Research in Veterinary Science 89 (2010) 214–222

Contents lists available at ScienceDirect

Research in Veterinary Science

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

Glycoconjugate histochemistry in the small and large intestine of normal and *Solanum glaucophyllum*-intoxicated rabbits

C.N. Zanuzzi [a,b,*], C.G. Barbeito [a,b], M.L. Ortíz [a], F.A. Lozza [a], P.A. Fontana [a], E.L. Portiansky [a], E.J. Gimeno [a]

[a] Institute of Pathology, "Prof. Dr. Bernardo Epstein", School of Veterinary Sciences, National University of La Plata, La Plata, Buenos Aires, Argentina
[b] Department of Histology and Embryology, School of Veterinary Sciences, National University of La Plata, La Plata, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Accepted 1 March 2010

Keywords:
Vitamin D_3
Glycosylation
Intestine
Differentiation
Histochemistry
Lectins

ABSTRACT

Vitamin D participates in mineral homeostasis, immunomodulation, cell growth and differentiation. The leaves of *Solanum glaucophyllum* contain high levels of 1,25-dihydroxyvitamin D3 as glycoside derivatives and their chronic ingestion generates a hypervitaminosis D-like state. We analyzed changes on carbohydrate expression as a cell differentiation indicator on samples of the small and large intestine of *S. glaucophyllum*-intoxicated rabbits, using conventional and lectin histochemistry. Male *New Zealand* white rabbits were intoxicated with *S. glaucophyllum* during two or four weeks and killed the day after. A group of animals ("possibly recovered group") were intoxicated during 15 days and killed at day 45 of the beginning of the experiment. We found changes in the lectin binding pattern in the small and large intestine of the intoxicated rabbits. Some of these changes were reverted in the possibly recovered group. Vitamin D could be a new regulator factor of the intestinal glycosylation process.

1. Introduction

Solanum glaucophyllum (synonym *S. malacoxylon*) is a calcinogenic plant responsible for the enzootic calcinosis of ruminants in South America, a disease that causes considerable economic losses in Argentina, Brazil and Uruguay (Carrillo and Worker, 1967; Worker and Carrillo, 1967; Puche and Bingley, 1995). This plant contains high levels of 1,25-dihydroxyvitamin D_3 as glycoside derivatives in its leaves (Gil et al., 2007). Hydrolytic enzymes from bacterial flora in rumen, intestine or other tissues cleave the sugar residue from the glycoside and release the steroidal fragment, the hormone 1,25-dihydroxyvitamin D_3. The chronic ingestion of the leaves generates a hypervitaminosis D-like state. The excess of vitamin D hormone stimulates the synthesis of mucosal calcium binding proteins (CaBPs), the absorption of calcium and phosphate, and produce hypercalcemia and/or hyperphosphatemia (Wasserman, 1974; Walling and Kimberg, 1975; Schneider and Schedl, 1977; Mello, 2003), resulting in soft tissue mineralization, especially in the heart, arteries, lungs, kidneys, and erosions at the joins. Chronically intoxicated animals present stiffness, painful gait, kyphosis, anorexia, loss of body condition, and in the most severe cases advance cachexia (Worker and Carrillo, 1967). The causes of anorexia and cachexia in clinically affected animals remain unknown.

Vitamin D receptors (VDR) are present in multiple tissues. The 1,25-dihydroxyvitamin D_3 regulates the expression of several genes in all these target organs. These genes have specific DNA sequences or vitamin D response elements (VDREs). The VDREs are located within their promoter regions and represent high affinity binding sites for the vitamin D receptor. The activation of vitamin D receptors can increase or decrease specific gene transcription and, consequently, modify the secretion of the products coded by them (Wesley et al., 2006). In addition to the well known effects of vitamin D on mineral homeostasis, it participates in immunomodulation, and in the processes of cell growth and differentiation (Gimeno et al., 2000; Bikle, 2007; Fontana et al., 2009).

Vitamin D enhances the efficiency to absorb dietary calcium and phosphate in the intestine (Wesley et al., 2006; Bikle, 2007). It also participates in enterocyte proliferation and differentiation (Suda et al., 1990; Ménard et al., 1995; Holt et al., 2002). High doses of vitamin D_3 inhibit cell proliferation in colonic neoplasms, whereas lower doses stimulate epithelial differentiation (Newmark and Lipkin, 1992). More recently, vitamin D_3 has been implicated in intestinal detoxification (Kutuzova and DeLuca, 2007) and in the preservation of the mucosal barrier integrity (Kong et al., 2008).

Little is known about intestinal changes associated with hypervitaminosis D state (Razzake and Lanske, 2006), and there are few studies on cell differentiation changes in plant-induced hypervitaminosis D in domestic animals (Barros and Gimeno, 2000; Gimeno et al., 2004; Gomar, 2006; Zanuzzi et al., 2008; Fontana et al., 2009).

* Corresponding author. Address: Instituto de Patología, Facultad de Ciencias Veterinarias, UNLP. Calle 60 y 118, 1900, La Plata, Argentina. Tel.: +54 221 4236663x426; fax: +54 221 423 7980.
E-mail addresses: carozanuzzi@fcv.unlp.edu.ar, carozanuzzi@gmail.com (C.N. Zanuzzi).

0034-5288/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2010.03.002

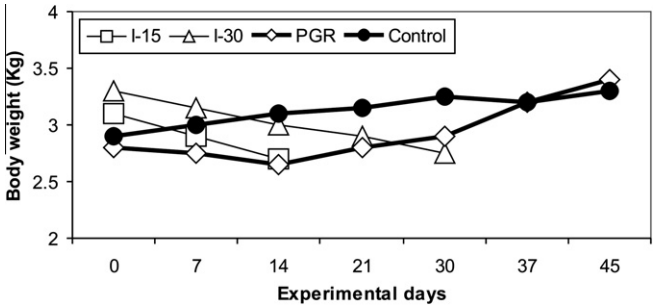

Fig. 1. Body weight variations in *S. glaucophyllum*-intoxicated animals. All the intoxicated animals showed a drop weight with intoxication time. PRG recovered their weight reaching those of the control group at the end of the experiment. Values expressed as mean body weight ± SD.

Table 1
Clinical signs during intoxication time.

Experimental Groups	Diarrhoea	Conjunctivitis Rhinitis/Otitis	Anorexia	Asthenia
I-1515	–	–	+	+
I-3030	+	+	+	+
PRG-1545	+*	+*	+*	+*
Control	–	–	–	–

*Signs shown only during the intoxication period.

The differential carbohydrate expression of the cells is of great value as a cellular differentiation indicator. The terminal glycosylation sequences expressed by cells reflect the expression of the corresponding glycosyltransferases and glycosidases (Biol-N'garagba et al., 2002) and, consequently, carbohydrates are considered a secondary product of the gene expression (Taylor, 2003).

Different glycoproteins are produced by the epithelial cells in the intestine. Thus, goblet cells synthesize and secrete mucin, such as MUC2, MUC3, MUC4, MUC5 and MUC6. The secretion of these cells together with that from Brunner's gland at the duodenum serves as an important physical pre-epithelial defence against luminal constituents (Biol-N'garagba and Louisot, 2003; Schumacher et al., 2004). In addition, most membrane-bound glycoproteins of

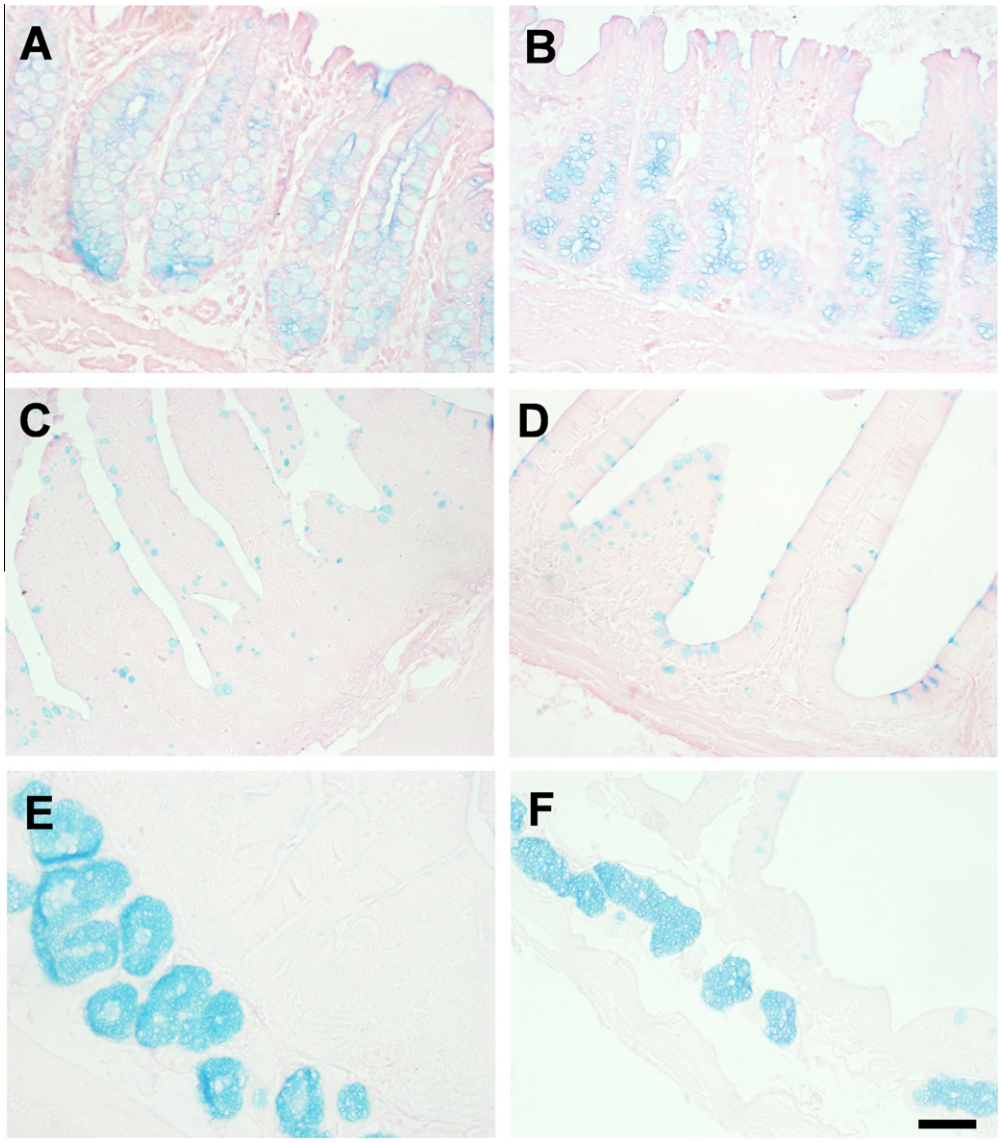

Fig. 2. Alcian blue (AB) staining in *S. glaucophyllum*-intoxicated rabbits. AB 0.5 staining of control (A) and I-1515-intoxicated rabbits (B) in the rectum; AB 1.0 staining of control (C) and I-1515-intoxicated rabbits (D) in the ileum; AB 2.5 staining of control (E) and I-3030-intoxicated rabbits (F) in the duodenum. Bar = 50 μm.

the enterocyte microvilli are of great biological importance, since they play a key role for diverse processes, such as digestion, transport, detoxification or defence (Biol-N'garagba and Louisot, 2003).

The identification of different types of glycoconjugates in tissue sections can be studied using conventional histochemistry. Besides, lectins, a heterogeneous group of proteins or glycoproteins of plant and animal origin, bind to specific terminal or internal sugars and oligomers in complex carbohydrates (Spicer and Schulte, 1992). Several studies have shown that lectin histochemichal techniques are useful to study the process of cell differentiation in different cell lineages of the intestine (Etzler and Branstrator, 1979; Gelbert et al., 1992; Falk et al., 1994, 1995; Jepson et al., 1995).

Since working with bovines for experimental purposes carries out economical and management difficulties, we have chosen rabbits as experimental models for this study due to their high susceptibility to *S. glaucophyllum* intoxication as has been shown in previous studies (Dallorso et al., 2001; Gimeno et al., 2004).

The aim of the present work was to study, using conventional and lectin histochemistry, the effect of *S. glaucophyllum* hypervitaminosis D-induced state on the carbohydrate expression in the small and large intestine of rabbits.

2. Materials and methods

2.1. Animals

Twenty-one three-month-old *New Zealand* male rabbits were used. All animals were clinically healthy at the beginning of the

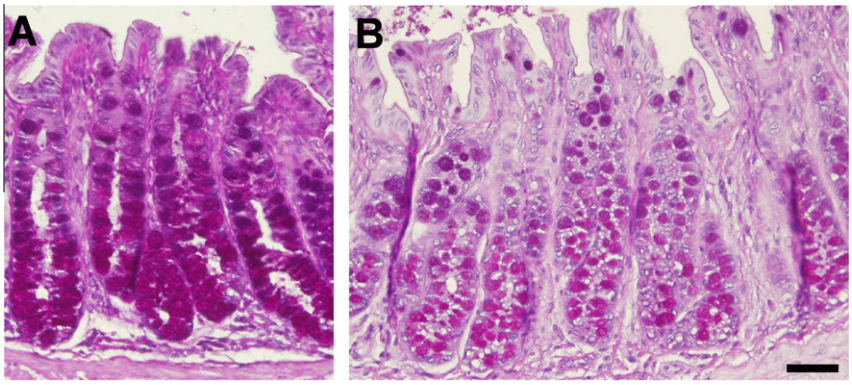

Fig. 3. PAS staining in *S. glaucophyllum*-intoxicated rabbits. PAS staining of control (A) and I-3030-intoxicated rabbits (B) in the rectum. Bar = 50 μm.

Table 2
Lectin-binding pattern in duodenum.

Lectins	Groups	Villi				Crypts			Brunner Glands	
		E			GC	E			Mucous cell	Serous cell
		AG	AC	BC		AG	AC	BC		
DBA	Control	3	–	–	–/2	2	–	–	–	–
	I-1515	1	–	–	–/3	–	–	–	–	–
	I-3030	1	1	–	–/1	2	–	–	–	1
	PRG-1545	3	1	–	–/2	2	–	–	–	–
SBA	Control	3	–	–	–/2	3	–	–	1	1
	I-1515	1	–	–	–/2	2	–	–	1	–
	I-3030	1	1	–	–/2	2	–	–	1	1
	PRG-1545	2	1	–	–/2	2	–	–	1	1
UEA-1	Control	–	–	–	–	1	–	–	–	2
	I-1515	3	–	–	1	2	–	–	–	1
	I-3030	2	–	–	1	1	–	–	–	2
	PRG-1545	2	–	–	1	2	–	–	–	1
PNA	Control	2	–	–	–/1	2	–	–	–	2
	I-1515	1	–	–	–/1	2	–	–	–	3
	I-3030	1	–	–	–/1	1	–	–	–	3
	PRG-1545	1	–	–	–/1	1	–	–	–	2
Con-A	Control	2	1	1	–	1	1	1	–	1
	I-1515	3	1	1	–	1	1	1	–	1
	I-3030	3	1	1	1	1	1	1	1	1
	PRG-1545	2	1	1	–	1	1	1	1	1
RCA-1	Control	3	–	–	1	2	–	–	2	1
	I-1515	3	–	–	2	2	–	–	2	1
	I-3030	3	1	–	1	2	–	–	1	1
	PRG-1545	3	1	1	2	1	–	–	1	1
WGA	Control	3	–	–	1	2	–	–	1	2
	I-1515	3	–	–	1	1	–	–	2	1
	I-3030	3	–	–	1	1	–	–	1	1
	PRG-1545	2	–	–	1	1	–	–	1	2

AG, apical glycocalix; AC, apical cytoplasm; BC, basal cytoplasm; E, enterocytes; GC, goblet cells; –, null staining; 1, weak staining; 2, moderate staining; 3, strong staining.

experiment. They were fed with a standard diet and water *ad libitum*. Every animal was housed in an individual cage. All the procedures were carried out in accordance to the "Guide for the Care and Use of Laboratory Animals" of the National Research Council (National Academy Press, 1996, Washington, USA).

2.2. Intoxication with S. glaucophyllum

Ten animals were experimentally intoxicated *per os* with 125 mg/animal of powdered *S. glaucophyllum* leaves twice a week until they were killed. Five of them were killed 15 days after the beginning of the intoxication (I-1515 group), whereas the other five were left for other 15 days (I-3030 group). Five more animals were intoxicated for 15 days but killed after 45 days ("possibly recovered group": PRG-1545). Six rabbits were used as controls. The body weight of each animal was recorded once a week. Clinical signs were observed and recorded every day during the entire study.

2.3. Histological and histochemical studies

All the rabbits were carefully necropsied and samples of duodenum, jejunum, ileum, colon and rectum of each animal were rinsed in phosphate buffer solution (PBS) 0.2 M, fixed in 10% neutral buffered formalin and embedded in paraffin. Slices of 5 μm were stained with Haematoxylin and Eosin, PAS (Periodic acid Schiff reaction), alcian blue (AB) or used for the lectin histochemical study.

2.4. Conventional histochemistry

We used two classic mucin techniques, the periodic acid Schiff reaction (PAS) and AB (pH 0.5, 1.0 and 2.5), to analyze the carbohydrate composition of the mucin produced by the cells of the surface and glandular intestinal epithelium (Bancroft and Stevens, 1990).

PAS technique method: sections were dewaxed, rehydrated, treated with periodic acid for 5 min, and washed several folds with distilled water. Slides were then covered with Schiff's solution for 15 min, and then washed in running tap water for 5–10 min. Hill's haematoxylin was used for counterstaining. Slides were dehydrated in alcohol, cleared in xylene and finally mounted.

AB technique method: sections were dewaxed, rehydrated and then stained in freshly filtered AB, pH 0.5, 1.0 and 2.5 for 40 min, washed in distilled water and counterstained with light eosin. Slides were then dehydrated in alcohol, cleared in xylene and finally mounted.

Goblet cells and enterocytes (glycocalix and apical cytoplasm) of the small and large intestine, Paneth cell granules, and mucous and serous cells of duodenal Brunner's glands were evaluated.

The intensity of the labelling was scored using a semi-quantitative scale from 0 to 3, being 0: negative, 1: weak, 2: moderate and 3: strong.

2.5. Lectin histochemistry

Mounted slices were dewaxed, rehydrated and then incubated with 0.03% H_2O_2 in methanol (purum $\geqslant$99.0%) for 30 min at room temperature to inhibit endogenous peroxidase activity. Slides were

Table 3
Lectin-binding pattern in jejunum.

Lectins	Groups	Villi				Crypts			
		E			GC	E			PC
		AG	AC	BC		AG	AC	BC	
DBA	Control	3	–	–	1–2	3	–	–	1
	I-1515	–	–	–	–/2	–	–	–	–
	I-3030	–	1	–	–/1	2	–	–	–
	PRG-1545	2	–	–	–/1	2	–	–	2
SBA	Control	3	–	–	1	2	–	–	1
	I-1515	1	–	–	1–2	2	–	–	1
	I-3030	1	1	–	1–2	2	–	–	1
	PRG-1545	2	1	–	1–2	2	–	–	2
UEA-1	Control	–	–	–	–	–	–	–	–
	I-1515	2	–	1	1	2	–	–	–
	I-3030	2	–	–	1	–	–	–	–
	PRG-1545	2	–	–	1	2	–	–	–
PNA	Control	3	–	1	–/1	1	–	–	–
	I-1515	1	–	–	–/1	2	–	–	1
	I-3030	1	1	–	–	1	–	–	–
	PRG-1545	1	–	–	–	1	–	–	–
Con-A	Control	3	1	–	–	2	–	–	2
	I-1515	3	1	–	–	2	–	–	–
	I-3030	2	1	1	–	2	1	1	1
	PRG-1545	2	1	1	–	2	1	1	1
RCA-1	Control	3	–	–	2	3	–	–	1
	I-1515	3	–	–	1	2	–	–	1
	I-3030	3	1	–	1	2	1	1	1
	PRG-1545	3	1	–	1	2	1	1	1
WGA	Control	2	–	–	1	2	–	–	–
	1515	3	–	–	–	2	–	–	–
	I-3030	3	–	–	–	1	–	–	–
	PRG-1545	2	–	–	1	2	–	–	–

AG, apical glycocalix; AC, apical cytoplasm; BC, basal cytoplasm; E, enterocytes; GC, goblet cells; PC, Paneth cells; –, null staining; 1, weak staining; 2, moderate staining; 3, strong staining.

Table 4
Lectin-binding pattern in ileum.

Lectins	Groups	Villi				Crypts			
		E			GC	E			PC
		AG	AC	BC		AG	AC	BC	
DBA	Control	3	–	–	1–2	2	–	–	2
	I-1515	1	–	–	–	–	–	–	–
	I-3030	1	–	–	–/2	1	–	–	1
	PRG-1545	3	–	–	–/2	2	–	–	2
SBA	Control	2	–	–	–/1	2	–	–	1
	I-1515	1	–	–	–/2	2	–	–	1
	I-3030	1	–	–	–/3	1	–	–	2
	PRG-1545	2	–	–	–/2	2	–	–	2
UEA-1	Control	2	–	–	–/1	1	–	–	1
	I-1515	2	–	–	1–2	2	–	–	2
	I-3030	2	–	–	–/2	1	–	–	1
	PRG-1545	2	–	–	–/2	1	–	–	1
PNA	Control	–	–	–	–	2	–	–	–
	I-1515	1	–	–	1	1	–	–	–
	I-3030	2	–	–	1	1	–	–	–
	PRG-1545	1	–	–	1	1	–	–	–
Con-A	Control	1	1	1	–	–	1	1	1
	I-1515	1	1	1	–	–	1	1	1
	I-3030	1	1	1	–	–	1	1	1
	PRG-1545	1	1	1	–	–	1	1	1
RCA-1	Control	2	–	–	1	2	–	–	–
	I-1515	2	–	–	2	2	1	–	2
	I-3030	2	1	1	2	2	1	1	1
	PRG-1545	2	–	–	2	2	–	–	1
WGA	Control	2	–	–	1	2	–	–	1
	I-1515	1	–	–	1	1	–	–	–
	I-3030	2	–	–	1	1	–	–	–
	PRG-1545	2	–	–	1	1	–	–	1

AG, apical glycocalix; AC, apical cytoplasm; BC, basal cytoplasm; E, enterocytes; GC, goblet cells; PC, Paneth cells; –, null staining; 1, weak staining; 2, moderate staining; 3, strong staining.

then treated with bovine serum albumin (BSA) 1% in PBS 0.2 M for 30 min and incubated at 4 °C overnight with biotinylated lectins. The seven used lectins (Lectin Kit BK 1000, Vector Laboratories, Inc., Burlingame, CA, USA) with different carbohydrate specificity were the following: Con-A (*Canavalia ensiformis,* specifically binding α-D-Man and α-D-Glc); DBA (*Dolichos biflorus*, with binding specificity to α-D-GalNAc); SBA (*Glicine maximus*, binding specificity to α-D-GalNAc, β-D-GalNAc and α-and β-Gal); PNA (*Arachis hypogea*, that specifically binds β-D-Gal and (β1–3) GalNAc); RCA-1 (*Ricinus communis*-1, binding specificity β-D-Gal and α-D-Gal); UEA-1 (*Ulex europaeus*-1, binding specificity α-L-Fuc) and WGA (*Triticum vulgaris,* binding specificity α-D-GlcNAc and NeuNAc) (Goldstein and Hayes, 1978). The optimal lectin concentration was 30 μg/ml in PBS for all lectins, except for PNA (10 μg/ml). The horseradish peroxidase streptavidin SA-5704 (Vector Laboratories, Inc., Burlingame, CA, USA), used as a detection system, was incubated during 30 min at room temperature. Slides were rinsed three folds in PBS during 5 min each time. Liquid 3,3′-diaminobenzidine tetrahydrocloride (DAB) was used as chromogen (DakoCytomation, Carpinteria, CA, USA). Negative controls for lectin staining included exposure to horseradish peroxidase and substrate medium without lectin. The dark, golden brown DAB–H_2O_2 reaction product showed the positively stained structures. Hill's haematoxylin was used for counterstaining.

The lectin binding pattern of goblet cells and enterocytes (glycocalix and apical cytoplasm) of the small and large intestine, Paneth cell granules, and mucous and serous cells of duodenal Brunner's glands were evaluated.

The intensity of lectin binding was scored using a semi-quantitative scale from 0 to 3, being 0: negative, 1: weak, 2: moderate and 3: strong. Lectin controls were performed by the addition of inhibitory sugars at a final concentration of 0.01 M.

3. Results

3.1. Clinical signs and macroscopic lesions

The intoxicated animals of I-1515 and I-3030 groups showed anorexia 24–48 h after the beginning of the intoxication. A gradual asthenia state and body weight reduction was present and became more pronounced with intoxication time (Table 1). Some animals showed other clinical signs, such as diarrhoea, rhinitis, conjunctivitis and otitis. Animals from the PRG-1545 showed clinical signs only during intoxication time, recovering their body weight during the remaining 30 days (Fig. 1).

At necropsy, animals from I-1515 and I-3030 groups showed reduction on adipose tissue, muscular atrophy, and calcification of the vascular system, lungs and renal cortices. In the PRG-1545 the only lesions found were those associated with soft tissue mineralization.

3.2. Histochemical studies

In the duodenum PAS and AB pH 2.5 staining was weak or moderate in the glycocalix of villi and crypt enterocytes. The mucin of goblet cell was moderately stained with PAS, and heterogeneously with AB solutions. The mucin of Brunner's gland cells were weakly labelled with PAS and moderate to strongly with AB solutions, whereas the apical granules of serous cells resulted weakly labelled with PAS and negative with AB solutions (Fig. 2E and F).

In the jejunum and ileum apical glycocalix of villi enterocytes were moderate to strongly stained with PAS and weak to moderately stained with AB at pH 1.0 or 2.5 (Fig. 2C and D). The mucin of goblet cells was moderately stained throughout the epithelium

Table 5
Lectin-binding pattern in colon.

Lectins	Groups	Superficial epithelium				Superficial glands				Middle glands				Deep glands			
		E			GC	E			GC	E			GC	E			GC
		AG	AC	BC		AG	AC	BC		AG	AC	BC		AG	AC	BC	
DBA	Control	3	–	–	–/2	3	–	–	–/2	3	1	1	–/2	3	–	–	–/2
	I-1515	1	–	–	–/2	–	–	–	–	–	–	–	–/3	3	–	–	–/2
	I-3030	1	–	–	–/2	–	–	–	–/2	–	–	–	–/2	3	–	–	–/2
	PRG-1545	3	–	–	–	2	–	–	–/2	2	–	–	–/2	2	–	–	–/2
SBA	Control	3	1	–	–/2	–	–	1	2–3	–	–	–	–/1	2	–	–	–/2
	I-1515	1	1	–	–	–	–	–	–/1	–	–	–	–/1	1	–	–	–/1
	I-3030	2	–	–	–/2	2	–	–	–/2	2	–	–	–/1	2	–	–	–/2
	PRG-1545	2	–	–	–/1	2	1	–	–	1	–	–	–/2	2	–	–	–/2
PNA	Control	1	–	–	–	1	–	–	–	1	–	–	–	2	–	–	–
	I-1515	1	–	–	–	1	–	–	–	1	–	–	1	2	–	–	1
	I-3030	2	–	–	–	–	–	–	–	1	–	–	–	1	–	–	1
	PRG-1545	2	–	–	–	2	–	–	–	2	–	–	–	2	–	–	1
UEA-1	Control	1	–	–	–/2	2	–	–	–/1	1	–	–	–/1	–	–	–	–/2
	I-1515	3	–	–	–/1	2	–	–	–/1	1	–	–	–/2	–	–	–	–/2
	I-3030	2	–	–	–/2	2	–	–	–/1	–	–	–	–/1	–	–	–	–/1
	PRG-1545	3	–	–	–/1	2	–	–	–/1	–	–	–	–/1	–	–	–	–/2
WGA	Control	3	–	–	–	2	–	–	–	2	–	–	–	2	–	–	–
	I-1515	3	–	–	–	2	–	–	–	2	–	–	–	2	–	–	–
	I-3030	3	–	–	–	1	–	–	–	2	–	–	1	–	–	–	–
	PRG-1545	3	–	–	–	2	–	–	–	1	–	–	1	1	–	–	1
RCA-1	Control	3	–	–	–/2	2	–	–	–/2	1	–	–	–/	1	–	–	–/
	I-1515	3	–	–	–/1	2	–	–	–/1	2	–	–	–/1	2	–	–	–/2
	I-3030	3	–	–	–/2	3	–	–	–	1	–	–	–/1	2	–	–	–/1
	PRG-1545	3	–	–	–	3	–	–	–	1	–	–	–/1	2	–	–	–/1
Con-A	Control	3	1	1	2	1	1	1	–/2	2	1	1	1	2	1	1	1
	I-1515	3	1	1	–/2	2	1	1	–/1	2	1	1	1	2	1	1	1
	I-3030	3	1	1	–/1	2	1	1	–/1	1	1	1	1–2	2	1	1	–/1
	PRG-1545	3	1	1	–/1	2	–	–	–/1	1	–	–	–/1	2	–	–	–/1

AG, apical glycocalix; AC, apical cytoplasm; BC, basal cytoplasm; E, enterocytes; GC, goblet cells; –, null staining; 1, weak staining; 2, moderate staining; 3, strong staining.

with both techniques. Paneth cells granules were PAS positive in the jejunum.

The surface epithelium of the colon showed a weak to moderate reactivity with both techniques. Superficial, middle and deep crypts were more weakly labelled. Similar structures were labelled in the rectum, although PAS intensity was higher than in the colon. The mucin of goblet cells of both colon and rectum was heterogeneously stained both with PAS and AB. In some

Table 6
Lectin-binding pattern in rectum.

Lectins	Groups	Superficial epithelium				Superficial glands				Middle glands				Deep glands			
		E			GC	E			GC	E			GC	E			GC
		AG	AC	BC		AG	AC	BC		AG	AC	BC		AG	AC	BC	
DBA	Control	3	2	2	–/3	–	1	–	–	–	1	–	1–2	–	1	–	–/3
	I-1515	1	–	–	–/3	–	–	–	–	–	–	–	–/2	–	–	–	–/2
	I-3030	1	–	–	1–2	–	–	–	–	–	–	–	–/1	–	–	–	–/2
	PRG-1545	2	1	1	–/2	–	1	–	–/1	–	1	–	–/2	–	1	–	–/2
SBA	Control	3	1	1	–/2	–	–	–	–/2	–	–	–	–/2	–	–	–	–/2
	I-1515	3	–	–	1–3	–	–	–	–	–	–	–	–/2	–	–	–	–/2
	I-3030	3	1	1	–/2	–	–	–	–/2	–	–	–	–/2	–	–	–	–/2
	PRG-1545	2	1	1	–	–	1	1	–/	–	1	–	–/1	–	1	–	–/2
PNA	Control	1	–	1	–/2	–	1	1	–/1	1	–	–	–/2	–	–	–	–/2
	I-1515	1	–	–	–/2	–	–	–	–/1	–	–	–	–/2	–	–	–	–/2
	I-3030	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	PRG-1545	1	–	–	–	–	1	–	–	1	–	–	–	–	–	–	–
UEA-1	Control	–	–	–	–/1	–	–	–	–	–	–	–	–/2	–	–	–	–/2
	I-1515	2	–	–	–/2	–	–	–	–/2	–	–	–	–/2	–	–	–	–/3
	I-3030	1	–	–	–/2	–	–	–	–/2	–	–	–	–/2	–	–	–	–/2
	PRG-1545	2	–	–	–/2	–	–	–	–/2	–	–	–	–/2	–	–	–	–/2
WGA	Control	2	–	–	–	–	–	–	–/	–	–	–	–/1	–	–	–	–/2
	I-1515	2	–	–	–	–	–	–	–/1	–	–	–	–/1	–	–	–	–/1
	I-3030	2	–	–	–	–	–	–	–	–	–	–	–/2	–	–	–	–/2
	PRG-1545	2	–	–	–	–	–	–	–	–	–	–	–/1	–	–	–	–/1
RCA-1	Control	2	–	–	–/1	–	–	–	–/2	–	–	–	–/2	–	–	–	–/2
	I-1515	3	–	–	–/	–	–	–	–/1	–	–	–	–/1	–	–	–	–/1
	I-3030	2	–	–	–/2	–	–	–	–/2	–	–	–	–/2	–	–	–	–/2
	PRG-1545	2	–	–	–/1	–	–	–	–/2	–	–	–	–/2	–	–	–	–/2
Con-A	Control	2	1	1	–	–	–	–	–/1	–	–	–	–/	–	–	–	1
	I-1515	1	1	1	–	–	–	–	–/1	–	–	–	–/1	–	–	–	–/1
	I-3030	2	1	1	–	–	–	–	–/1	–	–	–	–/1	–	–	–	–/1
	PRG-1545	2	1	1	–	–	–	–	–/1	–	–	–	–/1	–	–	–	–/1

AG, apical glycocalix; AC, apical cytoplasm; BC, basal cytoplasm; E, enterocytes; GC, goblet cells; –, null staining; 1, weak staining; 2, moderate staining; 3, strong staining.

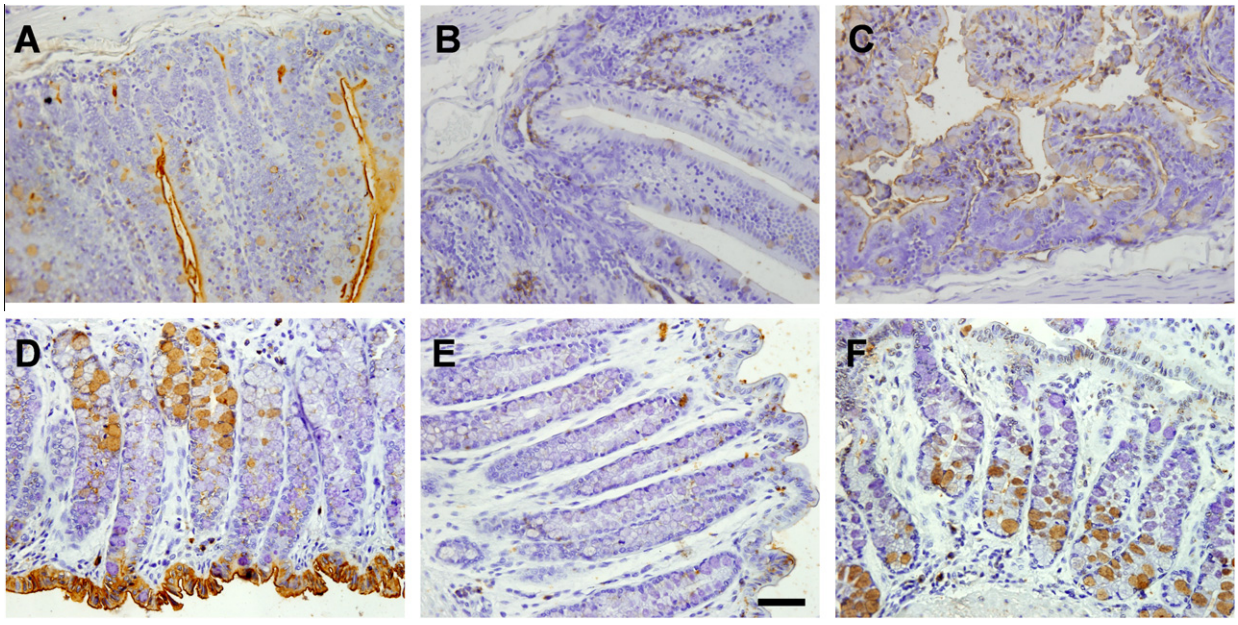

Fig. 4. Lectin histochemical staining in in *S. glaucophyllum*-intoxicated rabbits. DBA binding pattern in the jejunum of control (A), I-3030-intoxicated rabbits (B) and PRG-intoxicated animals (C); DBA binding pattern in the rectum of control (D), I-3030-intoxicated rabbits (E) and PRG-intoxicated animals (F). Bar = 50 μm.

intoxicated animals these cells had a foamy aspect (Figs. 2 and 3A and B).

Neither in the small intestine nor in the large intestine PAS or AB techniques showed differences between the studied groups.

3.3. Lectin histochemistry

The complete pattern of the lectin histochemistry analysis is shown in Tables 2–6. In the following paragraphs we summarised the most relevant results.

The binding of DBA and SBA to the glycocalix of the crypts and villi of the small intestine of the intoxicated animals was weaker than in control group (Fig. 4A–C). In the PRG-1545 the intensity of the labelling was variable in the duodenum and jejunum. However, in the ileum resembled that of control group. In the colon and rectum there was also a reduction in DBA binding pattern at the glycocalix of the surface epithelium and that of the crypts in both intoxicated groups (Fig. 4D–F).

PNA binding pattern was weaker in the I-3030 group and in the PRG than control group, in the duodenum. The labelling in the jejunum of the I-3030 group and in the PRG-1545 was higher than in the control group at the jejunum, whereas the results were variable in the ileum.

WGA showed stronger binding to the epithelium of the jejunum in the intoxicated animals. The results were variable in the duodenum.

UEA-1 labelling of the glycocalix of the crypt and villi was null in the duodenum and jejunum of control animals, whereas it showed a stronger reactivity in both intoxicated groups. The PRG-1545 showed a variable reactivity in both small intestinal segments. UEA-1 reactivity varied from null to strong between groups in the colon, whereas the binding pattern was null in control group and variable in other groups in the rectum. Goblet cells were heterogeneously labelled both in the small and large intestine.

4. Discussion and conclusions

Cell differentiation and maturation processes in tissues undergoing constant renewal, like the intestinal epithelium, can be efficiently studied by classic carbohydrate histochemistry or lectin histochemistry (Pang et al., 1987; Falk et al., 1994). We used these techniques to analyze the glycoconjugate expression in the intestinal epithelium of rabbits under a hypervitaminosis D state induced by the chronic ingestion of *S. glaucophyllum*.

PAS and AB are conventional techniques that distinguish macromolecules that contain carbohydrates in tissue sections, and provide information for histochemical classification of such constituents. The PAS positive reactions indicate the presence of neutral glycoproteins, whereas AB reactions indicate the presence of acidic mucins (Bancroft and Stevens, 1990). Additional and complementary information of the carbohydrate composition is obtained using lectin histochemistry (Lueth et al., 2005; Liquori et al., 2007). The goblet cell mucin in the *S.glaucophyllum*-intoxicated animals showed a variable but conserved carbohydrate composition in most of the intestinal sections, without changes during the intoxication time. Brunner's gland mucin reacted with RCA-1, Con-A, SBA and WGA, lectins with the same binding specificity observed by Schumacher et al. (2004). Histochemical changes in goblet cell and Brunner's gland mucin frequently occur in different pathological conditions. Most studies on intestinal mucin composition are referred to inflammation, infectious conditions or age-related modifications (Ehsanullah et al., 1982; Morrissey et al., 1983; Reid et al., 1984; Koninkx et al., 2008; Mantle et al., 1991; Itagaki et al., 1994; Sharma and Schumacher, 1995a,b; Sharma et al., 1995; Schumacher et al., 2004). However, in our study we did not detect changes with PAS or AB techniques. Further mucin characterization by means of other biochemical techniques (Mantle et al., 1991) would be necessary.

Mucin secretory cells did not show variations in the composition of their secretion using conventional histochemistry, while lectin histochemical changes were present in the glycocalix of villi, crypts and surface epithelium of both small and large intestines. The most relevant changes found in the present study were the reduction in DBA and SBA bindings since they were present in all the intestinal sections studied. Taking into account the terminal carbohydrate specificity of these lectins these changes could reflect a decrease on α-D-GalNAc, since this is the sugar residue that bind both lectins.

Lectin binding pattern of the intestinal epithelium varies with the species, the anatomical section considered, the period of life (Pang et al., 1987; Falk et al., 1994; Chae and Lee, 1995) and under physiological and pathological conditions. Changes in the intestine of pigs and rats under bacterial infection or parasitosis (Sohn and Chae, 2000; Yamauchi et al., 2006; Thomsen et al., 2006), in young rabbits mucoid enteropathy (Itagaki et al., 1994) and enterotoxemia (Jelinek, 1996) are only some examples. In addition, it has been reported that modifications on nutritional factors and diverse hormonal stimuli may be involved in the glycosylation process (Biol et al., 1992; Sharma et al., 1995; Biol-N'garagba et al., 2002; Biol-N'garagba and Louisot, 2003). Since the intestinal epithelium is continuously exposed to dietary components, indigenous flora and multiple other microorganisms it is difficult to specify the role of each one both under physiological and pathological conditions. Besides, in most cases they are synergistically acting. Thus, changes on intestinal carbohydrate composition depend on a great diversity of factors.

The molecular pathways that trigger or regulate the glycosylation process of the intestine are not still completely defined. The regulation of the morphological and functional maturation of the mammalian small intestine is under the control of multiple hormonal and nutritional factors during postnatal life (Dauca et al., 1990; Jaswal et al., 1990; Biol et al., 1998; Biol-N'garagba and Louisot, 2003; Chaudhry et al., 2008). Among the hormonal factors, glucocorticoids modulate the gene transcription of glycosyltransferases, such as fucosyltransferases. Thus, these hormones trigger precocious changes in the sialylation, galactosylation and fucosylation processes of the intestinal epithelium during suckling (Biol-N'garagba and Louisot, 2003). Thyroid hormones, other hormones with intracellular receptors, are also involved as regulatory factors (Mahmood and Torres-Pinedo, 1985; Chaudhry et al., 2008). In addition, undernutrition in suckling rats negatively impact on the carbohydrate composition on enterocytes glycocalix, but the administration of cortisone and insulin revert the glycosylation pattern acquired under that state (Jaswal et al., 1990). Besides, changes on diet composition can alter the intestinal flora homeostasis and its interaction with the intestinal epithelium and, consequently, produce alterations on the expression of carbohydrate at the brush border enterocytes (Sharma and Schumacher, 1995a,b; Sharma et al., 1995).

We suggest that vitamin D, another steroidal hormone as glucocorticoid, may also participate in the glycosylation process. We propose two possible pathways for this observation: it may regulate the transcription of certain glycosyltransferases and glycosidases that might alter the attachment or removal of carbohydrates of the intestinal glycoproteins and, consequently, change the lectin binding pattern in the small and large intestine of the intoxicated animals. On the other hand, vitamin D could also be acting via the polyamine way. Polyamines are policationic substances that participate in the processes of enterocyte proliferation and differentiation (Suda et al., 1990). Vitamin D would stimulate the synthesis of enzymes involved in polyamine metabolism, such

as ornithine decarboxylase and spermidine N-acetyltransferase Biol-N'garagba et al., 2002).

The final stages of digestion of dietary proteins and sugar absorption occur in the enterocyte glycocalix, which showed the more remarkable changes in the lectin binding in the present study. This specialised glycocalix actively participates in the digestion and absorption processes of luminal nutrients. These processes can occur due to the activity of several substances present in the brush border membrane such as peptidases, several transport systems, and glycoproteins involved in detoxification or infectious processes (Biol-N'garagba and Louisot, 2003; Ganapathy et al., 2006; Wright et al., 2006). The changes in the lectin binding pattern found in the intestine of the intoxicated animals might be explained by the observed anorexia. Our present data support the idea that changes in particular lectins and in specific sections of the small intestine (DBA, SBA and PNA in duodenum and jejunum, and WGA in jejunum) are present only during *S. glaucophyllum* intoxication. Moreover, those lectin binding changes were not observed in nutritionally restricted animals (data not shown).

In most of the analyzed intestinal sections, the PRG-1545 had identical binding pattern to that found in control animals, indicating a reversal effect of the intoxication. However, in some animals the results resembled those observed in the intoxicated group. Variations in the hormonal and metabolic profile of animals from PRG-1545 during and after the period of intoxication, as well as differences in the mechanism of response, adaptation or recovery of each intestinal section could be responsible for the heterogeneous results observed between intestinal sections as well as between members of the same group. It is known that the activity of certain disaccharidases changes during starvation, considering age and intestinal section, showing a variable recovery response after refeeding (Pathak et al., 1981, 1982; Gorastiza et al., 1985; Firmansyah et al., 1989; Ortega et al., 1996).

The data showed in the present study would support a possible role of vitamin D in the glycosylation process of the intestine, independently of the starving effect. Additional studies are needed to investigate whether the vitamin D or another active principle of the plant may be directly involved in the pathogenesis of the anorexia, or if the latter is secondarily triggered after the impairment of the general health state. Although it has been the scope of many studies, as far as we know no other toxic principle different from vitamin D and its metabolites has been found in the plant. We suggest that both the vitamin D and the anorexia, which may be induced by the own hypervitamin D state, could be acting simultaneously. The anorexia that showed the rabbits of our study was previously documented in the cattle (Carrillo et al., 1971; Tokarnia et al., 1974), and it was initially proposed as responsible for the body weight lose due to the reduction in feed intake (Carrillo et al., 1971; Noseda et al., 1976). However, we should not underestimate the idea of possible changes in the glycosylation pattern of the intestinal epithelium as responsible for alterations in the digestive and absorption processes. The body weight regulation depends on multiple factors. It is known that high calcium levels induce lower body fat and/or body weight, and reduced weight gain at midlife (Davies et al., 2000; Heaney et al., 2002). *S. glaucophyllum* intoxication induces a hypercalcemic state in the intoxicated animals. Calcemia in I-1515 and I-3030 groups showed a progressive increase with intoxication time. In PRG-1545 calcemia also increased during the intoxication period but then gradually reached normal values (Fontana et al., 2009). Thus, calcium itself would also be clearly involved in the gradual and progressive lose of weight and body condition.

In addition to the particular clinical signs that characterize the disease, the intoxicated animals secondarily showed otitis, rhinitis, conjunctivitis or diarrhoea. Vitamin D has immunomodulatory effects. This hormone suppresses the adaptive immunity and stimulates the innate immunity (Bikle, 2008, 2009). In previous studies we documented morphological and functional changes in primary and secondary lymphoid organs of cattle and rabbits intoxicated with *S. glaucophyllum*, consisting in atrophy of those organs as well as changes in their lymphoid cell kinetics (Fontana and Zanuzzi, 2007; Fontana et al., 2009; Fontana, 2009). We also demonstrated Paneth cells hyperplasia and hypertrophy in the small intestine of the intoxicated animals (Zanuzzi et al., 2008). As Paneth cells have a critical role in intestinal innate defence (Porter et al., 2002; Eckman, 2005) this finding might indicate a stimulatory or compensatory response of the innate immune system, perhaps to protect the intestine from potential infectious processes. Although we did not evaluate the specific impact of the hypervitaminosis D state on the adaptive and innate immunity in the intoxicated animals, we speculate that the secondary signs shown by them could be associated with a local or systemic immunological impairment.

In conclusion, based on the discussed data we proposed a possible effect of vitamin D as a glycosylation regulator. It remains as an open question the mechanism by which it could be exerting that action.

Acknowledgements

We thank Mr. Rubén Mario, Mrs. Rosa Villegas and Miss Maria Guadalupe Guidi for their technical assistance. Financial support was provided in part by grants from Agencia Nacional de Promoción Científica (ANPCyT) (PICT 08-08573), Consejo Nacional de Investigaciones Científicas (CONICET) and Academia Nacional de Agronomía y Veterinaria (ANAV), Argentina. CNZ, CGB, ELP and EJG are members of CONICET (National Scientific Research Council). MLO and FAL are fellows of the ANPC y T.

References

Bancroft, J.D., Stevens, A., 1990. Theory and Practice of Histological Techniques, third ed. Churchill, Livingstone, New York. pp. 639–640.
Barros, S.S., Gimeno, E.J., 2000. Cell differentiation and bone protein synthesis in the lungs of sheep with spontaneous calcinosis. J. Comp. Pathol 123, 270–277.
Bikle, D.D., 2007. What is new in vitamin D? Curr. Opin. Rheumatol. 19, 383–388.
Bikle, D.D., 2008. Vitamin D and the immune system: role in protection against bacterial infection. Curr. Opin. Nephrol. Hyperten. 17, 348–352.
Bikle, D.D., 2009. Vitamin D and immune function: understanding common pathways. Curr. Osteoporos. Rep. 7, 58–63.
Biol, M.C., Lenoir, D., Hugueny, I., et al., 1992. Hormonal regulation of glycosylation process in rat small intestine: responsiveness of fucosyl-transferase activity to hydrocortisone during the suckling period, unresponsiveness after weaning. Biochim. Biophys. Acta 1133, 206–212.
Biol, M.C., Lenir, D., Greco, S., et al., 1998. Role of insulin and nutritional factors in intestinal glycoprotein fucosylation during postnatal development. Am. J. Physiol. 275, 936–942.
Biol-N'garagba, M.C., Greco, S., George, P., et al., 2002. Polyamine participation in the maturation of glycoprotein fucosylation, but not sialylation, in rat small intestine. Pediatr. Res. 51, 625–634.
Biol-N'garagba, M.C., Louisot, P., 2003. Regulation of the intestinal glycoprotein glycosilation during postnatal development: role of hormonal and nutritional factors. Biochimie. 85, 33–52.
Carrillo, B.J., Worker, N.A., 1967. Enteque seco: arteriosclerosis y calcificación metastásica de origen tóxico en animales a pastoreo. Rev. Invest. Agropec. INTA 4, 9–30.
Carrillo, B.J., Tilley, J.M., Garcés, N.E., et al., 1971. Intoxicación experimental de bovinos con *Solanum malacoxylon*. Gac. Vet. 23, 468–484.
Chae, C., Lee, Y.S., 1995. Age-related lectin histochemical changes in the porcine small intestine. J. Vet. Med. Sci. 57, 883–889.
Chaudhry, K.K., Mahmood, S., Mahmood, A., 2008. Hormone induced expression of brush border lactase in suckling rat intestine. Moll. Cell Biochem. 312, 11–16.
Dallorso, M.E., Gil, S., Pawlak, E., Lema, F., Marquez, A., 2001. 1,25 $(OH)_2$ vitamin D concentration in the plasma of Solanum glaucophyllum-intoxicated rabbits. Aust. Vet. J. 79, 419–423.
Dauca, M., Bouziges, F., Colin, S., et al., 1990. Development of the vertebrate small intestine and mechanism of cell differentiation. Int. J. Dev. Biol. 34, 205–218.
Davies, K.M., Heaney, R.P., Recker, R.R., et al., 2000. J. Clin. Endocrinol. Metab. 85, 4635–4638.
Eckman, L., 2005. Defence molecules in intestinal innate immunity against bacterial infections. Curr. Opin. Gastroenterol. 21, 147–151.
Ehsanullah, M., Filipe, M.I., Gazzard, B., 1982. Mucin secretion in inflammatory bowel disease: correlation with disease activity and dysplasia. Gut. 23, 485–489.

Etzler, M.E., Branstrator, M.L., 1979. Cell surface of intestinal cells and their relationship to cellular differentiation. Ciba Found Symp. 70, 51–68.
Falk, P., Roth, K.A., Gordon, J., 1994. Lectins are sensitive tools for defining the differentiation programme of mouse gut epithelial cell lineages. Gastrointest. Liver Phisiol. 29, 987–1003.
Falk, P., Lorenz, R.G., Sharon, N., et al., 1995. *Moluccella laevis* lectin, a marker for cellular differentiation program in mouse gut epithelium. Am. J. Physiol. 268, 553–567.
Firmansyah, A., Suwandito, L., Penn, D., et al., 1989. Biochemical and morphological changes in the digestive tract of rats after prenatal and postnatal malnutrition. Am. J. Clin. Nutr. 50, 261–268.
Fontana, P., 2009. Evaluación morfológica y funcional del sistema inmune y células asociadas al mismo, en animales intoxicados con *Solanum glaucophyllum*. Doctoral Thesis, School of Veterinary Sciences, National University of La Plata.
Fontana, P.A., Zanuzzi, C.N., 2007. Cambios estructurales y funcionales de órganos y células del sistema inmune de bovinos experimentalmente intoxicados con *Solanum glaucophyllum*. Rev. Med. Vet. 88, 47–56.
Fontana, P.A., Zanuzzi, C.N., Barbeito, C.G., et al., 2009. Evaluation of immunotoxic effects of plant-induced hypervitaminosis D on cattle thymus. Pesq. Vet. Bras. 27, 267–275.
Ganapathy, V., Gupta, N., Martindale, R.G., 2006. Protein digestion and absorption. In: Johnson, L. (Ed.), Physiology of the gastrointestinal tract, fourth ed. Elsevier Academic Press, Amsterdam, pp. 1668–1692.
Gelbert, H., Whiteley, H., Ballard, G., et al., 1992. Temporal lectin histochemical characterization of porcine small intestine. Am. J. Vet. Res. 53, 1873–1880.
Gil, S., Dallorso, M., Horst, R., 2007. Screening of vitamin D activity (VDA) of *Solanum glaucophyllum* leaves measured by radioimmunoassay (RIA). Steroid. Biochem. Mol. Biol. 103, 483–486.
Gimeno, E.J., Costa, E.F., Gomar, M.S., et al., 2000. Effects of plant-induced hypervitaminosis D on cutaneous structure, cell differentiation and cell proliferation in cattle. J. Vet. Med. A 47, 201–211.
Gimeno, E.J., Portiansky, E.L., Gomar, V., et al., 2004. Calcinosis in ruminants due to plant poisoning. Contributions on the pathogenesis. In: Acamovic, T., Stewart, C.S., Pennycott, T.W. (Eds.), Poisonous plants and related toxins (CAB-International): Commonwealth Agricultural Bureau, Cambridge, MA, USA, pp. 84–89.
Goldstein, I.J., Hayes, C.E., 1978. The lectins: carbohydrate-binding proteins of plants and animals. Adv. Carbohydr. Chem. Biochem. 35, 127–340.
Gomar, M.S., 2006. Efectos de la hipervitaminosis D vegetal en la diferenciación y proliferación celulares cutáneas, aórticas y pulmonares del conejo". Doctoral Thesis, School of Veterinary Sciences, National University of La Plata, 2006.
Gorastiza, E., Poullain, M.G., Marche, C., et al., 1985. Effect of fasting and reeding on the adaptation of the small intestine in rats. A model for physiopathologic studies. Gastroenterol. Clin. Biol. 9, 790–796.
Heaney, R.P., Davies, K.M., Barger-Lux, M.J., 2002. Calcium and weight: clinical studies. J. Am. Coll. Nutr. 21, 152–155.
Holt, P., Arber, N., Halmos, B., et al., 2002. Colonic epithelial cell proliferation decreases with increasing levels of serum 25-hydroxyvitamin D. Cancer Epidemiol. Biomarkers. Prev. 11, 113–119.
Itagaki, S., Perfumo, S., Petrucelli, C., et al., 1994. Lectin histochemical changes of colon goblet cell mucin in rabbit mucoid enteropathy. Lab. Anim. Sci. 44, 82–84.
Jaswal, V.M., Babbar, H.S., Mahmood, A., 1990. Effect of malnutrition and hormone treatments on intestinal microvillus membrane glycosylation in suckling rats. Ann. Nutr. Metab. 34, 155–162.
Jelinek, F., 1996. Changes of glycid composition and binding of some lectins in the caecum of rabbits with experimentally induced enterotoxaemia. Vet. Med. 41, 219–223.
Jepson, M.A., Mason, C.M., Simmons, N.L., et al., 1995. Enterocytes in the follicle-associated epithelia of rabbit small intestine display distinctive lectin-binding properties. Histochem. Cell. Biol. 103, 131–134.
Kong, J., Zhang, Z., Musch, M.W., et al., 2008. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. Am. J. Physiol. Gastrointest. Liver. Phisiol. 294, 208–216.
Koninkx, J.F.J.G., Mirck, M.H., Hendriks, H.G.C.J.M., et al., 2008. *Nippostrongylus brasiliensis*: histochemical changes in the composition of mucins in goblet cells during infection in rats. Exp. Parasitol. 65, 84–90.
Kutuzova, G.D., DeLuca, H.F., 2007. 1,25-Dihydroxyvitamin D3 regulates genes responsible for detoxification in intestine. Toxicol. Appl. Pharmacol. 218, 37–44.
Liquori, G.E., Mastrodonato, M., Zizza, S., et al., 2007. Glycoconjugate histochemistry of the digestive tract of *Triturus carnifex* (Amphibia, Caudata). J. Mol. Hist. 38, 191–199.
Lueth, M., Sturegärd, E., Sjunnesson, H., et al., 2005. Lectin histochemistry of the gastric mucosa in normal and *Helicobacter pylori* infected guinea-pigs. J. Mol. Hist. 36, 51–58.
Mahmood, A., Torres-Pinedo, R., 1985. Effect of hormone administration on the sialylation and fucosylation of intestinal microvillus membranes of suckling rats. Pediatr. Res. 19, 899–902.
Mantle, M., Atkins, E., Kelly, J., et al., 1991. Effects of *Yersinia enterocolitica* infection on rabbit intestinal and colonic goblet cells and mucin: morphometrics, histochemistry, and biochemistry. Gut 32, 1131–1138.
Mello, J.R.B., 2003. Calcinosis-calcinogenic plants. Toxicon 41, 1–12.
Ménard, D., Levy, E., Delvin, E.E., 1995. Effects of calcitriol on proliferation and differentiation of human fetal jejunum. Biol. Neonate 68, 157–162.
Morrissey, S.M., Ward, P.M., Jayaraj, A.P., et al., 1983. Histochemical changes in mucus in duodenal ulceration. Gut 24, 909–913.
Newmark, H.L., Lipkin, M., 1992. Calcium, vitamin D and colon cancer. Cancer Res. 52, 2067–2070.
Noseda, R.P., Cumba, S.A., Bardón, J.C., et al., 1976. Reproducción experimental de enteque seco por duraznillo blanco en bovinos, su tratamiento y posterior estudio comparativo de animales tratados y no tratados. Gaceta Vet. 38, 105–112.
Ortega, M.A., Núnez, N.C., Suárez, M.D., et al., 1996. Age related response to severe starvation and refeeding in rats. Ann. Nutr. Metab. 40, 351–358.
Pang, K.Y., Bresson, J.L., Walker, W.A., 1987. Development of gastrointestinal surface. VIII. Lectin identification. Am. J. Physiol. 252, 685–691.
Pathak, R.M., Mahmood, A., Dudeja, P.K., et al., 1981. Intestinal brush border membrane structure and function: effect of early postnatal undernutrition. Pediatr. Res. 15, 112–114.
Pathak, R.M., Dudeja, P.K., Ansari, 1982. Alterations in intestinal function in response to thyroxine and cortisone administration in undernourished rats. Ann. Nutr. Metab. 26, 331–336.
Porter, E.M., Bevins, C.L., Ghosh, D., et al., 2002. The multifaceted paneth cell. Cell. Mol. Life. Sci. 59, 156–170.
Puche, R.C., Bingley, J.B., 1995. Calcinosis of cattle in Argentina (first eng. ed.). Universidad Nacional de Rosario Editora, Rosario. 1995..
Razzake, V., Lanske, B., 2006. Hypervitaminosis D and premature aging: lessons learned from Fgf23 and Klotho mutant mice. Trends. Mol. Med. 12, 298–305.
Reid, P.E., Culling, C.F.A., Dunn, W.L., et al., 1984. Chemical and histochemical studies of normal and diseased human gastrointestinal tract. II. A comparison between histologically normal small intestine and Crohn's disease of the small intestine. Histochem. J. 16, 253–264.
Schneider, L.E., Schedl, H.P., 1977. Effects of *Solanum malacoxylon* on duodenal calcium binding protein in the diabetic rat. Endocrinology 100, 928–933.
Schumacher, U., Duku, M., Katoh, M., et al., 2004. Histochemical similarities of mucins produced by Brunner's glands and pyloric glands: a comparative study. Anat. Rec. 278, 540–550.
Sharma, R., Schumacher, U., 1995a. Morphometric analysis of intestinal mucins under different dietary conditions and gut flora in rats. Dig. Dis. Sci. 40, 2532–2539.
Sharma, R., Schumacher, U., 1995b. The influence of diets and gut microflora on lectin binding patterns of intestinal mucins in rats. Lab. Invest. 73, 558–564.
Sharma, R., Schumacher, U., Ronaasen, V., et al., 1995. Rat intestinal mucosal responses to a microbial flora and different diets. Gut 36, 209–214.
Sohn, Y.S., Chae, C., 2000. Lectin-binding capacity of glycoconjugates in *Escherichia coli* 09:K103:NM, 987P+ST+-infected porcine lower small intestines. J. Vet. Med. Sci. 5, 543–547.
Spicer, S.S., Schulte, B.A., 1992. Diversity of cell glycoconjugates shown histochemically: a perspective. J. Histochem. Cytochem. 40, 1–48.
Suda, T., Shinki, T., Takahashi, N., 1990. The role of vitamin D in bone and intestinal cell differentiation. Annu. Rev. Nutr. 10, 195–211.
Taylor, M.E., Drickamer, K., 2003. Introduction to Glycobiology. Oxford University Press, p. 207.
Thomsen, L.E., Knudsen, K.E., Hedemann, V., et al., 2006. The effect of dietary carbohydrates and *Trichuris suis* infection on pig large intestine tissue architecture, epithelial cell proliferation and mucin characteristics. Vet. Parasitol. 142, 112–122.
Tokarnia, C.H., Döbereiner, J., Peixoto, P.V., 1974. Aspectos clinico-patológicos complementares da intoxicação por algumas plantas toxicas brasileiras. Pesq. Vet. Bras. 14, 111–122.
Walling, M.W., Kimberg, D.V., 1975. Effects of 1 α-25-dihydroxyvitamin D and *Solanum glaucophyllum* on intestinal calcium and phosphate transportand on plasma Ca, Mg and P levels in the rat. Endocrinology 97, 1567–1576.
Wasserman, R.H., 1974. Calcium absorption and calcium binding protein synthesis: *Solanum malacoxylon* reverses strontium inhibition. Science 183, 1092–1094.
Wesley, J.P., Watanuki, M., Shevde, N.K., 2006. Vitamin D_3: synthesis, actions, and mechanisms in the intestine and colon. In: Johnson, L. (Ed.), Physiology of the Gastrointestinal Tract, fourth ed. Elsevier Academic Press, Amsterdam, pp. 1753–1771.
Worker, N.A., Carrillo, B.J., 1967. "Enteque seco": calcification and wasting in grazing animals in Argentina. Nature 215, 72–74.
Wright, E.M., Loo, D.D., Hirayama, B., et al., 2006. The sugar absorption. In: Johnson, L. (Ed.), Physiology of the gastrointestinal tract, fourth ed. Elsevier Academic Press, Amsterdam, pp. 1654–1665.
Yamauchi, J., Kawai, Y., Yamada, M., et al., 2006. Altered expression of goblet cell- and mucin glycosylation-related genes in the intestinal epithelium during infection with the nematode *Nippostrongylus brasiliensis* in rat. A.P.M.I.S. 114, 270–278.
Zanuzzi, C.N., Fontana, P.A., Barbeito, C.G., et al., 2008. Paneth cells: histochemical and morphometric study in control and *Solanum glaucophyllum*-intoxicated rabbits. Eur. J. Histochem. 52, 93–100.