# Intestinal histology of newborn goat kids fed lyophilized bovine colostrum

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ABSTRACT: Enteric histology of newborn goat kids fed lyophilized bovine colostrum (LBC) was studied. At 0, 7, and 14 h of life 15 male newborns received 5% of body weight of lyophilized bovine colostrum and 14 male newborns goat colostrum (GC), both with 55 mg/ml of IgG. Samples of duodenum, jejunum, and ileum were collected at 18, 36, and 96 h of life for analyses of villus height, crypt depth, muscle layer thickness, partial volume of the absorptive mucosa (Vv), density of the absorptive mucosa (Sv), and quantification of goblet cells. Three animals were sampled without colostrum intake (0 h). The histomorphometry was not different between GC and LBC in all segments. In the jejunum, the villus height differed in sampling times (36 h > 0 h and 18 h). The maximum villus height was observed in the jejunum. In the jejunum, crypt depth differed in the sampling times (96 h > 0 and 18 h). Interaction between intestinal segment and sampling times was observed to crypt depth (duodenum 18 h > jejunum 18 h and duodenum 96 h > ileum 96 h). In the ileum, the muscle layer thickness differed in the sampling times (36 h > 0 and 18 h and 96 h > 0 h). The greatest thickness of muscle layer was observed in the duodenum and at 96 h the muscle layer was thicker than at 18 h. The ileum showed the highest Vv at 36 h. The Vv was the highest in the jejunum and higher at 36 h than at 96 h. In the jejunum, an interaction between the treatment and sampling times to goblet cells number (LBC 18 h < GC 18 h and GC 96 h > LBC 96 h) was observed. The ileum showed the greatest number of goblet cells. The ingestion of lyophilized bovine colostrum did not determine any consequences for enteric histology in the first 4 days of goat kids' life.

**Keywords**: lacteal secretion; intestinal morphometry; goblet cells; newborn kids; stereology

During the period of passive immunity acquisition, first 24–48 h of life, the ruminants' gastrointestinal tract has special characteristics that allows immunoglobulin uptake from colostrum (Bessi et al., 2002a, b; Pauletti et al., 2007; Kindlein et al., 2008). Thus, this lacteal secretion is important to newborn survival, influencing the intestine development and is responsible for providing, in addition to immunoglobulin, significant amounts of nutrients, hormones, and bioactive factors, such as insulin-like growth factor-I (IGF-I) (Bühler et al., 1998; Kráčmar et al., 1999, 2004; Blättler et al., 2001; Křenková and Čanderle, 2001; Blum and Baumrucker, 2002; Pauletti et al., 2007; Kindlein et al., 2008; Penchev Georgiev, 2008a, b). The intake

of these growth factors is related to growth and maturation of the intestinal epithelium that influences the rate of epithelial cell renewal, as well as digestive and absorptive functions (Hammon and Blum, 2002; Kelly and Coutts, 2000; Blum and Baumrucker, 2002).

In spite of the importance and beneficial effects of colostrum intake, in goat kids this colostrum can be a vehicle of pathogens transmission such as *caprine* arthritis-encephalitis viruses (CAE). These pathogens transmitted to the small ruminant cause health problems with consequent losses in animal performance and high rates of morbidity and mortality in the system production (Greenwood, 1995; Blacklaws et al., 2004; Peterhans et al.,

2004). Thus, to prevent this problems and confer adequate passive immunity transfer, alternative methods of immunoglobulin G (IgG) supply to small ruminants, such as a bank of bovine colostrum (refrigerated, frozen) and artificial colostrum supply, have been evaluated (Argüello et al., 2004; Lima et al., 2009; Moretti et al., 2010a, b; Nordi et al., 2012; Moretti et al., 2012a, b).

The supply of lyophilized goat and bovine colostrum has been used and considered a promising alternative management to newborn small ruminants (Castro et al., 2005; Lima et al., 2009; Moretti et al., 2010a, b, 2012a). The homology structure of immunoglobulins between the ruminants' species enables the substitution of goat maternal colostrum by bovine colostrum, providing adequate amounts of IgG to newborns. Additionally, this lacteal secretion is easy to obtain, it usually has high immune quality, allows storage for prolonged periods, and prevents the transmission of pathogens in the goat herd (Curtain and Fudenberg, 1973; Greenwood, 1995; Lima et al., 2009; Nordi et al., 2012; Moretti et al., 2012b).

Using scanning electron microscopy technique, Moretti et al. (2012a) investigated morphological characteristics of enteric tissues of goat kids fed lyophilized bovine colostrum during the period of passive immunity acquisition. The authors observed that in the first four days of goat kids' life the small intestine structure was unaffected by different sources of colostrum, goat or lyophilized bovine, and by the replacement of fetal enterocytes, which are able to absorb macromolecules, by adult-type ones. Considering these results, the hypothesis of the present study is that the supply of lyophilized bovine colostrum as an alternative source of immunoglobulins does not modify the histological and histomorphometric intestinal characteristics of newborn goat kids.

The objective was to evaluate the influence of feeding newborn goat kids with lyophilized bovine colostrum on histological and histomorphometrical characteristics of small intestine epithelium.

#### MATERIAL AND METHODS

# Animals, feeding, and experimental procedures

This study comprised thirty-two male Saanen × Boer newborn goat kids. The animals from In-

tensive System of Sheep and Goats Production (SIIPOC/ESALQ/USP) were kept, maintained, and treated in adherence to accepted standards for humane treatment of animals (authorized by ESALQ/USP ethics committee). After birth, prior to nursing, the animals were separated from their dams, weighed and identified. At 0, 7, and 14 h of life, 14 newborn goat kids received 5% of body weight of goat colostrum (GC group) and 15 newborn goat kids received 5% of body weight of lyophilized bovine colostrum (LBC group), both containing 55 mg/ml of IgG, by bottle. Immediately after birth, three animals were sampled without colostrum ingestion, used as the control group (0 h group). There was no difference (P > 0.05) in birth weight of newborn goat kids between the groups  $(3.84 \pm 0.642, 3.7 \pm 0.644,$ and  $4.3 \pm 0.360$ kg to GC, LBC, and 0 h group, respectively). Following the colostrum meals period, all goat kids were fed 250 ml of Holstein cow milk twice a day.

## Colostrum procedures

Pools of colostrum were prepared using approximately 8 l of bovine colostrum and 12 l of goat colostrum of the first milk secretion. The colostrum was homogenized to form a unique pool of goat colostrum and another of bovine colostrum and stored at -20°C. The IgG concentration in colostrum was determined through the radial immunodiffusion method (Mancini et al., 1965; Besser et al., 1985). The bovine colostrum was lyophilized, and the powder obtained was blended and stored in a tightly sealed container at -20°C. Prior to feeding, the goat colostrum was diluted with whole cow milk (100 ml of goat colostrum was added to 100 ml of milk, corresponding to 1:1 ratio) to reach a concentration of 55 mg/ml of IgG.

The lyophilized bovine colostrum, however, was resuspended in water until it reached the original chemical composition of the pool taken to the lyophilization process and, subsequently, diluted with whole cow milk (114.7 ml of bovine colostrum was added to 100 ml of milk, corresponding to 1:1.15 ratio) to reach a concentration of 55 mg/ml of IgG. The colostrum was thawed in water bath (54°C) prior to nursing. Samples of final meals of goat and lyophilized bovine colostrum were collected for the determination of their composition using the standard procedures in AOAC (2000). The IGF-I concentration in goat and lyophilized bovine

<i>C</i>	Treatment				
Composition	goat colostrum	lyophilized bovine colostrum			
Dry matter (%)	20.1 ± 0.2	18.9 ± 0.2			
Fat (%)	$7.8 \pm 0.1$	$4.0 \pm 0.13$			
Crude protein (%)	$9.8 \pm 0.0$	$9.4 \pm 0.0$			
IGE-I (ng/ml)	356 3 +1 0	1587 + 230			

Table 1. Chemical composition and insulin-like growth factor-I (IGF-I) concentration (means ± standard errors) of goat and lyophilized bovine colostrum fed to goat kids

colostrum was quantified by immunoradiometric assays after extraction procedure, on triplicate, using the kit IGF-I EIASIA KAP1581 (DIASource ImmunoAssays S.A., Louvain-La-Neuve, Belgium). Colostrum chemical composition and IGF-I (ng/ml) concentration are shown in Table 1.

# Histomorphometry of the intestine epithelium

The duodenum, medium jejunum, and ileum were removed at 0, 18, 36, and 96 h of life. The segments were opened, washed with 0.9% NaCl solution and cold phosphate-buffered solution (PBS) (0.1 mol, pH 7.2) and pre-fixed for 30 min in 4% phosphate-buffered paraformaldehyde solution. The fixative was then washed out with PBS and crosssectional pieces were excised from each sample, dehydrated in alcohol concentrations (30, 50, 70, 90, and 100%) and embedded in glycol methacrylate (JB-4; Polyscience, Inc., Warrington, USA). For each animal and segment, 20 non-serial sections containing adjacent crypts and villi and muscle layer oriented were taken and stained with 0.05% toluidine-blue O (Sigma-Aldrich, St. Louis, USA) solution and mounted with coverslip and synthetic resin. Villus height, crypt depth, and muscle layer thickness were evaluated in images taken using a light microscope Top Light B2 coupled to a BEL Micro Image Explorer analysis system (both BEL Engineering srl, Monza, Italy).

Additionally, the histomorphometric analysis was done using stereological methodology (Baddeley et al., 1986) to analyze partial volume of the absorptive mucosa surface (Vv) and density of the absorptive mucosa surface (Sv) in five fields by intestinal segments (magnification 10×) (Van Ginneken et al., 2002). This variable was estimated using a point counting grid type cycloid with 35 arc

cycloids and 70 test points overlapped, according to Baddeley et al. (1986).

The volume of the absorptive mucosa surface (Vv) of the intestinal mucosa was calculated by the following formula (%):

 $Vv = \Sigma P \text{ (mucosa)}/\Sigma P \text{ (referencial volume)} \times 100$ 

where:

 $\Sigma P$  (mucosa) = number of test points falling on the region of interest (specific layer)

 $\Sigma P$  (referential volume) = number of test points in the reference volume (grid)

The density of the absorptive mucosa surface (Sv) of the intestinal mucosa was calculated by the following formula (mm<sup>2</sup>/mm<sup>3</sup>):

 $Sv = 2 \times \Sigma I \text{ (mucosa)/I(p)} \times \Sigma P \text{ (mucosa)}$ 

where:

 $\Sigma I$  = number of intersections of the test lines with the epithelium of the absorptive mucosa

I(p) = test line length (mm) associated with a point of the grid

 $\Sigma P$  = number of test points falling on the reference volume (intestinal wall) (Figure 1)

# Number of goblet cells

For each animal and segment, 15 non-serial sections containing oriented villi were taken (magnification 10×) and used to goblet cells quantification (Mashimo et al., 1996). The sections were placed on slides, stained with 0.05% toluidine-blue solution (Sigma-Aldrich, St. Louis, USA), buffered with citric acid and anhydrate bi-phosphate solution (pH 4.5), and mounted with coverslip and synthetic resin. Blue staining goblet cells in each oriented villi were counted using a light microscope (Top Light B2), coupled to a BEL Micro Image

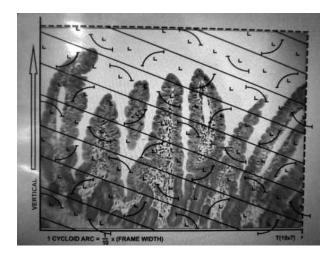


Figure 1. Point counting grid type cycloid used to stereological analysis

Explorer analysis system (both BEL Engineering srl, Monza, Italy).

# Statistical analysis

A completely randomized design was used. The statistical analysis was performed using SAS software (Statistical Analysis System, Version 9.2., 2008). Histomorphometric variables and goblet cells were arranged in a  $2 \times 3$  factorial scheme. Colostrum group and sampling time were considered as the main effects. The 0 h group data were analyzed by orthogonal contrasts using the General Linear Models (GLM) Procedure of SAS. The Analysis of Variance (ANOVA) (F test at 5% probability) was performed using the MIXED Procedure of SAS. Differences between means were analyzed by Tukey's test ( $\alpha = 0.05$ ) and values expressed as means and standard errors of means (SEM).

The morphometric values were also analyzed regardless of the treatment (goat or lyophilized bovine colostrum) in a  $4 \times 3$  factorial scheme. The intestinal segments (duodenum, jejunum, and ileum) and sampling times (0, 18, 36, and 96 h of life) were considered as main effects. The variable was submitted to analysis of variance (F test at 5% probability) using the MIXED Procedure of SAS. The normality of data was tested by Shapiro-Wilk test and it was not attended in relation to the villus height in the duodenum and in the number of goblet cells in the jejunum. Transformation (log base 10) was used when the normality presupposition was not attended and the results are presented as retransformed Least Squares Means ( $x^2$ ).

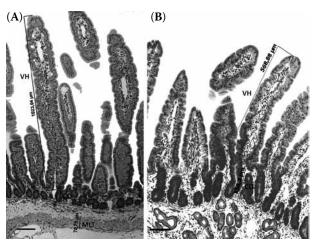


Figure 2. Intestinal histomorphometry: villus height (VH), crypt depth (CD), and muscle layer thickness (MLT) in the duodenum (**A**) and jejunum (**B**) segments of goat kids (bar =  $100 \mu m$ )

#### **RESULTS**

There were no effects of colostrum feeding treatments in histomorphometric variables (Figure 2) as villus height, crypt depth, muscle layer thickness, Vv, and Sv (P > 0.05) in the duodenum, jejunum, and ileum segments (Tables 2–4). In the duodenum and ileum, the villus height did not differ in the sampling times (P > 0.05). The jejunum showed differences in the sampling times to villus height (P < 0.05), at 36 h villus was higher than at 0 and 18 h. Regardless of the treatments, the maximum villus height was observed in the jejunum (860.1  $\pm$  42.8  $\mu$ m) compared to duodenum (595.9  $\pm$  33.5  $\mu$ m) and ileum (649.9  $\pm$  36.8  $\mu$ m) (P < 0.05).

In the duodenum, an interaction was observed between treatment and sampling times to crypt depth (P < 0.05). However, the Tukey's test did not detect the differences. In the jejunum, crypt depth differed only in the sampling times, at 96 h crypts were deeper than at 0 and 18 h (P < 0.05). In the ileum, the crypt depth did not differ in the sampling times (P > 0.05). Evaluating the crypt depth regardless of the treatments, an interaction between intestinal segment and sampling times was verified (P < 0.05) (Figure 3). Duodenum and ileum crypt depth did not differ in the sampling times and, in the jejunum crypt depth it increased at 96 h  $(154.9 \pm 8 \mu m)$ . At 18 h, the duodenum  $(153.6 \pm$ 8.4 µm) showed crypts deeper than the jejunum and at 96 h, the crypts were deeper in the duodenum  $(168.8 \pm 8.0 \,\mu\text{m})$  than in the ileum  $(129.7 \pm 8.4 \,\mu\text{m})$ .

Table 2. Morphometric features in the duodenum (Least Squares Means  $\pm$  standard errors) of newborn goat kids fed goat and lyophilized bovine colostrum

Traits	Treatment	Sampling times (h)				Effect		
		0	18	36	96	T	ST	$T \times ST$
Villus height (μm) <sup>a</sup>	GC		424.6 ± 87.3	474.2 ± 87.3	495.5 ± 88.1	ns	ns	ns
	LBC		$644.2 \pm 87.0$	$553.3 \pm 78.4$	$529.7 \pm 78.0$			
	GM	$862.1 \pm 103.8$	$522.4 \pm 61.5$	$510.5 \pm 58.9$	$514.0 \pm 59.1$			
Crypt depths (µm)	GC		158.3 ± 11.1	178.5 ± 11.2	$157.3 \pm 6.4$	ns	ns	*
	LBC		$148.7 \pm 9.9$	$160.0 \pm 9.9$	$159.8 \pm 5.9$			
	GM	$156.5 \pm 29.3$	$153.9 \pm 7.8$	$153.5 \pm 7.5$	$169.3 \pm 7.5$			
$\begin{array}{c} & GC \\ Muscle layer \\ thickness (\mu m) \end{array}  \begin{array}{c} GC \\ LBC \\ GM \end{array}$	GC		166.2 ± 26.9	$245.7 \pm 27.0$	$225.0 \pm 27.2$	ns	ns	ns
	LBC		$174.2 \pm 26.9$	$152.7 \pm 24.3$	$217.0 \pm 24.1$			
	GM	$202.2 \pm 34.6$	$170.2 \pm 19.0$	$199.2 \pm 18.2$	$221.0 \pm 18.3$			
Vv (%)	GC		$39.2 \pm 8.2$	$45.0 \pm 6.9$	$46.7 \pm 7.0$	ns	ns	ns
	LBC		$52.8 \pm 6.2$	$52.8 \pm 6.2$	$40.2 \pm 6.2$			
	GM	$65.9 \pm 8.4$	$46.0 \pm 5.1$	$53.8 \pm 4.7$	$43.4 \pm 4.8$			
Sv (mm <sup>2</sup> /mm <sup>3</sup> )	GC		$0.072 \pm 0.002$	$0.068 \pm 0.002$	$0.067 \pm 0.002$	ns	ns	ns
	LBC		$0.071 \pm 0.002$	$0.067 \pm 0.002$	$0.068 \pm 0.002$			
	GM	$0.068 \pm 0.003$	$0.071 \pm 0.001$	$0.067 \pm 0.000$	$0.067 \pm 0.001$			

T = treatment, ST = sampling time,  $T \times ST$  = interaction between treatment and sampling time, GC = goat colostrum, LBC = lyophilized bovine colostrum, GM = general mean, Vv = volume of the absorptive mucosa, Sv = density of the absorptive mucosa, Sv = not significant

In the duodenum and jejunum, the muscle layer did not differ in the sampling times (P > 0.05). The ileum showed differences in the sampling times

to muscle layer (P < 0.05), the layer was thicker at 36 h than at 0 and 18 h of life and at 96 h than at 0 h. Regardless of the treatments, the highest

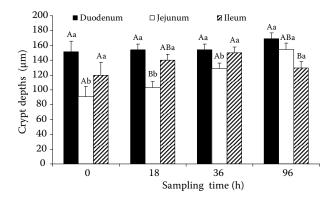


Figure 3. Crypt depth (Least Squares Means ± standard errors) in small intestinal segments of goat kids fed lyophilized bovine or goat colostrum

<sup>ab</sup>means with different small letters in the same intestinal segment differ among sampling times by Tukey's test (P < 0.05) <sup>AB</sup>means with different capital letters in the same sampling time differ among intestinal segments by Tukey's test (P < 0.05)

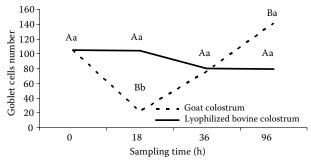


Figure 4. Goblet cells number (Least Squares Means) in the jejunum segment of goat kids fed goat or lyophilized bovine colostrum

 $^{\rm ab}$  means with different small letters in the same colostrum treatment are different by Tukey's test (P < 0.05)

 $^{\mathrm{AB}}$ means with different capital letters in the same sampling time are different by Tukey's test (*P* < 0.05)

<sup>\*</sup>P < 0.05

aretransformed data ( $x^2$ )

Table 3. Morphometric features in the jejunum (Least Squares Means  $\pm$  standard errors) of newborn goat kids fed goat and lyophilized bovine colostrum

	т , .	Sampling times (h)				Effect		
Traits	Treatment	0	18	36	96	T	ST	$T \times ST$
Villus height (μm)	GC		864.5 ± 62.9	1.149.7 ± 69.6	934.0 ± 79.0	ns	杂	ns
	LBC		$878.0 \pm 69.7$	$1024.5 \pm 70.0$	$920.5 \pm 69.9$			
	GM	$825.2 \pm 92.2$	$871.2 \pm 49.4^{\rm b}$	$1087.1 \pm 49.4^{a}$	$927.3 \pm 53.1^{ab}$			
Crypt depths (µm)	GC		95.8 ± 11.0	$128.0 \pm 11.0$	$152.3 \pm 12.5$	ns	非	ns
	LBC		$109.3 \pm 11.0$	$127.8 \pm 11.0$	$158.4 \pm 11.0$			
	GM	$88.1 \pm 14.1$	$102.5 \pm 7.8^{b}$	$127.9 \pm 7.8^{ab}$	$155.4 \pm 8.3^{a}$			
Muscle layer thickness (μm)	GC		$80.8 \pm 16.6$	125.3 ± 16.6	$139.2 \pm 18.8$	ns	ns	ns
	LBC		$117.2 \pm 16.6$	$112.5 \pm 16.6$	$139.9 \pm 16.6$			
	GM	$84.1 \pm 21.4$	99.0 ± 11.7	$118.9 \pm 11.8$	139.6 ± 12.6			
Vv (%)	GC		77.6 ± 3.5	$83.0 \pm 3.4$	$68.9 \pm 3.9$	ns	ns	ns
	LBC		$83.6 \pm 3.8$	$81.6 \pm 3.5$	$79.3 \pm 3.4$			
	GM	$67.6 \pm 4.4$	$80.6 \pm 2.6$	$82.3 \pm 2.4$	$74.1 \pm 2.6$			
Sv (mm <sup>2</sup> /mm <sup>3</sup> )	GC		$0.068 \pm 0.001$	$0.068 \pm 0.001$	$0.069 \pm 0.001$	ns	ns	ns
	LBC		$0.064 \pm 0.001$	$0.070 \pm 0.001$	$0.070 \pm 0.001$			
	GM	$0.069 \pm 0.002$	$0.068 \pm 0.000$	$0.069 \pm 0.000$	$0.069 \pm 0.001$			

T = treatment, ST = sampling time,  $T \times ST$  = interaction between treatment and sampling time, GC = goat colostrum, LBC = lyophilized bovine colostrum, GM = general mean, Vv = volume of the absorptive mucosa, Sv = density of the absorptive mucosa, Sv = not significant

thickness of the muscle layer was observed in the duodenum (197.9  $\pm$  8.5  $\mu$ m) compared with jejunum (111.4  $\pm$  8.3  $\mu$ m) and ileum (83.9  $\pm$  9.4  $\mu$ m) (P < 0.05). In the sampling times, the muscle layer was thicker at 96 h (153.7  $\pm$  8.2) than at 18 h of life (117.4  $\pm$  7.8  $\mu$ m).

In the duodenum and jejunum, the Vv did not differ in the sampling times (P > 0.05). The ileum showed the highest Vv (P < 0.05) at 36 h of goat kids' life. The Vv was the highest in the jejunum ( $76.3 \pm 2.3\%$ ) compared to duodenum ( $53.0 \pm 2.4\%$ ) and ileum ( $58.8 \pm 2.6\%$ ). In the sampling times, the Vv (P < 0.05) was higher at 36 h than at 96 h.

In the duodenum and ileum, the number of goblet cells did not differ between treatments and in the sampling times (P > 0.05). In the jejunum segment, a significant interaction between treatment and sampling times was observed (P < 0.05) (Figure 4). In LBC group, the number of goblet cells did not differ in the sampling times and, in GC group, the lowest number was observed at 18 h. The number of goblet cells in GC (22.4) was smaller than in LBC (104.7) at 18 h and higher at 96 h (174.2 in GC and

79.1 in LBC). The ileum showed the greatest number of goblet cells (P < 0.05) (Figure 5) compared to duodenum and jejunum (379.3, 134.9, and 80.7, respectively).

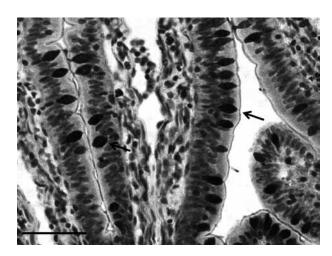


Figure 5. Goblet cells (arrow) in the ileum segment of goat kids (bar = 100  $\mu m)$ 

<sup>&</sup>lt;sup>a,b</sup>means within a row with different superscripts are different by Tukey's test

<sup>\*</sup>P < 0.05

Table 4. Morphometric features in the ileum (Least Squares Means  $\pm$  standard errors) of newborn goat kids fed goat and lyophilized bovine colostrum

Traits	Treatment	Sampling times (h)				Effect		
		0	18	36	96	T	ST	$T \times ST$
Villus height (µm)	GC		638.5 ± 72.8	667.6 ± 72.7	721.7 ± 115.0	ns	ns	ns
	LBC		$680.3 \pm 72.7$	$829.9 \pm 72.9$	$627.7 \pm 73.3$			
	GM	586.9 ± 113.6	$659.4 \pm 51.4$	$748.7 \pm 51.5$	$674.7 \pm 68.0$			
Crypt depths (µm)	GC		$142.8 \pm 11.2$	153.5 ± 11.2	$126.8 \pm 14.5$	ns	ns	ns
	LBC		$138.4 \pm 11.2$	$147.6 \pm 11.2$	$129.0 \pm 11.3$			
	GM	$122.8 \pm 17.5$	$140.6 \pm 7.9$	$150.6 \pm 7.9$	$127.9 \pm 9.2$			
Muscle layer thickness (μm)	GC		$74.3 \pm 6.2$	$103.4 \pm 6.9$	$88.8 \pm 8.0$	ns	非	ns
	LBC		$87.9 \pm 6.2$	$98.9 \pm 6.2$	$106.8 \pm 6.2$			
	GM	$60.8 \pm 9.7$	$81.1 \pm 4.4^{\rm bc}$	$101.2 \pm 4.7^{a}$	$97.8 \pm 5.1^{ac}$			
Vv (%)	GC		$60.2 \pm 5.3$	$72.9 \pm 5.3$	49.9 ± 6.9	ns	赤	ns
	LBC		$60.2 \pm 5.3$	$73.6 \pm 5.9$	$59.8 \pm 5.3$			
	GM	$46.8 \pm 8.3$	$60.2 \pm 3.8^{b}$	$73.3 \pm 4.0^{a}$	$54.8 \pm 4.4^{\rm b}$			
Sv (mm <sup>2</sup> /mm <sup>3</sup> )	GC		0.069 ± 0.001	$0.068 \pm 0.001$	0.069 ± 0.001	ns	ns	ns
	LBC		$0.068 \pm 0.001$	$0.070 \pm 0.001$	$0.070 \pm 0.001$			
	GM	$0.069 \pm 0.002$	$0.068 \pm 0.000$	$0.069 \pm 0.000$	$0.070 \pm 0.001$			

T = treatment, ST = sampling time,  $T \times ST$  = interaction between treatment and sampling time, GC = goat colostrum, LBC = lyophilized bovine colostrum, GM = general mean, Vv = volume of the absorptive mucosa, Sv = density of the absorptive mucosa, Sv = not significant

### **DISCUSSION**

The development, absorption, and maturation of the gastrointestinal tract in the first days after birth is greatly influenced by colostrum intake (Bessi et al., 2002a, b; Moretti et al., 2010a, b; Machado-Neto et al., 2011; Nordi et al., 2012). In the present study the goat kids' intestinal histomorphometry was unaffected by different sources of colostrum, goat or lyophilized bovine. Similar to the results observed in this study, Moretti et al. (2012a) using scanning electron microscopy, did not verify morphological differences in the villi of goat kids fed goat or lyophilized bovine colostrum.

The histomorphometric analysis revealed deeper crypt and higher muscle layer thickness in the duodenum, compared with other intestinal segments. The deepest crypt was linked with cell proliferation and renovation process after the colostrum ingestion (Bittrich et al., 2004; Godlewski et al., 2005). A higher percentage of mitosis process and an increase in crypts depth were detected on the

lambs' duodenum at 24 h after feeding on bovine colostrum (Moretti et al., 2010a; Machado-Neto et al., 2011). According to those authors, higher concentrations of bioactive factors present in bovine colostrum probably influenced the proliferation, differentiation activity, and growth of the duodenal mucosa. In the current study, IGF-I concentration in goat and lyophilized bovine colostrum was low, which may be responsible for the absence of differences in the enteric histomorphometry features between the colostrum sources. Similar results were found in villi density in goat kids fed goat or lyophilized bovine colostrum (Moretti et al., 2012a). Corroborating these results, Bühler et al. (1998) and Pauletti et al. (2007) did not find changes in the intestinal morphometric characteristics of calves ingesting different amounts of IGF-I.

As expected, the villus height was greater in the goat kids' jejunum segment. The same feature was found in calves by Blättler et al. (2001), Bittrich et al. (2004), and Sauter et al. (2004). In this segment, in the first hours of goat kids' life it is possible

<sup>&</sup>lt;sup>a-c</sup>means within a row with different superscripts are different by Tukey's test

<sup>\*</sup>P < 0.05

to verify enterocytes containing vacuoles filled with colostrum and IgG immunostaining that are related with intense activity of IgG uptake during the passive acquisition period (Moretti et al., 2012a; Nordi et al., 2012).

The enteric and absorptive capacity could be related with villus height, volume and density of the mucosa surface. As verified by Pauletti et al. (2007) in calves and Van Ginneken et al. (2002) in pigs, the jejunum showed the highest Vv, probably because of the highest villus or more numerous structures compared to other intestinal segments. A correlation between the villus height and Vv was verified (r = 0.72), suggesting a reflection about the capacity of the absorptive mucosa area. In this study, the Sv was unaffected by different sources of colostrum, goat or lyophilized bovine, indicating that despite the increase of mucosal layer volume, the absorptive area per mucosa unit remains the same. Differences in absorptive mucosa area and intestine development are related with intense absorption of macromolecules and total solids obtained from colostrum, presence of villus with varying sizes, different stages of maturity enteric, and changes in rates of nutrient transport by the absorptive Ouvir cells (Pácha, 2000; Bessi et al., 2002a; Van Ginneken et al., 2002; Pauletti et al., 2007; Machado-Neto et al., 2011).

The mucins are synthesized and secreted by exocytosis by the goblet cells into the gut lumen compounding a mucus layer, lubricating and protecting the intestinal epithelium (Corfield et al., 2001; Deplancke and Gaskins, 2001). Many substances, including dietary nutrients, hormones, acidification of intestinal contents, osmolality, and inflammatory mediators can regulate mucins synthesis and induce their discharge by exocytosis (Specian and Neutra, 1980; Fontaine et al., 1996; Corfield et al., 2001; Deplancke and Gaskins, 2001). In this study, the number of goblet cells filled with mucin was low at 18 h of life in GC group compared to 0 h group. Probably the ingestion of homologous colostrum did not stimulate mucus production by the goblet cells, and likewise, the exocytosis process. Blättler et al. (2001), like in the present work, did not observe significant differences in the number of goblet cells in the ileum of calves supplemented with homologous colostrum. After the colostrum ingestion period (14 h of life), all goat kids received whole cow milk twice a day, which can be related with the increase in the number of goblet cells filled with mucin in GC group at 96 h of life. The supply of lyophilized bovine colostrum did not determine alterations in the goblet cell number during the first hours of life, reaffirming that bovine lacteal secretion can stimulate the production and secretion of mucin in the intestinal tract.

#### CONCLUSION

The ingestion of lyophilized bovine colostrum did not apparently determine any consequences for enteric histology and histomorphometric features in the first 4 days of goat kids' life. Thus, the results indicate that this lacteal secretion can be successfully used as an alternative source of IgG, substituting goat colostrum in newborn goat kids.

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#### REFERENCES

AOAC (2000): Official Methods of Analysis. 17<sup>th</sup> Ed. Association of Official Analytical Chemists, Gaithersburg, USA.

Argüello A., Castro N., Zamorano M.J., Castroalonso A., Capote J. (2004): Passive transfer of immunity in kid goats fed refrigerated and frozen goat colostrum and commercial sheep colostrum. Small Ruminant Research, 54, 237–241.

Baddeley A., Gundersen H.J.G., Cruz-Orive L.M. (1986): Estimation of surface area from vertical sections. Journal of Microscopy, 42, 259–276.

Besser T.E., Garmedia A.E., McGuire T.C., Gay C.C. (1985): Effect of colostral immunoglobulin G1 and immunoglobulin M concentrations on immunoglobulin absorption in calves. Journal of Dairy Science, 68, 2033–2037.

Bessi R., Pauletti P., d'Arce R.D., Neto R.M. (2002a): Colostral antibodies absorption in calves. II. Distal small intestine study. Revista Brasileira de Zootecnia, 31, 2325–2331. (in Portuguese)

Bessi R., Pauletti P., d'Arce R.D., Neto R.M. (2002b): Colostral antibodies absorption in dairy calves. I. Proximal small intestine study. Revista Brasileira de Zootecnia, 31, 2314–2324. (in Portuguese)

- Bittrich S., Philipona C., Hammon H.M., Romé V., Guilloteau P., Blum J.W. (2004): Preterm as compared with full-term neonatal calves are characterized by morphological and functional immaturity of the small intestine. Journal of Dairy Science, 87, 1786–1795.
- Blacklaws B.A., Berriatua E., Torsteinsdottir S., Watt N.J., Andres D., Klein D., Harkiss G.D. (2004): Transmission of small ruminant lentiviruses. Veterinary Microbiology, 101, 199–208.
- Blättler U., Hammon H.M., Morel C., Philipona C., Rauprich A., Romé V., Le Huërou-Luron I., Guilloteau P., Blum J.W. (2001): Feeding colostrum, its composition and feeding duration variably modify proliferation and morphology of the intestine and digestive enzyme activities of neonatal calves. Journal of Nutrition, 131, 1256–1263.
- Blum J.W., Baumrucker C.R. (2002): Colostral and milk insulin-like growth factors and related substances: mammary gland and neonatal (intestinal and systemic) targets. Domestic Animal Endocrinology, 23, 101–110.
- Bühler C., Hammon H., Rossi G.L., Blum J.W. (1998): Small intestinal morphology in eight-day-old calves fed colostrum for different durations or only milk replacer and treated with long-R3-insulin-like growth factor I and growth hormone. Journal of Animal Science, 76, 758–765.
- Castro N., Capote J., Álvarez S., Argüello A. (2005): Effects of lyophilized colostrum and different colostrum feeding regimens on passive transfer of immunoglobulin G in majorera goat kids. Journal of Dairy Science, 88, 3650–3654.
- Corfield A.P., Carroll D., Myerscough N., Probert C.S.J. (2001): Mucins in the gastrointestinal tract in health and disease. Bioscience, 6, 1321–1357.
- Curtain C.C., Fudenberg H.H. (1973): Evolution of the immunoglobulin antigens in the ruminantia. Biochemical Genetics, 8, 301–308.
- Deplancke B., Gaskins E.R. (2001): Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. American Journal of Clinical Nutrition, 73, 1131–1141.
- Fontaine N., Meslin J.C., Lory S., Andrieux C. (1996): Intestinal mucin distribution in the germ-free rat and in the heteroxenic rat harbouring a human bacterial flora: effect of inulin in the diet. British Journal of Nutrition, 75, 881–892.
- Godlewski M.M., Slupecka M., Wolinski J., Skrzypek T., Skrzypek H., Motyl T., Zabielski R. (2005): Into the unknown the death pathways in the neonatal gut epithelium. Journal of Physiology and Pharmacology, 56, 7–24.
- Greenwood P.L. (1995): Effects of caprine arthritis-encephalitis virus on productivity and health of dairy goats in New South Wales, Australia. Preventive Veterinary Medicine, 22, 71–87.
- Hammon H.M., Blum F.W. (2002): Feeding different amounts of colostrum or only milk replacer modify receptors of in-

- testinal insulin-like growth factors and insulin in neonatal calves. Domestic Animal Endocrinology, 22, 155–168.
- Kelly D., Coutts A.G.P. (2000): Development of digestive and immunological function in neonates: role of early nutrition. Livestock Production Science, 66, 161–167.
- Kindlein L., Pauletti P., Bagaldo A.R., Rodrigues A.P.O., Machado-Neto R. (2008): Effects of enriched colostrum supply in intestinal mucosa morphology of newborn calves. Acta Scientiae Veterinarie, 36, 31–34. (in Portuguese)
- Kráčmar S., Gajdůšek S., Jelínek P., Zeman L., Kozel V., Kozlová M., Kráčmarová E. (1999): Changes in amino acid composition of goat's colostrum during the first 72 hours after birth. Czech Journal of Animal Science, 44, 541–545.
- Kráčmar S., Jankovská R., Šustová K., Kuchtík J., Zeman L. (2004): Analysis of amino acid composition of sheep colostrum by near-infrared spectroscopy. Czech Journal of Animal Science, 49, 177–182.
- Křenková L., Čanderle J. (2001): Leptin in colostrum and blood serum: Is there any relation to milk production and weight gain of piglets in Large White sows? Czech Journal of Animal Science, 46, 375–376.
- Lima A.L., Pauletti P., Susin I., Machado-Neto R. (2009): Fluctuation of serum variables in goats and comparative study of antibody absorption in new-born kids using cattle and goat colostrum. Revista Brasileira de Zootecnia, 38, 2211–2217. (in Portuguese)
- Machado-Neto R., Grigolo I.H., Moretti D.B., Kindlein L., Pauletti P. (2011): Intestinal histology of Santa Ines lambs fed bovine or ovine colostrum. Czech Journal of Animal Science, 56, 465–474.
- Mancini G., Carbonara A.O., Heremans J.F. (1965): Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2, 235–254.
- Mashimo H., Wu D.C., Podolsky D.K., Fishman M.C. (1996): Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. Science, 274, 262–265.
- Moretti D.B., Pauletti P., Kindlein L., Machado-Neto R. (2010a): Enteric cell proliferation in newborn lambs fed bovine and ovine colostrum. Livestock Science, 127, 262–266.
- Moretti D.B., Kindlein L., Pauletti P., Machado-Neto R. (2010b): IgG absorption by Santa Ines lambs fed Holstein bovine colostrum or Santa Ines ovine colostrum. Animal, 4, 933–937.
- Moretti D.B., Nordi W.M., Lima A.L., Pauletti P., Susin I., Machado-Neto R. (2012a): Goat kids' intestinal absorptive mucosa in period of passive immunity acquisition. Livestock Science, 144, 1–10.
- Moretti D.B., Nordi W.M., Lima A.L., Pauletti P., Susin I., Machado-Neto R. (2012b): Lyophilized bovine colostrum as a source of immunoglobulins and insulin-like growth factor for newborn goat kids. Livestock Science, 145, 223–229.

- Nordi W.M., Moretti D.B., Lima A.L., Pauletti P., Susin I., Machado-Neto R. (2012): Intestinal IgG uptake by small intestine of goat kid fed goat or lyophilized bovine colostrum. Livestock Science, 144, 205–201.
- Pácha J. (2000): Development of intestinal transport function in mammals. Physiology Reviews, 80, 234–238.
- Pauletti P., Bagaldo A.R., Kindlein L., Machado Neto R. (2007): Insulin-like growth factor-I, passive immunity transfer, and stereological characteristics of small intestine of newborn calves. Animal Science Journal, 78, 631–638.
- Penchev Georgiev I. (2008a): Differences in chemical composition between cow colostrum and milk. Bulgarian Journal of Veterinary Medicine, 11, 3–12.
- Penchev Georgiev I. (2008b): Effect of colostrum insulinlike growth factors on growth and development of neonatal calves. Bulgarian Journal of Veterinary Medicine, 11, 75–88.
- Peterhans E., Greenland T., Badiola J., Harkiss G., Bertoni G., Amorena A., Eliaszewicz M., Juste R.A., Kraßnig R.,

- Lafont J., Lenihan P., Pétursson G., Pritchardl G., Thorley J., Vitu C., Mornex J., Pépin M. (2004): Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. Veterinary Research, 35, 257–274.
- Sauter S.N., Roffler B., Philipona C., Morel C., Romé V., Guilloteau P., Blum J.W., Hammon H.M. (2004): Intestinal development in neonatal calves: effects of glucocorticoids and dependence on colostrum feeding. Biology of the Neonate, 85, 94–104.
- Specian R.D., Neutra M.R. (1980): Mechanism of rapid mucus secretion in goblet cells stimulated by acetylcholine. Journal of Cell Biology, 85, 626–640.
- Van Ginneken C., Van Meir F., Sys S., Weyns A. (2002): Stereologic characteristics of pig small intestine during normal development. Digestive Diseases and Science, 47, 868–878.

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