Livestock Science 157 (2013) 125–131

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Goblet cell mucin distribution in the small intestine of newborn goat kids fed lyophilized bovine colostrum

R. Machado-Neto *, M.C.F. Pontin, W.M. Nordi, A.L. Lima, D.B. Moretti

Department of Animal Science, University of São Paulo, Avenue Pádua Dias 11, Piracicaba, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 17 December 2012
Received in revised form
16 May 2013
Accepted 21 June 2013

Keywords:
Goat
Colostrum
Intestinal mucosa
Goblet cells
Mucin

ABSTRACT

The number of goblet cells containing neutral and acidic mucins, including sulphomucins and sialomucins, was investigated in the small intestine of goat kids fed with lyophilized bovine colostrum in the period of passive immunity acquisition. At 0, 7 and 14 h of life, 15 male newborns received 5% of body weight of lyophilized bovine colostrum (LBC) and 14 male newborns received goat colostrum (GC), both with 55 mg/mL of IgG. Three additional animals were sampled at birth, without colostrum intake. Duodenum, jejunum and ileum samples were collected at 18, 36 and 96 h of life. Histological stains, periodic acid-Schiff, 1% alcian blue pH 2.5 and 1% alcian blue pH 1.0 were used to identify neutral and acidic mucins and acidic sulphated mucins, respectively. The number of goblet cells containing neutral and acidic mucins, including sulphomucins and sialomucins, does not differ in the duodenum ($P > 0.05$). In the jejunum, LBC showed a higher number of goblet cells containing sialomucins compared to GC ($P < 0.05$). The highest number of goblet cells containing acidic and neutral mucins and total number of goblet cells were observed at 96 h ($P < 0.05$). In this segment, vacuoles of colostrum were present at 18 and 36 h mainly in the upper region of the villi, while the goblet cells were located at the bottom. At 96 h, vacuoles of colostrum were not detected, only goblet cells distributed throughout the villi. In the ileum, the number of goblet cells containing sulphomucins was higher ($P < 0.05$) at 96 h than at 18 h. The LBC group showed higher ($P < 0.05$) number of goblet cells containing sulphomucins at 96 h and total number of goblet cells at 36 and 96 h than the 0-h group. The present work revealed that the greater the absorption of colostrum in the goat kids' jejunum epithelium, the smaller the number of goblet cells. Considering this segment, feeding newborns with heterologous colostrum caused alteration in the number of goblet cells containing sialomucin. This condition suggested a reaction of the intestinal epithelium with increasing secretion due to the presence of non-recognized substances from the lyophilized bovine colostrum.

1. Introduction

In small ruminants, the substitution of maternal colostrum with bovine colostrum has been used as an alternative to ensure appropriate acquisition of immunoglobulin by the newborns (Lima et al., 2009; Machado-Neto et al., 2011; Moretti et al., 2010, 2012b). The intake of this first mammary secretion with the potential presence of foreign elements and pathogens, hormones, bioactive factors and inflammatory mediators can stimulate exocytosis of secretory granules in goblet cells distributed in the intestinal epithelium (Antunović et al., 2005; Corfield et al., 2001; Deplancke and Gaskins, 2001).

The secretory granules contain glycoproteins, including mucins, which are classified into two types, neutral and acidic. The latter can be further differentiated in sulfated

* Correspondence to: Avenue Pádua Dias, 11, 13418-900, Piracicaba, São Paulo, Brazil. Tel.: +55 19 3429 4260; fax: +55 19 3429 4883.
E-mail address: raul.machado@usp.br (R. Machado-Neto).

1871-1413/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.livsci.2013.06.033

(sulphomucins) or non-sulfated (sialomucins) (Deplancke and Gaskins, 2001). The frequency of goblet cells containing neutral and acidic mucins in the epithelium is related to the gastrointestinal segment, the presence of harmful agents and the stage of development. The neutral mucin occurs in greater quantities in the gastric mucosa, whereas the acidic mucin predominates in the intestinal epithelium (Corfield et al., 2001; Deplancke and Gaskins, 2001). Acidic mucins are more resistant to degradation by bacterial glycosidases and host proteases and show higher viscosity and acidity compared with neutral mucins. Thus, the primary function of this mucin is associated with resistance to attack by microorganisms, justifying its greater presence in the large intestine (Deplancke and Gaskins, 2001; Fontaine et al., 1996).

The objective of this study was to evaluate the relationship between the number of goblet cells containing neutral and acidic mucins, including sulphomucins and sialomucins, in the small intestine of goat kids with the period of passive immunity acquisition and the supply of lyophilized bovine colostrum.

2. Materials and methods

2.1. Feed

Colostrum was collected from two Holstein cows and 14 goats from commercial dairy farms. The animals were milked manually and the lacteal secretions were homogenized to form a unique pool of bovine colostrum and goat colostrum, respectively. The colostrum pools were then stored at −20 °C. The frozen pool of bovine colostrum was lyophilized and the resulting powder was homogenized and stored in sealed containers at −20 °C.

Samples of each pool were collected to determine IgG concentration by radial immunodiffusion (Besser et al., 1985; Mancini et al., 1965). Antiserum to bovine immunoglobulin G was added to agarose solution 1% and standard bovine IgG or samples of the bovine colostrum (diluted 1:50 in phosphate-buffered solution) were placed in wells. The ring-shaped immunoprecipitates were measured after 24 h. The same procedure was repeated to evaluate the IgG concentration in goat colostrum using antiserum to goat immunoglobulin G.

At the time of meal offering, 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 the analysis of chemical composition, Table 1.

2.2. Experimental procedures

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

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

Composition	Lyophilized bovine colostrum	Goat colostrum
Humidity and volatile (%)	81.11 ± 0.19	79.88 ± 0.18
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 any maternal colostrum intake. Fifteen animals received 5% of body weight of lyophilized bovine 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).

Goat kids from LBC and GC were randomly slaughtered at 18, 36 and 96 h of life with anesthesia (0.3 mg/Kg of xylazine and 20 mg/Kg of ketamine) 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. The small intestine was separated into the duodenum, middle jejunum and ileum and samples were collected for histochemical analysis of goblet cells.

2.3. Histochemistry

The intestinal segments were fixed in buffered (0.1 M; pH 7.2), 4% p-formaldehyde solution, and subdivided into 5-mm sections, which were washed in phosphate-buffered saline (PBS; 0.1 M, pH 7.2) four consecutive times. The washed material was dehydrated by immersion in increasing ethanol concentrations (30%, 50%, 70%, 90% and 100%). The dehydrated material was blocked in glycolmethacrylate resin (JB-4; Polysciences, Inc., Warrington, PA, USA), and transverse non-sequential, 5-μm sections were obtained. For each animal and segment, oriented villi were taken (magnification, × 10) and used to characterize and quantify different mucins in the goblet cells (Mashimo et al., 1996).

Sections were stained with 1% Alcian blue (Ab, pH = 2.5)/periodic acid-Schiff (PAS) to detect neutral (pink) and acidic (blue) mucins. For identification of the subtypes of acidic mucins (sulphomucins and sialomucins), the tissue sections were stained with 1% Ab pH = 1.0 (only strong sulphated mucins were stained) and the goblets cells containing sulphomucins were counted. Thereafter, the same slides were counterstained with 1% Ab pH = 2.5 and the total number of acidic goblet cells were counted. The number of goblet cells containing sialomucins was obtained by determining the difference between the total number of acidic mucin and the number of sulphomucins (Kleessen et al., 2003).

Goblet cells in 20 oriented villi were counted using a light microscope (Top Light B2, BEL Engineering srl)