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Enzyme activity in the small intestine of goat kids during the period of passive immunity acquisition

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ABSTRACT

Enzyme activity of protein and carbohydrate degradation in small intestinal mucosa was investigated in goat kids fed with lyophilized bovine and goat colostrum. At 0, 7 and 14 h of life 15 male newborns received 5% of body weight of lyophilized bovine colostrum and 14 goat colostrum, both with 55 mg/mL of IgG. Duodenum, jejunum and ileum samples were collected at 18, 36 and 96 h of life. Three animals were sampled at birth, without colostrum intake. Activity of aminopeptidase N and A, dipeptidil peptidase IV, lactase, maltase and sucrase was determined as one international unit per gram of tissue. Intracellular enzymatic activity of acid phosphatase was observed by histochemistry in tissue section. Only the activity of aminopeptidase A in the ileum was affected by treatment, with a greater value for LBC than for GC ($P<0.05$). The aminopeptidase N activity was the highest at 36 h in the duodenum ($P<0.05$) and lowest at 96 h in the jejunum ($P<0.05$). Dipeptidil peptidase IV activity was highest at 36 h in the duodenum ($P<0.05$), lowest at 96 h in the jejunum ($P<0.05$) and higher at 36 h than at 96 h in the ileum ($P<0.05$). Aminopeptidase A activity in the ileum was highest at 36 h ($P<0.05$), followed by 18 and 96 h of life ($P<0.05$). Lactase activity in the duodenum increased from 18 to 36 h and from 36 to 96 h in the jejunum ($P<0.05$). Maltase activity increased only in the duodenum from 18 to 96 h ($P<0.05$). Sucrase activity in the jejunum decreased from 18 to 36 h and from 36 to 96 h in the ileum ($P<0.05$). At birth, activity of most enzymes was similar to that at later times ($P<0.05$). Histochemistry analyses showed a higher frequency of lysosomes with acid phosphatase activity in the duodenum, especially at 36 h of life. In the jejunum, the presence of lysosomes with acid phosphatase activity was the highest at 96 h, followed by 36 and 18 h of life. In the ileum, all samples showed low presence of lysosomes with acid phosphatase activity. These results indicate that lyophilized bovine colostrum, as a heterologous source of antibodies or nutrients, is a possible alternative management tool for goats. The present work also suggests that in the first 4 days of life, enzyme activity in the intestinal epithelium of goats is still not fully stimulated, which is an important characteristic for these animals that depend on macromolecule absorption to acquire passive protection after birth.

1. Introduction

In the first hours of life, the gastrointestinal tract, especially that of ruminants, undergoes major changes that will greatly impinge upon development of the animals (Šimůnek et al., 1995; Kelly and Coutts, 2000). Besides the replacement of fetal-type enterocytes for a second

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generation of cells, which do not have the capacity to absorb macromolecules, the activities of intracellular and extracellular enzymes are changed (Campbell et al., 1977; Smeaton and Simpson-Morgan, 1985; Kelly and Coutts, 2000; Bessi et al., 2002). The acid phosphatase enzyme found in lysosomes and used as a marker of intracellular digestive activity and intestinal epithelium maturation seems to be inactive in the first 24 h of life of bovine and swine (Blättler et al., 2001; Bittrich et al., 2004). Regarding extracellular enzyme activity, it is known that until weaning the lactase activity decreases while the activity of other disaccharidases, such as sucrase and maltase, as well as aminopeptidase increases. These changes result in adaptation from a liquid diet to a solid one (Zhang et al., 1997; Sangild et al., 2000; Sauter et al., 2004).

Two mechanisms can modify the pattern of enzyme activity, changes in a given population of enterocytes within their brief life-span and cell replacement. However, the cell replacement mechanism seems most likely (Henning, 1985). The activity of these enzymes, as well as the development of the intestinal epithelium, is controlled mainly by genetics and hormones such as cortisol, insulin, growth hormone and bioactive factors, but ingestion of substrates of these enzymes can also influence specific activity (Riecken et al., 1989; Odle et al., 1996; Kelly and Coutts, 2000).

Colostrum, a milk secretion responsible for providing antibodies to newborns, also has essential and non-essential nutrients and other components that are associated with development of the gastrointestinal tract, including presence of a high concentration of Insulin-Like Growth Factor type I (IGF-I) (Šimůnek et al., 1995; Odle et al., 1996; Georgieva et al., 2003; Argüello et al., 2006; Rudovsky et al., 2008). Also, deprivation of colostrum in the first hours of life can determine the decrease or delay in enzyme activity in the small intestine (Kelly and Coutts, 2000).

In some situations, such as transmission of Caprine Arthritis Encephalitis Virus (CAEV), provision of colostrum to the newborn goat kid is not recommended, so it is necessary to search for alternatives (East et al., 1993; Quigley Iii et al., 2002; Argüello et al., 2004; Castro et al., 2005; Lima et al., 2009). Bovine colostrum can provide antibodies to small ruminants and is also an alternative easy to obtain and with high immune quality (Lima et al., 2009; Moretti et al., 2010a,b). The processing of colostrum by lyophilization, unlike pasteurization, is another alternative management that allows storage for prolonged periods, thus ensuring the biological function and quantity of antibodies (Quigley Iii et al., 2002; Argüello et al., 2003; Castro et al., 2005).

The aim of this study was to investigate the potential of protein and carbohydrate degradation in the intestine of goat kids fed with lyophilized bovine and goat colostrum.

2. Materials and methods

2.1. Animals, feeding and experimental procedures

In this study, 32 Saanen × Boer male goat kids were available. The animals were kept, maintained and treated in adherence to accepted standards for humane treatment of animals (authorized by ESALQ/USP ethics committee).

Colostrum was collected from two Holstein cows and 14 goats on commercial dairy farms. The animals were milked manually and the lacteal secretions were stored in sterile containers. Next, the colostrum was homogenized to form a unique pool of bovine colostrum and another of goat colostrum. Thereafter, the colostrum pools were stored at −20 °C. Samples of each pool were collected for determination of IgG concentration by radial immunodiffusion (Mancini et al., 1965; Besser et al., 1985). The frozen pool of bovine colostrum was lyophilized and the resulting powder was homogenized and stored in sealed containers at −20 °C.

At the time of offering the meals, the pool of goat colostrum was diluted with whole milk until reaching a concentration of 55 mg/mL of IgG. Bovine colostrum powder, however, was resuspended in water until it reached the original chemical composition of colostrum taken to the lyophilization process and, subsequently, diluted with whole milk until reaching a concentration of 55 mg/mL of IgG. Samples of final meals were collected for analysis of chemical composition (Table 1).

Table 1
Chemical composition (mean ± standard deviation) of lyophilized bovine and goat colostrum fed to newborn goat kids.

	Lyophilized bovine colostrum	Goat colostrum
Dry matter (%)	18.9 ± 0.2	20.1 ± 0.2
Crude protein (%)	9.4 ± 0.1	9.8 ± 0.1
Fat (%)	4.0 ± 0.1	7.8 ± 0.1

The newborn goat kids were separated from their mothers immediately after birth, without maternal colostrum intake. Fifteen animals received 5% of body weight of bovine lyophilized colostrum (LBC group) and 14 animals received goat colostrum (GC group) at 0, 7 and 14 h of life. Three goat kids did not receive colostrum and were sampled just after birth (0-h group).

2.2. Sample collection

Goat kids from LBC and GC were randomly slaughtered at 18, 36 and 96 h of life with anesthesia and bled from the carotid arteries. Three animals were sampled immediately after birth without colostrum ingestion, constituting a 0-h group. After slaughter, the abdominal cavity was opened, and the gastrointestinal tract was removed within 5–10 min. Afterwards, the small intestine was separated into the duodenum, middle jejunum and ileum. Samples were immediately frozen in liquid nitrogen and kept at −180 °C until colorimetric analyses of extracellular enzymatic activity. Additional intestinal samples were collected for histochemical analysis of intracellular enzymatic activity. The tissue was fixed in 4% phosphate buffer paraformaldehyde solution and maintained in it until histological procedures.

2.3. Extracellular enzyme activity of peptidases

Samples of intestinal mucosa (approximately 1 g) were thawed and homogenized in distilled water (10 mL) using an ultra-Turrax unit (Polytron®, Kinematica GnbH, Kriens-Luzern, Switzerland) for 1 min. Glycine buffer (71 mM pH 8.0) containing 1.5 mM of the substrates L-leucyl-*p*-nitroanilide, glycyl-L-prolyl-*p*-nitroanilide hydrochloride and L-glutamic acid 1-(4-nitroanilide) (Sigma Chemical Co., St. Louis, MO, USA) were used for the enzyme activity of aminopeptidase N (EC 3.4.11.2), dipeptidyl peptidase IV (EC 3.4.14.5) and aminopeptidase A (EC 3.4.11.7), respectively (Maroux et al., 1973; Nagatsu et al., 1976). The enzyme activity was expressed as international units (IU) per gram of tissue (1 IU corresponds to 1 mol of substrate hydrolyzed per minute at 37 °C).

2.4. Extracellular enzyme activity of disaccharidases

Samples of intestinal mucosa (approximately 1 g) were thawed and homogenized in distilled water (10 mL) using an ultra-Turrax unit (Polytron®, Kinematica GnbH) for 1 min. The substrates D-lactose monohydrate, D-maltose monohydrate and sucrose (56 mM) (Sigma Chemical Co.) in maleate buffer (0.1 M, pH 6.0) were used for the enzyme activity of lactase (EC 3.2.1.23), maltase (EC 3.2.1.20) and sucrase (EC 3.2.1.48), respectively (Dahlqvist, 1964). The enzyme activity was calculated as noted above.

2.5. Intracellular enzyme activity of acid phosphatase

For the histological analysis of intracellular enzyme activity, the tissues stored in 4% paraformaldehyde solution were dehydrated with solutions of ethanol (30%, 50%, 70%, 90% and 100% of ethanol) and embedded in glycol methacrylate (JB-4, Polysciences, Inc., Warrington, PA, USA). For each animal and segment, 5 μm thick sections were taken.

For the location of the acid phosphatase activity, the sections were incubated in Gomori medium solution containing sodium acetate buffer (0.05 M, pH 0.5), lead nitrate and enzyme substrate (β-sodium glycerophosphate) for 90 min at 37 °C. The slides were then washed with distilled water and incubated with 1% ammonium sulfate for 2 min (Bessi et al., 2002). The tissues were post-stained with toluidine blue O and examined. Samples of a rat spleen were used as positive control.

2.6. Statistical analysis

The statistical analysis was performed using the SAS (v 9.1) program package (SAS Institute, Cary, NC, USA). The extracellular enzyme activity in each segment was analyzed based on a 2×3 completely randomized factorial design. The treatments (lyophilized bovine colostrum and goat colostrum) and sampling time (18, 36 and 96 h of life) were considered as the main effects. After the data were confirmed for normal distribution with the Shapiro–Wilk test, analysis of variance was performed using the PROC MIXED (SAS Institute Inc., 2008). The diagonal covariance structure was used. If the F value was significant, the Tukey test was used for multiple comparisons between pairs of means at 5% of probability. Furthermore, comparisons between the mean of the 0-h group and the mean of each treatment (LBC 18 h, LBC 36 h, LBC 96 h, GC 18 h, GC 36 h and GC 96 h) were performed by orthogonal contrasts using general linear model (SAS Institute Inc., 2008) at 5% of probability. The values are presented as least-square means and standard errors.

The enzyme activity was also analyzed in the three sampling times, 18, 36, and 96 h, regardless of the source of colostrum ingested, lyophilized bovine or goat colostrum, as a 3×6 completely randomized factorial design. Intestinal segments (duodenum, jejunum and ileum) and enzymes studied (aminopeptidase N, dipeptidil peptidase IV, aminopeptidase A, lactase, maltase, sucrase) were considered as the main effects. The diagonal covariance structure was used. The transformation of the values (log base 10) was used to obtain normal distributions of data and the results are presented as retransformed least-square means (10^x). The variables were submitted to analysis of variance using PROC MIXED procedure. If the F value was significant, a Tukey test was used for multiple comparisons between pairs of means at 5% of probability.

The Pearson correlation analysis was used between the variables, through the PROC CORR program.

3. Results

3.1. Extracellular enzyme activity of peptidases

The values for the activity of peptidases in the small intestine are presented in Table 2. In the duodenum, activity of aminopeptidase A was not observed. The activity of aminopeptidase N and dipeptidyl peptidase IV were affected by sampling time ($P < 0.05$), but there was no effect of treatment or interaction between treatment and sampling time ($P > 0.05$). The highest activity observed for both enzymes, aminopeptidase N and dipeptidyl peptidase IV, was at 36 h ($P < 0.05$). There were no differences between the 0-h group and the other treatments ($P > 0.05$). The correlation between the activity of enzymes aminopeptidase N and dipeptidyl peptidase IV was 0.76 ($P < 0.05$).

In the jejunum, the activity of aminopeptidase N and dipeptidyl peptidase IV was affected by sampling time ($P < 0.05$), but there was no effect of treatment or interaction between treatment and sampling time ($P > 0.05$). The lowest activity observed for both enzymes, aminopeptidase N and dipeptidyl peptidase IV, was at 96 h ($P < 0.05$). For aminopeptidase A, there was no effect of treatment, sampling time or interaction between treatment and sampling time ($P > 0.05$). For aminopeptidase N and A, no significant difference was found between the 0-h group and the other treatments ($P > 0.05$). However, the dipeptidil peptidase IV activity of 0-h group, 2.83 ± 0.44, differed from the mean of LBC 96 h, 1.12 ± 0.26 ($P < 0.05$). The correlation between the activity of aminopeptidase N and dipeptidyl peptidase IV was 0.75 ($P < 0.05$). The enzyme activity of aminopeptidase A did not show significant correlation ($P > 0.05$) with the other aminopeptidases.

In the ileum, an effect of treatment, sampling time, or interaction between treatment and sampling time was not observed for the activity of aminopeptidase N ($P > 0.05$). The activity of the enzyme dipeptidyl peptidase IV was affected by sampling time ($P < 0.05$), however there was no effect of treatment or interaction between treatment and the sampling time ($P > 0.05$). The value for the activity of this enzyme at 36 h was higher than at 96 h ($P < 0.05$). The activity of aminopeptidase A was affected by treatment and sampling time ($P < 0.05$), but there was no interaction ($P > 0.05$). The highest activity of this enzyme was observed at 36 h, followed by 18 and 96 h ($P < 0.05$). For the three enzymes, there were no differences between the 0-h group and other treatments ($P > 0.05$). The correlation between aminopeptidase N was 0.46 with dipeptidyl peptidase and 0.37 with aminopeptidase A ($P < 0.05$), and the correlation between dipeptidyl peptidase IV and aminopeptidase was 0.62 ($P < 0.05$).

3.2. Extracellular enzyme activity of disaccharidases

The values for the activity of disaccharidases in the small intestine are presented in Table 3. Sampling time affected activity of lactase and maltase IV in the duodenum, but there was no effect of treatment or interaction between treatment and sampling time ($P > 0.05$). Higher lactase activity was observed at 18 h than at 36 h ($P < 0.05$), while the maltase activity at 18 h was higher than at 96 h ($P < 0.05$). There was no effect of the treatment, sampling time or interaction between the treatment and sampling time for the activity of sucrase ($P > 0.05$). For the three enzymes, there were no differences between the 0-h group and other treatments ($P > 0.05$). Correlations between the activity of disaccharidases were not observed ($P > 0.05$).

In the jejunum, lactase and sucrase activity were affected by time of sampling ($P < 0.05$), but there was no effect of treatment or treatment × sampling time ($P > 0.05$). The highest lactase activity was observed at 96 h ($P < 0.05$) and the sucrase activity at 18 h was higher than at 36 h ($P < 0.05$). There was no effect of treatment, sampling time, or treatment × sampling time on maltase activity ($P > 0.05$). There were no differences between the 0-h group and other treatments ($P > 0.05$). Correlations between the activity of disaccharidases were not observed ($P > 0.05$).

In the ileum, activity of lactase and sucrase was affected by sampling time ($P < 0.05$), but there was no interaction

Table 2
Enzyme activity (IU/g tissue) of peptidases in the small intestine of goat kids (least-square means ± standard errors).

Segment	Enzyme	Treatment[b]	Time (h)				Effect[a]		
			0 h[c]	18 h	36 h	96 h	T	ST	T × ST
Duodenum	Aminopeptidase N	LBC		1.18 ± 0.28	1.66 ± 0.29	0.72 ± 0.28	NS	*	NS
		GC		1.07 ± 0.28	2.12 ± 0.28	0.77 ± 0.36			
		Overall mean	1.64 ± 0.36	1.13 ± 0.19b	1.89 ± 0.20a	0.75 ± 0.23b			
	Dipeptidil peptidase IV	LBC		0.33 ± 0.10	0.64 ± 0.11	0.27 ± 0.10	NS	*	NS
		GC		0.29 ± 0.10	0.79 ± 0.10	0.20 ± 0.13			
		Overall mean	0.43 ± 0.07	0.31 ± 0.07b	0.71 ± 0.07a	0.23 ± 0.08b			
	Aminopeptidase A[d]	LBC		–	–	–	–	–	–
		GC		–	–	–			
		Overall mean	–	–	–	–			
Jejunum	Aminopeptidase N	LBC		3.33 ± 0.26	3.40 ± 0.27	2.59 ± 0.26	NS	*	NS
		GC		3.50 ± 0.26	3.40 ± 0.26	2.62 ± 0.41			
		Overall mean	3.82 ± 0.73	3.41 ± 0.18a	3.40 ± 0.18a	2.60 ± 0.24b			
	Dipeptidil peptidase IV	LBC		2.03 ± 0.26	2.38 ± 0.26	1.12 ± 0.26y	NS	*	NS
		GC		2.38 ± 0.26	2.10 ± 0.26	1.69 ± 0.41			
		Overall mean	2.83 ± 0.44x	2.20 ± 0.18a	2.24 ± 0.18a	1.40 ± 0.24b			
	Aminopeptidase A	LBC		0.27 ± 0.08	0.39 ± 0.08	0.16 ± 0.08	NS	NS	NS
		GC		0.22 ± 0.08	0.40 ± 0.08	0.44 ± 0.18			
		Overall mean	0.38 ± 0.02	0.25 ± 0.05	0.40 ± 0.05	0.30 ± 0.10			
Ileum	Aminopeptidase N	LBC		2.30 ± 0.35	2.57 ± 0.36	2.30 ± 0.40	NS	NS	NS
		GC		2.32 ± 0.35	2.80 ± 0.39	1.70 ± 0.45			
		Overall mean	1.96 ± 0.19	2.31 ± 0.24	2.68 ± 0.27	2.00 ± 0.31			
	Dipeptidil peptidase IV	LBC		2.27 ± 0.38	3.16 ± 0.40	1.58 ± 0.45	NS	*	NS
		GC		2.49 ± 0.38	3.01 ± 0.43	1.62 ± 0.61			
		Overall mean	2.19 ± 0.70	2.38 ± 0.27ab	3.08 ± 0.29a	1.60 ± 0.38b			
	Aminopeptidase A	LBC		0.43 ± 0.06	0.57 ± 0.07	0.12 ± 0.08	*	*	NS
		GC		0.19 ± 0.06	0.48 ± 0.06	0.08 ± 0.08			
		Overall mean	0.30 ± 0.06	0.31 ± 0.04b	0.53 ± 0.05a	0.10 ± 0.06c			

*$P < 0.05$; NS = $P > 0.05$; means within rows without common letters (a, b, c) differ ($P < 0.05$); means without common letters (x, y) differ from 0-h ($P < 0.05$). IU – international units, one mol of substrate hydrolyzed per minute at 37 °C.

[a] T = treatment; ST = sampling time; T × ST = interaction between treatment and sampling time.

[b] LBC = goat kids that received lyophilized bovine colostrum; GC = goat kids that received goat colostrum.

[c] Comparisons between the 0-h group mean and the mean of each treatment were performed by orthogonal contrasts.

[d] Enzyme activity of aminopeptidase A was not observed in this segment.

between treatment and sampling time ($P > 0.05$). The highest lactase activity was observed at 36 h ($P < 0.05$) and the sucrase activity at 36 h was higher than at 96 h ($P < 0.05$). There was no effect of the treatment, sampling time or interaction between treatment and sampling time for the activity of maltase ($P > 0.05$). For lactase and maltase, no significant difference was found between the 0-h group and the other treatments ($P > 0.05$). However, sucrase activity of the 0-h group, 0.09 ± 0.06, differed from that of LBC at 18 h, 0.33 ± 0.06 and LBC at 36 h, 0.43 ± 0.06 ($P < 0.05$). The correlation between activity of lactase and maltase was 0.36 and that between lactase and sucrase was 0.44 ($P < 0.05$). No correlation was observed between maltase and sucrase ($P > 0.05$).

3.3. Activity of extracellular enzymes in intestinal segments regardless of the source of colostrum ingested

There was an interaction between intestinal segment and enzyme at each sampling time ($P < 0.05$). The results are presented in Figs. 1–3. Aminopeptidase activity was predominant in the jejunum and ileum segments. For the disaccharidases, sucrase and maltase, the three intestinal segments made similar contributions to enzymatic activity. The enzyme lactase, in turn, showed intense activity in the duodenum and jejunum segments.

3.4. Intracellular enzyme activity of acid phosphatase

The acid phosphatase activity did not differ between the animals that received lyophilized bovine or goat colostrum. The duodenum was the segment that showed the highest acid phosphatase activity, especially at 36 h of life (Fig. 4). In the jejunum, the presence of lysosomes with acid

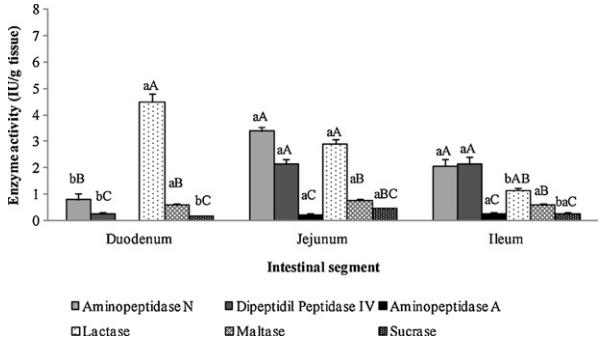

Fig. 1. Activity of six enzymes in small intestinal segments of goats at 18 h after birth. [ab]Means with different small letters in the same enzyme studied differ by Tukey's test ($P < 0.05$); [ABC]means with different capital letters in the same intestinal segment differ by Tukey's test ($P < 0.05$); IU – international units, one mol of substrate hydrolyzed per minute at 37 °C; data retransformed (10^x).

Table 3
Enzyme activity (IU/g tissue) of disaccharidases in the small intestine of goat kids (least-square means ± standard errors).

Segment	Enzyme	Treatment[b]	Time (h)				Effect[a]		
			0 h[c]	18 h	36 h	96 h	T	ST	T × ST
Duodenum	Lactase	LBC		4.91 ± 0.65	6.51 ± 0.68	6.76 ± 0.66	NS	*	NS
		GC		4.29 ± 0.66	6.46 ± 0.65	4.97 ± 0.86			
		Overall mean	4.79 ± 0.33	4.60 ± 0.46b	6.49 ± 0.47a	5.86 ± 0.55ab			
	Maltase	LBC		0.61 ± 0.04	0.68 ± 0.04	0.75 ± 0.05	NS	*	NS
		GC		0.61 ± 0.04	0.70 ± 0.04	0.82 ± 0.06			
		Overall mean	0.63 ± 0.02	0.61 ± 0.03b	0.69 ± 0.03ab	0.79 ± 0.04a			
	Sucrase	LBC		0.25 ± 0.05	0.15 ± 0.06	0.22 ± 0.06	NS	NS	NS
		GC		0.18 ± 0.05	0.25 ± 0.05	0.34 ± 0.07			
		Overall mean	0.20 ± 0.08	0.21 ± 0.04	0.20 ± 0.04	0.28 ± 0.05			
Jejunum	Lactase	LBC		2.76 ± 0.76	4.30 ± 0.78	7.57 ± 0.77	NS	**	NS
		GC		3.13 ± 0.76	4.96 ± 0.76	9.32 ± 1.20			
		Overall mean	3.69 ± 0.23	2.95 ± 0.53b	4.63 ± 0.54b	8.45 ± 0.71a			
	Maltase	LBC		0.75 ± 0.06	0.76 ± 0.06	0.80 ± 0.07	NS	NS	NS
		GC		0.83 ± 0.06	0.79 ± 0.06	0.82 ± 0.09			
		Overall mean	0.72 ± 0.04	0.79 ± 0.04	0.77 ± 0.04	0.81 ± 0.06			
	Sucrase	LBC		0.56 ± 0.06	0.33 ± 0.06	0.31 ± 0.06	NS	*	NS
		GC		0.40 ± 0.06	0.31 ± 0.06	0.51 ± 0.13			
		Overall mean	0.28 ± 0.10	0.48 ± 0.04a	0.32 ± 0.04b	0.41 ± 0.07ab			
Ileum	Lactase	LBC		1.13 ± 0.20	1.76 ± 0.21	1.14 ± 0.28	NS	*	NS
		GC		1.24 ± 0.20	1.78 ± 0.20	0.87 ± 0.26			
		Overall mean	1.07 ± 0.28	1.18 ± 0.14b	1.77 ± 0.14a	1.00 ± 0.19b			
	Maltase	LBC		0.60 ± 0.06	0.65 ± 0.06	0.71 ± 0.07	NS	NS	NS
		GC		0.63 ± 0.06	0.69 ± 0.06	0.68 ± 0.08			
		Overall mean	0.60 ± 0.09	0.62 ± 0.04	0.67 ± 0.04	0.69 ± 0.05			
	Sucrase	LBC		0.33 ± 0.06y	0.43 ± 0.06y	0.14 ± 0.08	NS	*	NS
		GC		0.28 ± 0.06	0.23 ± 0.06	0.13 ± 0.08			
		Overall mean	0.09 ± 0.06x	0.30 ± 0.04ab	0.33 ± 0.04a	0.13 ± 0.06b			

*$P < 0.05$; **$P < 0.001$; NS = $P > 0.05$; means within rows without common letters (a, b, c) differ ($P < 0.05$); means without common letters (x, y) differ from 0-h ($P < 0.05$). IU – international units, one mol of substrate hydrolyzed per minute at 37 °C.

[a] T = treatment; ST = sampling time; T × ST = interaction between treatment and sampling time.

[b] LBC = goat kids that received lyophilized bovine colostrum; GC = goat kids that received goat colostrum.

[c] Comparisons between the 0-h group mean and the mean of each treatment were performed by orthogonal contrasts.

phosphatase activity was the highest at 96 h, followed by 36 and 18 h of life. In this segment, only one tissue sample collected at 18 h and another at 36 h showed high enzyme activity in cells that were absorbing colostrum, however these lysosomes were not fusing with the filled vacuoles. In the ileum, a low presence of lysosomes with enzymatic activity was observed. At 36 h of life, two animals showed empty vacuoles with a positive reaction. Acid phosphatase activity was observed in the brush border of enterocytes in all the segments and sampling times studied.

4. Discussion

Postnatal development of the gastrointestinal tract is affected by intake of several bioactive factors from colostrum as verified by Odle et al. (1996), Zhang et al.

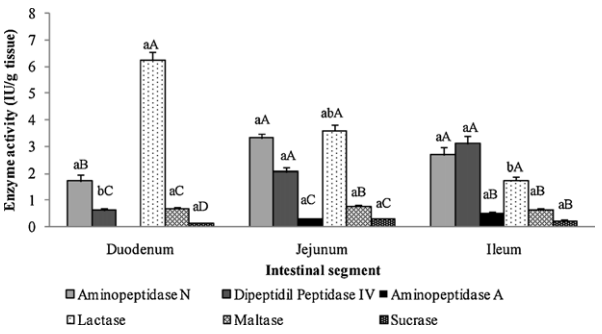

Fig. 2. Activity of six enzymes in small intestinal segments of goats at 36 h after birth. [ab]Means with different small letters in the same enzyme studied differ by Tukey's test ($P < 0.05$); [ABCD]means with different capital letters in the same intestinal segment differ by Tukey's test ($P < 0.05$); IU – international units, one mol of substrate hydrolyzed per minute at 37 °C; data retransformed (10^x).

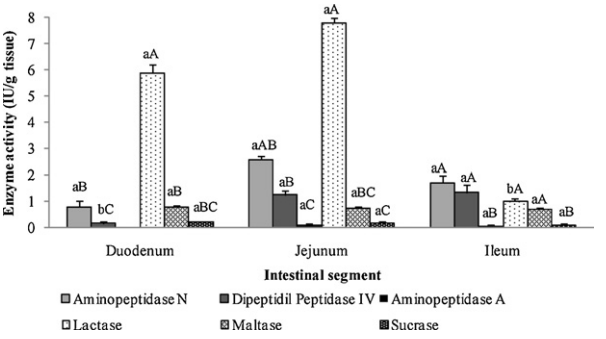

Fig. 3. Activity of six enzymes in small intestinal segments of goats at 96 h after birth. [ab]Means with different small letters in the same enzyme studied differ by Tukey's test ($P < 0.05$); [ABC]means with different capital letters in the same intestinal segment differ by Tukey's test ($P < 0.05$); IU – international units, one mol of substrate hydrolyzed per minute at 37 °C; data retransformed (10^x).

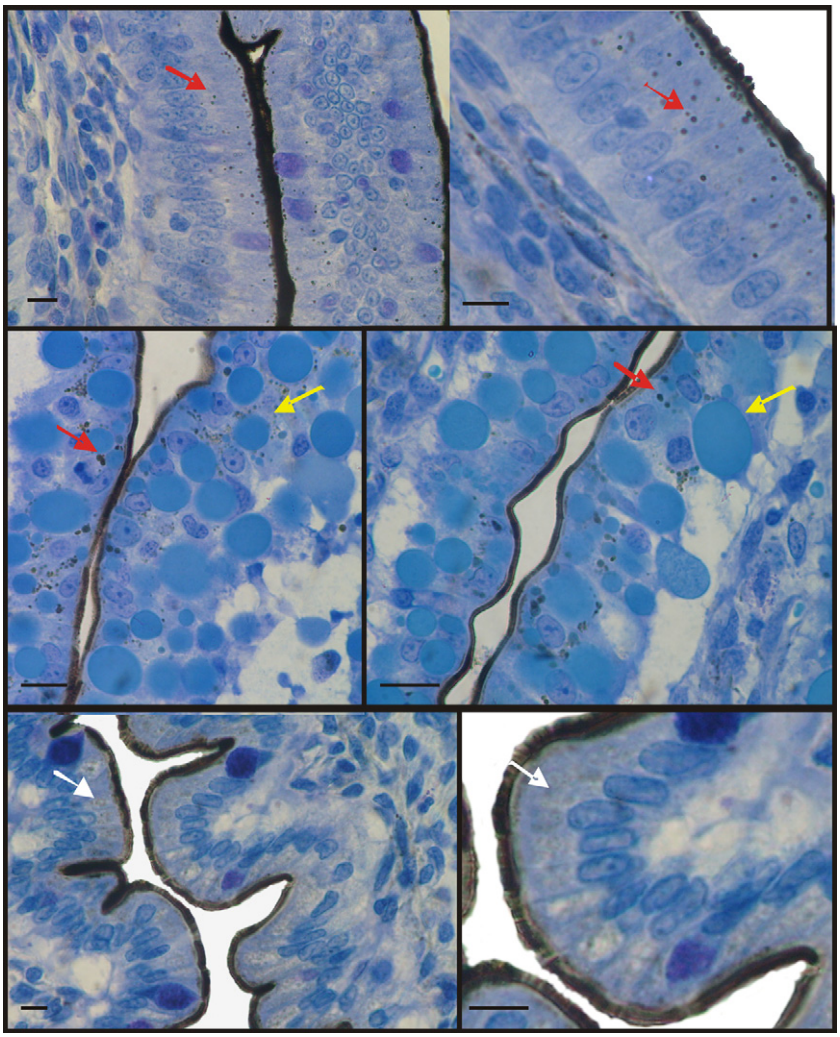

Fig. 4. (A) and (B) Phosphatase acid activity in the enterocytes of the duodenum of goat kids at 36 h of life; (C) and (D) phosphatase acid activity in the enterocytes of the jejunum of goat kids at 36 h of life; (E) and (F) phosphatase acid activity in the enterocytes of the ileum of goat kids at 36 h of life; red arrows: phosphatase acid reaction; yellow arrows: vacuoles with colostrum; white arrows: phosphatase acid reaction in vacuoles; Bar = 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(1997), Bühler et al. (1998) and Playford et al. (2000), as well as by presence of nutrients in this lacteal secretion (Sauter et al., 2004). In the current study, the activity of most enzymes in the three intestinal segments was significantly higher at 96 h than at 36 h. This behavior may be related to the fact that between 18 and 36 h, enterocytes found in the intestinal villi are progressively increasing the activity of aminopeptidases in the cell membrane due to several stimuli, such as food proteins. Sauter et al. (2004) suggested that increased enzyme activity may be a response to nutritional components of colostrum, such as protein, or non-nutritional factors, such as growth factors and hormones. The reduction in the enzyme activity that occurred at 96 h may be attributed to the enterocyte loss and replacement by a new cell type that, despite not having the same characteristics of fetal-type enterocytes, such as the ability to absorb macromolecules, is not yet fully mature and stimulated.

The activity of aminopeptidase A, a zinc-dependent membrane-bound aminopeptidase that catalyzes the cleavage of glutamic and aspartic amino acid residues from the N-terminus of polypeptides, was influenced by colostrum feeding. This finding suggests that the concentration of these amino acids in bovine colostrum was higher than in the goat colostrum or that aminopeptidase A-specific stimulating components are found in the heterologous colostrum.

The activity of disaccharidases, in turn, had a different behavior between the segments and enzymes. Lactase, the enzyme responsible for degradation of the main sugar in milk secretion, lactose, increased its activity between 18 and 36 h in the duodenum and ileum and between 36 and 96 h in the jejunum, revealing the increased capacity of degradation of this sugar in the intestinal epithelium of goat kids in the first hours of life. Zhang et al. (1997) also observed increased activity of lactase in pigs until

24 h of life. The ileum is the only segment that showed decreased lactase activity between 36 and 96 h of life, which could have resulted from the lesser amount of lactose that reaches this intestinal segment, resulting in a lower stimulus for the new generation of epithelial cells. These outcomes suggest that the activity of these disaccharidases is stimulated in the first days of life of goat kids.

The activity of maltase increased in the duodenum between 18 and 96 h of life, however, it did not change in the other intestinal segments. Sucrase activity was not detected in the duodenum and decreased between 18 and 36 h in the jejunum and between 36 and 96 h in the ileum. The smaller amount of substrates, maltose and sucrose, found in lacteal secretions, may have provided a low stimulus for the activation of these disaccharidases, which was also observed by Zhang et al. (1997).

Differences between the 0-h group and other treatments were not observed for the majority of enzymes in different intestinal segments, revealing that until the fourth day of life the digestive capacity of goat kids is not fully developed. The emergence of a new generation of cells does not necessarily indicate a mature cell condition. These enterocytes still need to be stimulated to develop the digestive capacity. Thus, greater care must be taken in this period, since the early supply of solids or a diet rich in sugars can cause diarrhea in animals (Zhang et al., 1997).

A different behavior for the activity of each enzyme in the intestinal segments was observed. The lactase enzyme showed intense activity in the duodenum and jejunum segments and the aminopeptidases in the jejunum and ileum segments. At 96 h of life, lactase activity was more pronounced and significantly higher than the activity of other enzymes in the duodenum and jejunum segment, an important characteristic in the first days of life of these animals that are fed with lactose-rich diets.

In the first 96 h of life, the intracellular activity was stimulated, especially in the proximal segments. The activity of acid phosphatase in the small intestine of goat kids was observed before and after ingestion of colostrum. These results show that intracellular digestive activity occurs in these small ruminants in the first hours of life. In swine and bovine, lysosomal activity has been observed after the first 24 h of life (Blättler et al., 2001; Bittrich et al., 2004). In the jejunum, where only two animals showed high acid phosphatase activity in cells absorbing colostrum, it seems that proteolytic activity was not significant and did not affect the process of macromolecules absorption from colostrum, an important characteristic especially for the animals that acquire passive immunity after birth.

5. Conclusion

These results indicate that lyophilized bovine colostrum, as a heterologous source of antibodies or nutrients, is a possible alternative management tool for goats. The present work also suggests that in the first 4 days of life, enzyme activity in the intestinal epithelium of goats is still not fully stimulated, which is an important characteristic for these animals that depend on macromolecule absorption to acquire passive protection after birth.

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