Intestinal IgG uptake by small intestine of goat kid fed goat or lyophilized bovine colostrum

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ABSTRACT

The immunoglobulin G (IgG) uptake and enterocyte nucleus position in the villous were studied in newborn goat kids fed goat or lyophilized bovine colostrum. Two groups of 15 newborn goat kids, each received 5% of body weight of goat colostrum (GC) or lyophilized bovine colostrum (LBC) containing 55 mg/mL of immunoglobulin G (IgG) at 0, 7 and 14 h of life. Three animals were sampled just after birth, receiving no colostrum intake, to be used as control. Samples of duodenum, medium jejunum and ileum were collected at 0, 18, 36 and 96 h of life. IgG vacuoles were not observed in the duodenum throughout the experiment regardless of all the experimental time points. In this segment, at 0, 18 and 36 h of life, nuclei were found in the apical, medial and basal positions in the enterocytes, and localized in the upper, medial and lower parts in the villous, respectively. At 96 h, a basal nuclei position was observed in the enterocytes, throughout the villous. In jejunum, IgG vacuoles were distributed along the villous at 18 and 36 h. In this segment at 0 h the nuclei were positioned predominantly apically in the enterocytes, throughout the villous. At 18 and 36 h, no consistent nuclei pattern was verified; however at 96 h, the nuclei were positioned basally in the enterocytes, throughout the jejunal villous. In the ileum at 0, 18 and 36 h, a great number of vacuoles without IgG were verified in the medial–apical part of the villous. In this segment, at 0 h of life and 96 h of life, the predominance of basal nuclei was observed. Nuclei were positioned in medial–apically part of the ileal enterocytes in the upper part of the villous at 18 and 36 h. It was found that the jejunal epithelium was the most important segment related to absorption process. The IgG absorption and nucleus position in the newborn goats were dependent on the small intestine segments and experimental time points, regardless of the colostrum source, GC or LCB. Considering the IgG uptake mechanism observed in the present study, the lyophilized bovine colostrum might be used instead of goat colostrum.

1. Introduction

The first hours of life are critical for goat kids, since their incomplete maturity of the immune system makes it susceptible to a new environment and survival to pathogenic microorganism attack. In this early stage of life newborn protection relies on mother immunoglobulin ingestion (Brambell, 1958).

The antibodies found in the colostrum are internalized by pinocytosis process in the small intestine absorptive epithelial cells and l transferred intact inside vacuoles into the bloodstream with high correlation between the IgG fed and IgG serum levels (Arguëllo et al., 2004; Jeffcott, 1972; Jochims et al., 1994; Kaup et al., 1996). Brambell (1958) proposed that process of IgG absorption in newborn rodents is a receptor mediated by Fc-fragment, a selective mechanism confirmed by Rodewald (1973). However, in more recent studies Bessi et al. (2002a) and Mayer et al. (2002) did not

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detect the evidence of Fc receptor participation in IgG absorption by cells of the small intestine in ruminants. In ruminants, the absorption of the maternal antibodies was correlated to the degree of vacuolation in the enterocytes and this vacuolation with the enterocytic tubular–vesicular system, compounded by invaginations of the membranes (Bessi et al., 2002a,b; Jochims et al., 1994; Kaup et al., 1996). The vacuolation in the small intestine is a common feature in young mammals (Bessi et al., 2002a,b; Clark and Hardy, 1971). After an initial period of intestinal permeability to IgG, normally during the first 48 h of life, "the gut close" as a consequence of a new generation of cells, adult-type enterocytes, is unable of absorbing macromolecules (Kaup et al., 1996; Kindlein et al., 2008a; Smeaton and Simpson-Morgan, 1985; Trahair and Robinson, 1989). The evidence that those cells is in a different stage of maturation is because of the basal nuclei position and the absence of vacuolization in the enterocytes (Bessi et al., 2002a; Machado-Neto et al., 2011). Several factors were related to intestinal mucosa development and maturity including colostrum factors, replacement of absorptive cells of the small intestine, hormones, physiological and environmental stressors factors, preterm birth, early release of gastric secretions, as well as, animal age (Bessi et al., 2002a,b; Jeffcott, 1972; Kelly and Coutts, 2000; Kindlein et al., 2008a; Kruse, 1983; Leece and Morgan, 1962; Moretti et al., 2010; Pauletti et al., 2007).

Goat colostrum ingestion by goat kids is associated with Caprine Arthritis–Encephalitis (CAE) contamination, a severe infectious disease that causes high rates of morbidity and mortality decreasing, consequently, the profitability of the commercial activity (Blacklaws et al., 2004; Callado et al., 2001; Peterhans et al., 2004). To minimize the disease incidence, especially in areas of risk, feeding newborn goat kids with bovine colostrum has been used as an alternative of immunoglobulin to prevent the newborn kids from infections of CAE colostrum or goat milk (Callado et al., 2001; Lima et al., 2009; Mellado et al., 2008; Sherman et al., 1990; Silva et al., 2007). Likewise, the management of bovine colostrum to small ruminants as an alternative should take into consideration pathogens like tuberculosis bovine, brucellosis, enzootic bovine leukosis (Lages et al., 2007). These pathogens can be transmitted to the small ruminants causing economic losses to the system. This study was carried out to investigate the absorption in the small intestine of goat and bovine immunoglobulin present in the colostrum and, also, the effect on the enterocyte nucleus position in different parts of the villous of goat kids in first 4 days of life.

2. Materials and methods

2.1. Animals, feeding and experimental procedures

Thirty-three male Saanen×Boer newborn goat kids were used in this study. The animals were removed, kept, maintained and treated in adherence to the accepted standards for 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, 15 animals received three meals, ranging from 139 mL to 184 mL, that correspond to 5% of body weight of goat colostrum (GC) and 15 animals received three meals

ranging from 134 mL to 191 mL, correspond to 5% of body weight of lyophilized bovine colostrum (LBC), containing 55 mg/mL of IgG by bottle. Immediately after birth, three kids, without colostrum ingestion, were used as control (time 0).

Pools of goat and bovine colostrum were prepared using around 20 l (8 l of the bovine colostrum and 12 l of the goat colostrum) of the first milk secretion from healthy goats and cows. The colostrum was homogenized and the immunoglobulin concentration determined through the radial immunodiffusion method (Mancini et al., 1965, modified by Besser et al., 1985). The bovine colostrum was lyophilized, and the powder obtained was blended and stored at −20 °C. Prior to feeding, the lyophilized bovine colostrum was resuspended in water and diluted with whole cow milk (114.7 mL of bovine colostrum and added 100 mL of milk, corresponding to 1:1.15 ratio) until reaching 55 mg/mL of IgG. Similarly, the goat colostrum was diluted with whole cow milk (100 mL of goat colostrum and added 100 mL of milk, corresponding to 1:1 ratio) until reaching 55 mg/mL of IgG. The colostrum was thawed in water bath (54 °C) prior to nursing.

2.2. Immunohistochemistry

Samples of duodenum, middle jejunum and ileum segments were obtained at 0 (for control group only), 18, 36 and 96 h of life, which corresponded to 4, 22 and 82 h after colostrum ingestion. 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 samples were, then, washed with PBS and cross-sectional pieces (5×5 mm) and excised from each sample. After successive dehydration with alcohol concentrations (30%, 50%, 70%, 90% and 100%), the segments were embedded in glycol methacrylate (JB-4; Polyscience, Warrington, PA, USA) and microsectioned (5 μm).

The protocol for IgG immunohistochemistry analysis was modified from Castro-Alonso et al. (2008) and Moretti et al. (2010). For the IgG analysis, the slices were incubated for 29 min with PBS (0.01 mol, pH 7.6) and Tween 20 at room temperature. The slices were then treated with pre-heated trypsin (40 º C) and maintained in a humidified chamber at 37 °C for 20 min. Tissues were then washed with PBS, incubated with 3% hydrogen peroxide and the reaction was stopped by adding goat serum (Vector Laboratories, Burlingame, CA) for incubation for 20 min at room temperature. The tissue sections were incubated with a polyclonal bovine or goat primary anti-IgG antibody developed in rabbit (diluted 1:500 in PBS/Tween 20/BSA; Sigma Chemicals Company, USA) for 15 h at 3 °C. A biotinylated secondary antibody against rabbit immunoglobulin, produced in goat (Vectastain ABC Kit PK-4002 — Vector Laboratories, Burlingame, CA) was then applied for 30 min at room temperature. Afterward, the slices were incubated with a preformed avidin and biotin complex (Vector Laboratories, Burlingame, CA) which was an irreversible interaction of the complex with biotinylated secondary antibody for 30 min. The DAB (3′3 diaminobenzidine — DAB Substrat Kit SK-4100 — Vector Laboratories Burlingame, CA) was used as a substrate for peroxidase.

Following color development, slices were counterstained with toluidine blue 0.05%, pH 4.5 for 30 s. To evaluate the enterocyte nucleus position in the villous, another group of slides were counterstained with toluidine blue 0.05%, pH 4.5 by 5 min. For both qualitative analysis, IgG uptake and enterocyte nucleus position in the villous, the slices were examined with a light microscope (Top Light B2), equipped with a computerized image analysis system (BEL Engineering srl — Italy), at magnifications 100×.

3. Results

3.1. Duodenum analysis

The IgG absorption and enterocyte nucleus position in the duodenal villous did not vary between animals fed GC and LBC.

IgG vacuoles were not detected inside of the enterocytes of the duodenum in the studied experimental time points. However, two animals presented fewer vacuoles at 18 and 36 h of life, corresponding to 4 and 22 h after colostrum ingestion.

In goat kids, at 0 h of life, the nuclei localization varied depending on enterocyte position in the villous. The nucleus was found in apical, medial and basal positions in the enterocytes, and localized in the upper, medial and lower parts of the villous, respectively. The same pattern was observed in animals at 18 and 36 h of life. At 96 h of life, basal nuclei position was observed in enterocytes in all extensions of the villous (Fig. 1).

3.2. Medium jejunum analysis

The IgG absorption and enterocyte nucleus position in the jejuna villous showed no variation between animals fed GC and LBC.

Abundant IgG vacuoles were detected during the period of high intestinal antibody permeability (until 36 h of life). Most animals evaluated at 18 h of life (corresponding to 4 h after colostrum ingestion) showed IgG vacuoles distributed throughout the villous (Fig. 2) and at 36 h of life (correspond to 22 h after colostrum ingestion), the IgG vacuoles were predominantly located in the medial–upper part of the villous

(Fig. 2). The IgG vacuoles were not observed in two animals, one at 18 h and the other at 36 h. In these periods, few enterocytes with vacuoles without IgG and large quantity of IgG vacuoles of several sizes were distributed in the basal and apical positions in the enterocytes. Evaluation of the epithelial cells at 0 and 96 h of life did not show IgG vacuoles.

In goat kids sampled at birth (time 0 h), apical nuclei positions were observed in the enterocytes, throughout the villous (Fig. 3). At 18 and 36 h of life, no consistent nuclei position pattern was verified (Fig. 3), with nuclei located at the citoplasmatic periphery of the cell, probably due to the presence of large amounts of IgG vacuoles formed after colostrum ingestion. At 96 h of life, nuclei were predominantly positioned basally in enterocytes throughout the jejunal villous.

3.3. Ileum analysis

The IgG absorption and enterocyte nucleus position in the ileal villous did not vary between animals fed GC and LBC.

At 0, 18 and 36 h of life, corresponding to 4 and 22 h after colostrum ingestion, a great number of vacuoles without IgG were verified throughout the enterocytes, in the mid to upper parts of the villous (Fig. 4). The size of the vacuoles decreased toward the lower part of the villous. A small number of IgG vacuoles inside the enterocytes were observed at 18 h in four and two animals fed LCB and GC, respectively, and one fed LCB at 36 h of life.

Basal nuclei position was observed in the enterocytes of the ileal villous of newborn goat kids at 0 h of life. At 18 and 36 h of life, the nuclei were medially–apically positioned in enterocytes and in the upper half of the villous and, at 96 h of life the predominance of basal nuclei was verified in the enterocytes throughout the villous (Fig. 5).

4. Discussion

In the duodenum, IgG vacuoles were not observed throughout the villous indicating absence of IgG absorption in this gut portion. According to Bainter (1994), Blum and Hammon (2000), Kaup et al. (1996) and Kelly and Coutts (2000), the ingestion of colostrum is important for morphological and functional developments of intestinal epithelium in ruminants and the presence of vacuoles is an indicator of
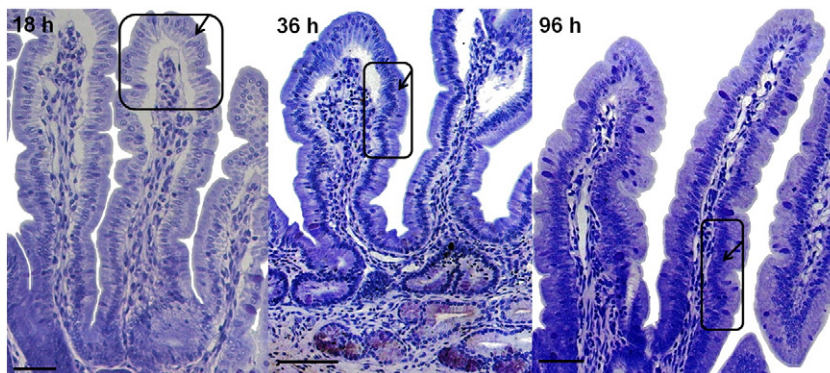

Fig. 1. Duodenum with apical, medial and basal nuclei positions in the enterocytes and localized in the upper, medial and lower part of the villous, at 18 and 36 h of life; and basal nuclei at 96 h of life (represented inside the rectangle form), respectively. Bars = 100 μm.
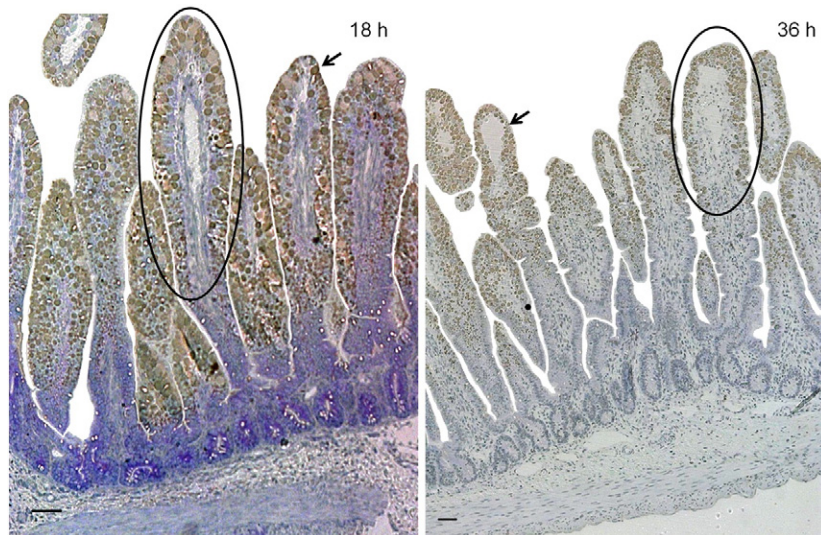
Fig. 2. Jejunum with IgG vacuoles in throughout the villous at 18 h of life; and IgG vacuoles in the medial–upper part of the villous at 36 h of life (represented by the oval form). Bars = 100 μm.

colostrum macromolecule uptake and transport by the enterocytes. Clark and Hardy (1971) found a positive correlation between [^{125}I] polyvinyl pyrrolidone absorption and the presence of vacuoles in the distal small intestine. Likewise, no enterocytes with colostrum filled vacuoles was found in the duodenum of newborn calves and lambs (Bittrich et al., 2004; Kaup et al., 1996; Machado-Neto et al., 2011). Contradicting these data, IgG internalization by enterocytes was found in the duodenum of one day-old goat kids (Castro-Alonso et al., 2008) and in lambs in the first 2 days of life (Smeaton and Simpson-Morgan, 1985).

No consistent pattern of nuclei position was verified in the jejunum during macromolecule absorptive period. This might be a consequence of the high number of IgG vacuoles present in the cells that move the nuclei to the cytoplasm periphery. The same characteristic was found in newborn calves by Bessi et al. (2002a,b). In the present study, a great number of cells containing large vacuoles with IgG reflect the intense activity of macromolecule uptake in this intestinal segment,

regardless of the source of colostrum, lyophilized bovine or goat. Since the lyophilized bovine colostrum did not affect the IgG uptake and, the enterocyte nucleus positions throughout the villous, it can be an interesting alternative to feed goat kids at birth.

During the first 36 h of life, period of high intestinal antibody permeability, a few vacuoles with IgG and a large number of vacuoles without IgG were observed in the ileum. The same characteristic was verified in newborn calves by Bittrich et al. (2004) and Kaup et al. (1996), indicating that absorption occurs in this segment. At 4 days of age, the goats did not show vacuoles in the ileum, suggesting "gut closure". Similarly, Bessi et al. (2002b) and Kindlein et al. (2008b), observed the absence of tubules, vesicles or vacuoles in the ileum of calves at 72 h.

In the current study, a large IgG uptake from colostrum was observed in the jejunal epithelium indicating that this segment is the most important intestinal region for immunoglobulin uptake. The decrease of IgG internalization by the
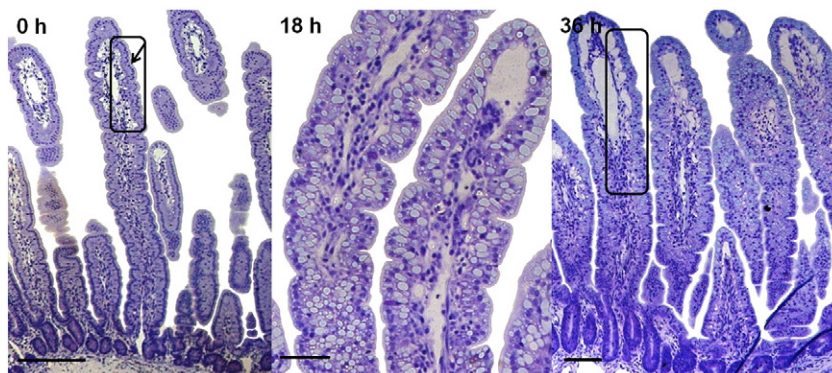

Fig. 3. Jejunum with apical nuclei in the enterocytes at 0 h of life and no nuclei position pattern at 18 and 36 h of life (represented in the rectangle form). Bars = 100 μm.
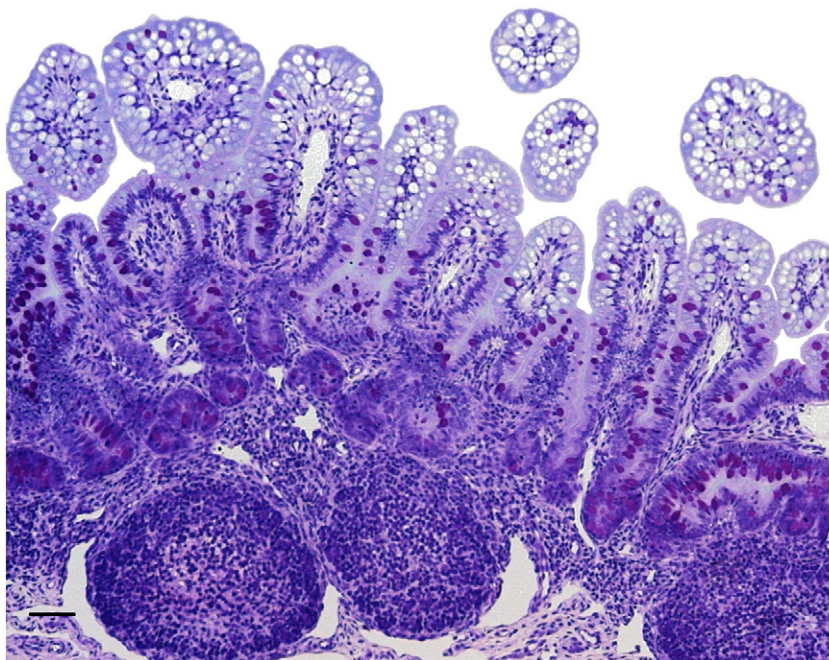
Fig. 4. Vacuoles without IgG (indicated by arrows) in the mid to upper part of the villous in ileum. Bars = 100 μm.

ileum enterocytes is probably due to a high absorption process by the jejunum enterocytes, reducing the amount of macromolecules reaching the ileum.

The basal nuclei position observed in the absorptive cells of the duodenum, jejunum and ileum at 96 h, indicates the presence of a new generation of cells, mainly adult-type, "impermeable" to macromolecules. This condition was also verified after the absorptive period in lambs and calves (Bessi et al., 2002a,b; Machado-Neto et al., 2011; Smeaton and Simpson-Morgan, 1985). The predominant basal nucleus position in the enterocytes, associated with the disappearance of vacuoles along the villous at 96 h of life, revealed the epithelial maturity in the small intestine segments. The findings of this experiment suggest that lyophilized bovine colostrum can be used for goat kids, since IgG uptake by enterocytes is similar to that found by goat colostrum.

Conflict of interest statement

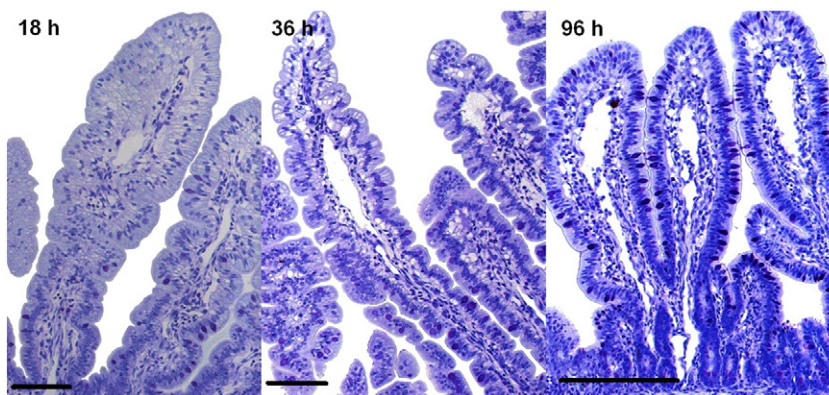I confirm that there is no conflict of interest related to this article.

Fig. 5. Ileum with medial–apical nuclei located in the enterocytes in the upper-half part of the villous at 18 and 36 h of life; and basal nuclei at 96 h of life. Bars = 100 μm.

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