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



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#### 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  $(2.39 \pm 0.09 \text{ g/dL})$  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  $(0.67 \pm 0.04 \text{ g/dL})$  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,  $0.24 \pm 0.04 \,\mathrm{g/dL}$ . 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

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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 ESALO/USP ethics committee).

Bovine and goat first milking colostrum from Holstein cows and Sannen  $\times$  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  $-20\,^{\circ}\mathrm{C}$  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  $-20\,^{\circ}\mathrm{C}$ .

At feeding, the frozen goat and bovine pools were thawed in warm water (up to  $50\,^{\circ}$ C) and diluted with whole milk until reaching a concentration of  $45-55\,\text{mg/mL}$  of IgG, constituting colostrum A, or  $15-25\,\text{mg/mL}$  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  $45-55\,\text{mg/mL}$  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 24h 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  $-20\,^{\circ}$ C.

**Table 1**Chemical composition of colostrum meals fed to newborn goat kids.

	Goat colostrum I appropriated	Goat colostrumlow	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	Compotision (%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 eletrophoretic profile

The total serum protein (TP) was determined by the biuret reaction (Reinhold, 1953). An electrophoretic analysis was performed with a sample of  $0.4\,\mu l$  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 ( $2.39 \pm 0.09 \text{ g/dL}$ ) showed lower value than GCA,

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