------------|
| Humidity and volatile (%) | 80.4                          | 83.99              | 86.37                         | 83.29                | 80.72                        |
| Dry matter (%)            | 19.5                          | 16.01              | 13.64                         | 16.71                | 19.29                        |
| Crude protein (%)         | 7.9                           | 5.59               | 7.10                          | 4.60                 | 9.20                         |
| Fat (%)                   | 6.9                           | 5.37               | 3.98                          | 4.69                 | 5.03                         |
| IgG (mg/mL)               | 45–55                         | 15–25              | 45–55                         | 15–25                | 45–55                        |

**Table 2**

Composition of concentrate consumed by the goat kids from birth to weaning.

| Ingredient                      | Composition (%DM) |
|---------------------------------|-------------------|
| Ground corn                     | 67.3              |
| Soybean meal                    | 25                |
| Sugar cane molasses             | 5.2               |
| Limestone                       | 1.4               |
| Mineral supplement <sup>a</sup> | 1.2               |

<sup>a</sup> Calcium – 19%; Phosphorus – 7.5%; Magnesium – 1%; Sulfur – 7%; Chlorine – 21.8%; Sodium – 14.3%; Manganese – 1100 ppm; Iron – 500 ppm; Zinc – 4600 ppm; Copper – 300 ppm; Cobalt – 405 ppm; Iodine – 80 ppm; Selenium – 15 ppm.

### 2.2. Total serum protein determination and electrophoretic profile

The total serum protein (TP) was determined by the biuret reaction (Reinhold, 1953). An electrophoretic analysis was performed with a sample of 0.4  $\mu\text{L}$  of serum in an agarose gel (CELMGEL). After 30 min of electrophoresis at 90 volts, the film was stained with 200 mL of 0.2% starch black (Amido Black 10B, CELM) for 5 min. Afterwards, a reading of the protein fractions in a densitometer (CELM DS35) with a wavelength of 520 nm was performed. The serum proteins were divided into the followings fractions: albumin, globulins, and gamma globulins. The relative percentage of each protein fraction was calculated in software (CELM SE-250) from the area under the curve created by the protein band.

### 2.3. Statistical analyses

A completely randomized design was used. The statistical analysis was performed using SAS software (SAS Institute Inc., 2004). The serum variables were analyzed as a repeated measure-over-time design, considering colostrum and sampling time as main effects. The goat kid effect was considered random, and the other effects were considered fixed in the model.

The data were submitted to analysis of variance using general linear mixed models (MIXED procedure). Means comparison were made based on differences in least-square means, with *P* values adjusted to multiple comparisons using Tukey option in the MIXED procedure ( $\alpha=0.05$ ). The results are presented as least-square means and standard errors.

Pearson and Spearman correlation analysis, through PROC CORR program from SAS software (SAS Institute Inc., 2004), were taken to verify associations between serum variables of interest.

## 3. Results

During the experimental period, none goat kid became ill or died in any of the colostrum groups. The serum TP concentration of goat kids showed interaction between the treatment and sampling time ( $P<0.05$ ), Table 3. In the last experimental days, 50 and 60, the TP values for all groups were higher than at 0 h ( $P<0.05$ ). Differences in the TP concentration were not observed after colostrum ingestion ( $P<0.05$ ).

The serum albumin concentration of goat kids was affected by the treatment and sampling time ( $P<0.05$ ). The LBC group ( $2.39 \pm 0.09$  g/dL) showed lower value than GCA,

**Table 3**

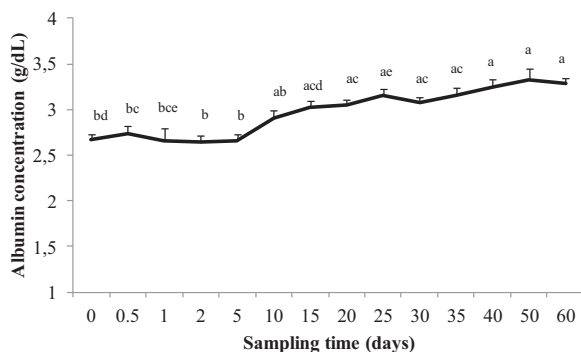
Total serum protein (g/dL) fluctuation in the treatments and sampling times (means and standard errors).

| Sampling times (days) | GCA           | GCB           | BCA           | BCB           | LBC            |
|-----------------------|---------------|---------------|---------------|---------------|----------------|
| 0                     | 4.60 ± 0.22b  | 4.46 ± 0.22b  | 4.83 ± 0.22b  | 4.71 ± 0.25b  | 4.72 ± 0.22b   |
| 0.5                   | 5.21 ± 0.31ab | 4.63 ± 0.31ab | 4.92 ± 0.31ab | 4.82 ± 0.35bc | 5.02 ± 0.31abc |
| 1                     | 5.26 ± 0.39ab | 5.34 ± 0.39ab | 4.77 ± 0.39ab | 4.66 ± 0.43bc | 5.30 ± 0.39abc |
| 2                     | 4.63 ± 0.26bc | 4.51 ± 0.26b  | 4.90 ± 0.26ab | 4.77 ± 0.29bc | 4.91 ± 0.26abc |
| 5                     | 5.05 ± 0.31ab | 4.76 ± 0.31ab | 5.27 ± 0.31ab | 4.55 ± 0.35bc | 4.83 ± 0.31bc  |
| 10                    | 5.91 ± 0.25a  | 5.13 ± 0.25ab | 4.98 ± 0.25ab | 5.05 ± 0.29bc | 5.16 ± 0.25abc |
| 15                    | 5.73 ± 0.24ac | 5.10 ± 0.24ab | 5.43 ± 0.24ab | 5.08 ± 0.27bc | 5.39 ± 0.24abc |
| 20                    | 5.78 ± 0.24ac | 5.17 ± 0.19ab | 5.32 ± 0.19ab | 5.22 ± 0.22bc | 5.42 ± 0.19abc |
| 25                    | 5.34 ± 0.21ab | 5.45 ± 0.21ab | 6.03 ± 0.21ab | 5.75 ± 0.24ab | 5.82 ± 0.21abc |
| 30                    | 5.41 ± 0.22ab | 5.29 ± 0.22ab | 5.77 ± 0.22ab | 5.55 ± 0.24ab | 5.57 ± 0.22abc |
| 35                    | 5.75 ± 0.25ab | 5.57 ± 0.25ab | 5.93 ± 0.25ab | 5.90 ± 0.29ab | 5.92 ± 0.25ac  |
| 40                    | 5.64 ± 0.22ac | 5.42 ± 0.22ab | 5.62 ± 0.22ab | 5.90 ± 0.25ac | 5.97 ± 0.22ac  |
| 50                    | 5.74 ± 0.14a  | 5.68 ± 0.14a  | 5.78 ± 0.14a  | 5.77 ± 0.16ac | 5.95 ± 0.14ac  |
| 60                    | 5.97 ± 0.20a  | 5.68 ± 0.20a  | 5.96 ± 0.20a  | 6.54 ± 0.22a  | 6.16 ± 0.20a   |

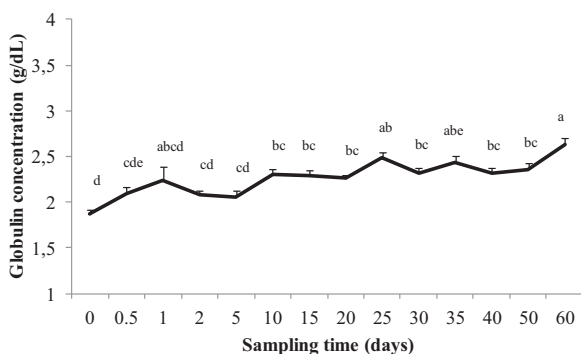
GCA: goat colostrum with 45–55 mg/mL of IgG; GCLB goat colostrum with 15–25 mg/mL of IgG; BCA: bovine colostrum with 45–55 mg/mL of IgG; BCB: bovine colostrum with 15–25 mg/mL of IgG; LBC: lyophilized bovine colostrum with 45–55 mg/mL of IgG; means without common letter (a, b and c) in the same column differ,  $P < 0.05$ .

GCB, BCA and BCB ( $3.20 \pm 0.09$ ,  $2.93 \pm 0.09$ ,  $3.25 \pm 0.09$  and  $3.07 \pm 0.10$  g/dL), respectively. In the initial sampling times, from birth to five days of life, albumin values were lower than in the final period, from 40 to 60 days of life, Fig. 1. Significant correlation between serum TP and albumin, for the entire experimental period was observed ( $r = 0.75$ ,  $P < 0.05$ ).

The serum globulin concentration of goat kids was affected only by sampling time ( $P < 0.05$ ), Fig. 2. At birth,



**Fig. 1.** Serum albumin (g/dL) fluctuation in the sampling times (means and standard errors). <sup>abcde</sup>Means without common letter differ,  $P < 0.05$ .



**Fig. 2.** Serum globulin (g/dL) fluctuation in the sampling times (means and standard errors). <sup>abcde</sup>Means without common letter differ,  $P < 0.05$ .

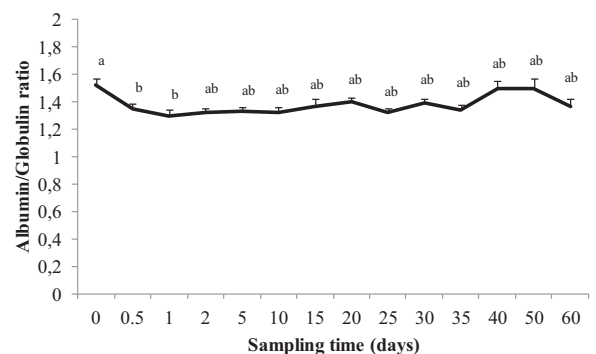
the value,  $1.87 \pm 0.05$  g/dL, was lower than 10 to 60 days of life ( $P < 0.05$ ).

The ratio albumin/globulin was affected by treatment and sampling time ( $P < 0.05$ ). The LBC group ( $1.25 \pm 0.04$ ) showed lower value than GCA, GCB, BCA and BCB ( $1.36 \pm 0.04$ ,  $1.38 \pm 0.04$ ,  $1.48 \pm 0.04$  and  $1.44 \pm 0.05$ , respectively). The value of albumin/globulin was higher at birth, with a mean concentration of  $1.52 \pm 0.05$ , than 0.5 and 1 day of life, Fig. 3.

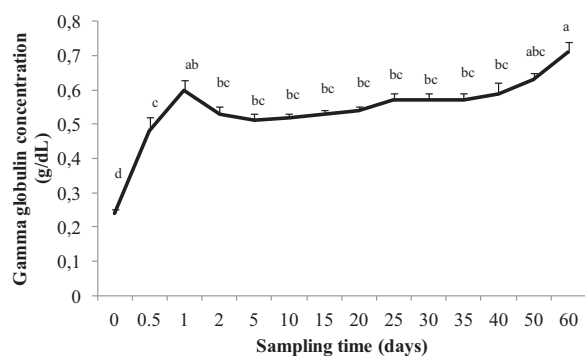
The serum gamma globulin was affected by the treatment and sampling time ( $P < 0.05$ ). The LBC group ( $0.67 \pm 0.04$  g/dL) showed higher value than GCB and BCB ( $0.48 \pm 0.04$  and  $0.50 \pm 0.04$  g/dL, respectively), and did not differ from GCA and BCA ( $0.62 \pm 0.04$  and  $0.50 \pm 0.04$  g/dL, respectively). The lowest value of serum gamma globulin concentration was observed at birth,  $0.24 \pm 0.04$  g/dL, Fig. 4.

#### 4. Discussion

The mean total protein concentration in the goat kids' blood serum at 60 days,  $6.06 \pm 0.09$  g/dL, is in accordance with that obtained by O'Brein and Sherman (1993), Silva et al. (2007) and Lima et al. (2009) who also studied goat kids in the first weeks of life. All the groups showed a gradual increase in serum TP up to 60 days of life. The low TP level observed at birth can be related with the lower



**Fig. 3.** Albumin/globulin ratio in the sampling times (means and standard errors). <sup>ab</sup>Means without common letter differ,  $P < 0.05$ .



**Fig. 4.** Serum gamma globulin (g/dL) fluctuation in the sampling times (means and standard errors). <sup>abcd</sup>Means without common letter differ,  $P < 0.05$ .

concentration or absence of the gamma globulin fraction in the serum of newborn goat kids. Moretti et al. (2010) working with lambs fed with bovine or ovine colostrum with high concentration of IgG, observed higher concentrations of serum TP after colostrum ingestion, suggesting that this result reflects the remarkable presence of exogenous IgG in the fluctuation of serum TP. In this study increased serum TP levels after colostrum ingestion were not observed, remaining below 6 g/dL in the first 50 days of life, probably due to the lower gamma globulin acquisition and their participation in serum TP.

The serum albumin that is produced in the liver has its synthesis influenced by nutrition, general liver condition, stress and extravascular concentration. This protein represents about 50–65% of serum TP and its functions are related to substance transport and regulation and maintenance of blood oncotic pressure (Kaneko et al., 1997). Albumin quantification in the serum is considered an indicator of an animal's proteic nutritional status; persistent low values suggest inadequate protein intake and alteration of hepatic function (Payne and Payne, 1987). In ruminants, changes in serum albumin concentration requires at least one month to be detected, due to the low degradation and synthesis rate of this protein (Payne and Payne, 1987). The serum albumin mean values observed in this study are consistent with those found by Simões et al. (2005) who worked with goat kids in the first weeks of life, and Kaneko et al. (1997) who established reference values between 2.70 and 3.90 g/dL for goats, showing appropriate proteic nutritional status in goat kids.

The serum albumin/globulin ratio allows the detection of changes in these proteins fractions, which, according to Kaneko et al. (1997), is the first indicator of liver and kidney diseases. The mean values of albumin/globulin concentrations observed in the present experiment are consistent with those described by Sharma et al. (2001), who found an average of 1.2 as normal for goats. However, the ratio difference at birth in relation to 0.5 and 1 day of life shows the influence of the immunoglobulins absorbed from colostrum. In the other sampling times, a balance between the albumin and globulin production was observed resulting in a constant ratio up to 60 days of life.

The low serum gamma globulin levels found at birth ( $0.24 \pm 0.04$  g/dL) confirm that the newborns did not suckle

their mothers. The mean gamma globulin concentration at 24 h of life was  $0.6 \pm 0.03$  g/dL, indicating failure of passive immunoglobulins transfer. This occurs when gamma globulins serum concentration is lower than 1.2 g/dL at 24 h of goat kids' life (O'Brien and Sherman, 1993). According to Castro et al. (2005), an intake of 4 g of immunoglobulin G/kg of body weight is sufficient to ensure adequate acquisition of passive protection. However, the GCA, BCA and LBC groups ingested in the first 24 h of life approximately 8 g of immunoglobulin G/kg of body weight and still had insufficient immunoglobulin absorption. The serum gamma globulin value at 96 h of life,  $0.71 \pm 0.03$  g/dL, was also lower than the expected. Thus, the present study indicates that goat kids fed with colostrum at IgG concentration below 55 mg/mL may present failure of passive immunity.

The serum gamma globulins concentration was lower in goat kids that received goat and bovine colostrum low in IgG when compared with those that received lyophilized bovine colostrum, showing a slight advantage of using this management. The lower values of albumin and albumin/globulin ratio in LBC group, compared to the other groups, also indicate a compensatory effect of the albumin fraction due to gamma globulins absorption. Besides being a rich immunoglobulins source, colostrum has a nutrients complex and a series of biologically active molecules related to gastrointestinal maturation and development, as well as elements protecting against protein degradation that allow these immune and growth factors to reach the intestine without degrading. Among these elements, there is trypsin and elastase inhibitor and  $\alpha$ 1-antichymotrypsin (Christensen et al., 1995; Stelwagen et al., 2009; Pandey et al., 2011). Lyophilization, a dehydration process that preserves the biochemical colostrum characteristics, allows storage at room temperature for extended periods and easily reconstitution in water and variation of immunoglobulin concentration. In the present work, colostrum storage in the lyophilized form may have positively influenced the preservation and activity of lacteal secretion resulting in greater immunoglobulins absorption in the LBC group compared to the GCB and BCB.

## 5. Conclusion

The fluctuation of serum fractions indicates that colostrum with IgG concentration up to 55 mg/mL may determine failure of passive immunity in goat kids. The fluctuation pattern of serum proteins in goat kids fed with goat and bovine colostrum ensures that the bovine lacteal secretion is an alternative source of passive immunity for goats and that the use lyophilized bovine colostrum is an effective and promising alternative management procedure.

## Acknowledgements

Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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