

Mucous Membrane of the Rumen of Ovines, Fed With Spineless, Forrage Cactus or Palm (Barbary Fig) (*Opuntia ficus indica* Mil): Histochemical Study by Means of Light Microscopy

Membrana Mucosa del Rumen de Ovinos, Alimentados con Forraje de Cactus Sin Espinas o Palmeras (Barbary Fig) (*Opuntia ficus indica* Mil): Estudio Histoquímico por Medio de Microscopía de Luz

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SUMMARY: The mucosa of the rumen performs the functions of absorption, transportation, metabolism and protection, due to the presence of microorganisms in this compartment of the digestive tract, which are responsible for the digestion of approximately 60% of the organic material apparently digested. The histological structure and the histochemical profile of glicoconjugate of the mucosa of the stomach of sheep that were being fed with forrage cactus or palm (Giant Palm - *Opuntia ficus indica* Mil)¹. Twelve sheep were used, with approximately 14 months old, which were oddly distributed in four diets: D₁ - 60.0% forrage palm + 40.0% concentrated ration; D₂ - 47.5% forrage palm + 12.5% elephant, napier grass + 40.0% concentrated ration, and D₃ - 34,8% forrage palm + 25.2% elephant, napier grass + 40.0% concentrated ration and D₄ - 60.0% elephant, napier grass + 40.0% concentrated ration. The experience period lasted 90 days, and, at the end, the animals were euthanized and fragments of different compartments of their stomachs were taken right after. The animals having consumed diets D₁, D₂ e D₃ presented more developed papillas, while the ones of diet D₄, presented less developed papillas. The stratum corneum of the epithelium of the rumen coating of the animals in diet D₁ presented a slight erosion, when compared to those in diets D₂, D₃ and D₄.

KEYWORDS: Mucous membrane; Histology; Histochemistry; Forrage palm; Forrage cactus; Ovine.

INTRODUCTION

The mucosa of the rumen performs the absorption, transportation, metabolism and protection. Due to the presence of microorganisms in this compartment of the digestive tract, which are responsible for the digestion of around 60% of the organic material which is apparently digested, and due to the constant changes in the fermentation products, the rumen mucous membrane have a great adaptation capability to internal and external conditions. In this compartment, the epithelium is stratified squamous keratinized, its width is varied and the layers are not well defined. The keratinized superior layer forms a protection shield against the rough and fibrous food, while the deeper layers metabolize the volatile fatty and short-chain acids,

especially the butyric, acetic and propionic, which are the main products of fermentation (Stinson *et al.*, 1982).

The internal surface of the rumen has small papillas with variable heights, which can reach 1.5 cm length in an adult bovine. The size and frequency of the papillas vary considerably according to the region of the rumen. They are generally more dense in the central parts of the dorsal and ventral bags, where we assume that there is a greater absorption. These papillas remain little developed while the animal is a suckling, and will rapidly increase, when rough food is included in the diet, and the fermentation starts in the rumen (Brownlee, 1956; Sander *et al.*, 1959).

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The development of the mucosa depends on the type of food the animal receives. Diets that are rich in carbohydrates, easily digested, and with a low fiber content, result in the production of a higher amount of propionic and butyric acids, which will trigger a greater development in young animals and a greater proliferation in adult animals.

Studies with goats show that, although the weight of the forestomachs is delayed by the prolonged feeding with milk, the normal histological changes of their age still followed their course at the rumen-reticulum; the omasum, although, remained in juvenile state until solid food was administered, and the histology the wall of the omasum changed rapidly, Tratmann em 1932, quoted by Wardrop (1961).

Although the production of fatty acids is responsible for the normal development of the papillas, the high concentration of acids in the diet, the exceeding production of lactic acid, associated to the low production of saliva and reduction of the tamponing capability can result in the incidence of parakeratosis. Chronic acidosis was also reported in the abomasum syndrome (van Soest, 1994).

Diets composed by forrage palm and concentrated ration are low in fiber and require a high digestion rate, which can probably explain the observed diarrhea, the reduction in the fat content of the milk, and the abdominal tympanism reported by cattle raisers who use the palm in the ration of their dairy cows. These diets, when administered for long periods, could also lead to the incidence of problems in the rumen's mucosa. Thus, the present work had the purpose to evaluate the histological structure and the histochemical profile of glycoconjugates of the mucosa of the rumen of sheep which consume rations with a high content of forrage palm.

MATERIAL AND METHOD

We used twelve sheep, with no defined breed, around 14 months old, which were oddly distributed in four groups of three each, with the diets: D₁- 60,0% forrage palm + 40,0% concentrated ration; D₂- 47,5% forrage palm + 12,5% elephant, napier grass + 40,0% concentrated ration and D₃ - 34,8% forrage palm + 25,2% elephant, napier grass + 40,0% concentrated ration and D₄ - 60,0% elephant, napier gras + 40,0% concentrated ration. The ration was divided in two equal parts, which were offered at 8 am and 4 pm. The experience period lasted 90 days, 20 of them for the adapting of the animals to the feed, and to its experimental handling, and 70 days of experimental diets. At the end, the animals were euthanized and, after the opening of the abdominal cavity, the different compartments of the stomach were identified (rumen, reticulum, omasum and abomasum) Fragments of the rumen were taken and fixed in Bouin liquid for 24 hours, dehydrated

in absolute alcohol, diaphanized in xylol, and included in paraffin wax. Sections of approximately 6µm width were submitted to Hematoxylin coloration - Eosyn and Picro -Sirius, according to the method of Junqueira & Carneiro (1990) for morphological studies. The following methods were used for the histochemical study of the mucins: 1. Periodic acid-Schiff (PAS), McManus (1946); 2. PAS after the enzymatic digestion by saliva amylase, Lison (1960); 3. PAS with block by acetillation and after saponification, McManus & Cason (1950); 4. Alcian Blue (A.B.) at 3% in acetic acid, Spicer (1960), before and after methylation, Fisher & Lillie (1954); followed by saponification, Lillie & Fullmer (1976); and Alcian Blue at 3% in HCl 0,1N, pH 0,5, Lev & Spicer (1964).

RESULTS

In this experiment, the animals having consumed all diets presented the general histological pattern for most of the mammals, with mucous, sub-mucous, muscular and serous tunics. The mucosa of the rumen, reticulum and omasum consisted of four cell strata: the basal, formed by cylindrical cells disposed in an only layer; the stratum spinosum, formed by polyedric cells, slightly larger than the basal cells, stratum granulosum, with flattened cells and granules of queratohyaline, present in the cytoplasm, and the stratum corneum, whose width varied from one to twenty cells. The cells had a squamous form, and their colorable nucleus could be present or not. The mucosa of the abomasum, at the fundic region, was constituted by simple cylindrical epithelium, and by its lamina propria of conjunctive tissue, filled in almost all its width by glands. The epithelium invaginated, forming small pits in which the glands emptied. The glands were tubular and finished near the muscularis mucosae. In the piloric region, the pits were deeper and the glands shorter, with a greater number of mucous cells.

Our results showed that, macroscopically, we could observe that the animals in diets with forrage palm + concentrated ration, (D₁, D₂ e D₃), presented more developed papillas and a yellow-colored mucosa, while those who consumed gras and concentrated ration (control group) presented smaller papillas and green-brownish mucosa. Under the light microscope, we verified that, in the groups that received diet D₄ (control) and D₁ (forrage palm + concentrated ration) the stratum granulosum of the papillar epithelium was slightly more developed and had a less eroded aspect compared to diets D₂ and D₃ (Figs. 1 and 4). In the animals in diets D₂ and D₃ (forrage palm + gras + concentrated ration) the stratum spinosum was less dense, while the stratum corneum was more eroded and presented quite vacuolated cells (Figs. 2 and 3).

In Tables I and II, we expose the histochemical results obtained in the four groups of studied animals.

Table I. Results of the histochemical reactions for the detection of the glicoconjugates in the rumen of ovine, submitted to different diets.

Fundic region	D ₁	D ₂	D ₃	D ₄
PAS	++	++	++	+
Amylase + PAS	++	++	++	+
Acetylation + PAS	-	-	-	-
Acetylation + saponification+PAS	++	++	++	+
A.B. pH = 2.5	+	+	+	++
A.B. pH = 0.5	±	±	±	±
Methylation + A.B. pH = 2.5	-	-	-	-
Methylation + saponification + A.B. pH = 2.5	+	+	+	++

D₁ - 60.0% forage palm + 40.0% concentrated ration. D₂ - 47.5% forage palm + 12.5% elephant gras + 40.0% concentrated ration. D₃ - 34.8% forage palm + 25.2% elephant gras + 40.0% concentrated ration. D₄ - 60.0% elephant gras + 40.0% concentrated ration. Qualitative degree of the reactions: (++) positive; (+) slightly positive; (-) negative; (±) slight traces.

Table II. Results of the histochemical reactions for the detection of the glicoconjugates in the rumen of ovines, submitted to different diets.

Piloric region	D ₁	D ₂	D ₃	D ₄
PAS	++	++	++	+
Amylase + PAS	++	++	++	+
Acetylation + PAS	-	-	-	-
Acetylation + saponification + PAS	++	++	++	+
A.B. pH = 2.5	+	+	+	++
A.B. pH = 0.5	±	±	±	±
Methylation + A.B. pH = 2.5	-	-	-	-
Methylation + saponification + A.B. pH = 2.5	+	+	+	++

D₁ - 60.0% forage palm + 40.0% concentrated ration. D₂ - 47.5% forage palm + 12.5% elephant gras + 40.0% concentrated ration. D₃ - 34.8% forage palm + 25.2% elephant gras + 40.0% concentrated ration. D₄ - 60.0% elephant gras + 40.0% concentrated ration. Qualitative degree of the reactions: (++) positive; (+) slightly positive; (-) negative; (±) slight traces.

DISCUSSION

The histological general pattern of the stomach of ovine is similar to most of the ruminants. The influence of the diet consumed by the animal, regarding changes in the mucosa, is directly related to the volatile fatty acids produced in the rumen. Diet changes result in favorable conditions for the growth of certain microorganisms, and consequently, the fermentation products will be different. The fatty acids, the propionic and the butyrate, perform a fundamental role in the changes of the rumen's mucosa, with the increase in the size of the papilla, in the width of the mucosa, including the incidence of papillar bodies and para-nuclear valvules (Wardrop, 1961; Tamate *et al.*, 1962; Harrison *et al.*, 1960; Sutton *et al.*, 1963). The changes observed in the gastric epithelium of animals in the different diets are caused by the diet-type differences. In

animals receiving palm + concentrated ration, the production of propionate was higher (Neiva, 1996), which justifies the greater development of the rumen mucosa in these animals. The vegetal food seems necessary to the total development of the histological structure of the rumen; different foods can alter the appearance of the surface of the mucosa of this organ (Brownlee, 1956), and can affect the histological structure of the granulosum and corneum strata. The most prominent abrasive materials in vegetal tissues are undoubtedly the "opal phytoliths" (Smithson, 1958; Baker *et al.*, 1961). Since the grasses have a relatively high content of silica, this may explain the difference in the erosion of the stratum corneum among the diets D₁, D₂, D₃ and D₄, as observed in the figures 1, 2, 3 e 4. Morphologically, the surface of the rumen epithelium of

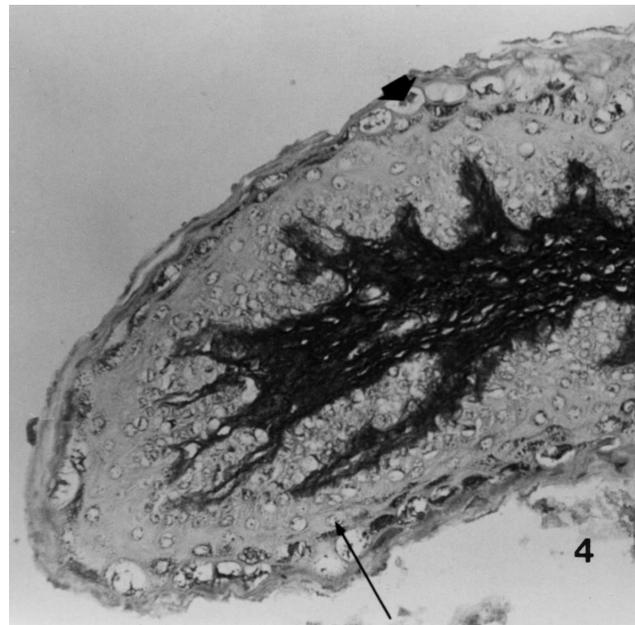
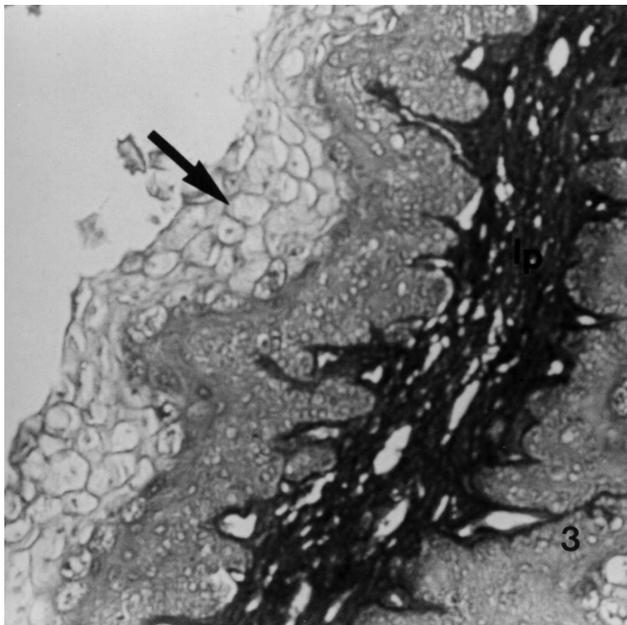
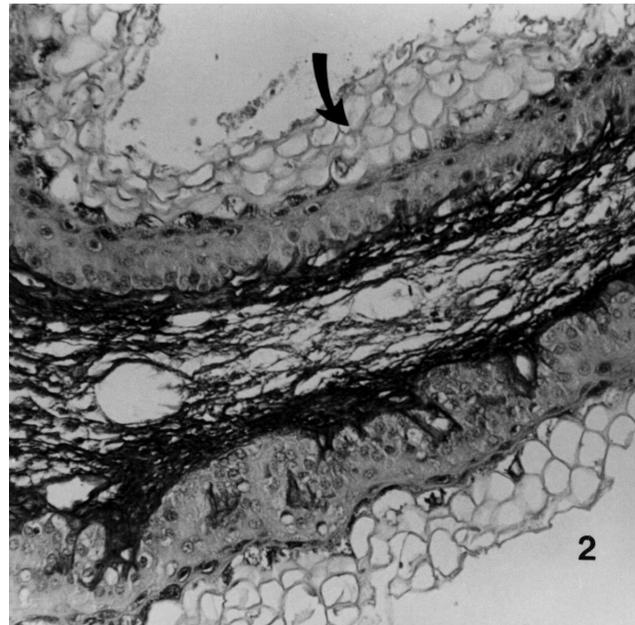
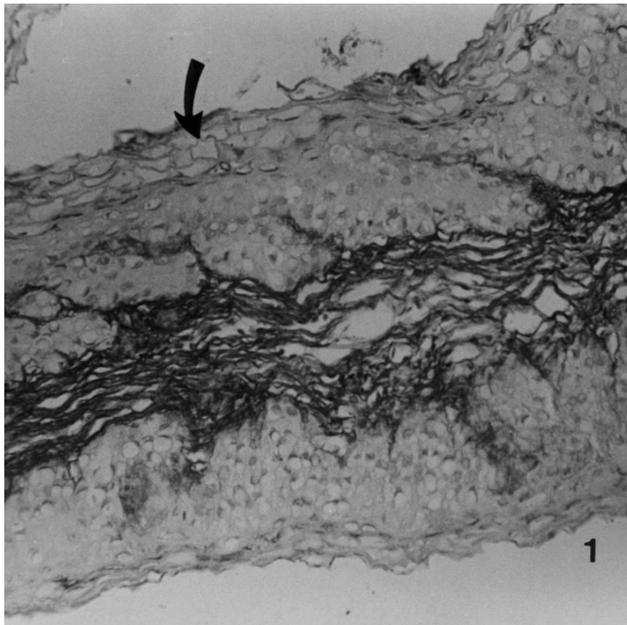


Fig. 1. Photomicrography of the rumen mucous in sheep under D_1 diet. Volumous cells on the granulous (arrow), keratinous layer with vacuolated cells and less scaling (arrow) as compared to previous Figures. Picosirius.

Fig. 2. Photomicrography of rumen mucous in the D_2 diet. In the rumen epithelium a large area of scaling on the keratinous layer (arrow). Own lamina (lp). Picosirius. 148x.

Fig. 3. Photomicrography of rumen mucous of sheep under D_3 diet. Rumen epithelium with intense scaling of cell on the keratinous layer (arrow). Picosirius. 148x.

Fig. 4. Photomicrography of rumen mucous in sheep under D_4 diet. Rumen epithelium with scaling cells on the keratinous layer (arrow). Picosirius. 148x.

the animals in diet D_1 was much less eroded than the one of the animals in diet D_4 . This probably happened due to the silica content of the grass, and its rougher physical structure, which provokes a greater friction among the

particles of food and the mucosa. Considering the very low fiber content in diet D_1 , we could expect the incidence of parakeratosis in the animals that consumed this diet, as it has been reported in animals receiving concentrated diets

(Orskov, 1986; Bull *et al.*, 1965). This didn't happen, though. It is possible that the silica content, or the content of other mineral substances in the forrage palm, have avoided the incidence of digestive problems. The intense erosion observed in the coating epithelium cells of the animals in diets D₂ and D₃, compared to the animals in diets D₁ and D₄, are possibly due to the association of the forrage palm and the elephant grass. Since these have a high silica content, and isolatedly, had an abrasive effect on the mucous membrane; when offered together, they may have increased this effect.

Histochemical comparative studies (Willems & Gerard, 1969; Sheahan & Jervis, 1976), reveals the presence of similar complexes of polyssaccharides (glicoconjugates) in the stomach of apes, dogs, cats, desert rats, baboons, rabbits, pigs, guinea pigs, hamsters, mice and humans. In marsupials (Krause *et al.*, 1976; Gemmel & Engelhardt, 1977; Carvalho *et al.*, 1987; Carvalho *et al.*, 1989) and also in the armadillo *Dasybus novemcinctus*

(Carvalho *et al.*, 1975) similar results with some differences in the intensity of the reactions were reported, probably due to the sensibility of the methodology to the fixation conditions and to the particularities and feeding habits of each species. Our findings suggest a possible change in the histochemical profile of the neutral mucosubstances, probably in response to the changes in the diet of the animals which received the forrage palm. Nevertheless, new studies are necessary to specifically feature these glicoproteins.

Based on the results of this work, we can conclude that:

1. Forrage palm in the ration of ovine triggered a greater development of the papillas.
2. The association of forrage palm and elephant grass in the ovine diet causes a greater erosion in the stratum corneum.
3. The influence of the diet on the structural pattern of the mucosa of the rumen was proved.

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RESUMEN: La mucosa del rumen actúa en la absorción, transporte, metabolismo y protección, debido a la presencia de microorganismos en este compartimiento del tracto digestivo, los cuales son responsables de la digestión de aproximadamente el 60% de material orgánico aparentemente digerido. Se analizó la estructura histológica y el perfil histoquímico de las estructuras de glicoconjugados de la mucosa del estómago de las ovejas que fueron alimentadas con forraje de cactus o palmeras (Giant Palm - *Opuntia ficus indica* Mil)1. Fueron utilizadas 12 ovejas, con aproximadamente 14 meses de edad, las cuales fueron distribuidas de manera impar en cuatro dietas: D₁ - 60.0% forraje de palmera + 40.0% ración concentrada; D₂ - 47.5% forraje de palmera + 12.5% hierba alta africana + 40.0% ración concentrada, y D₃ - 34,8% forraje de palmera + 25.2% hierba alta africana + 40.0% ración concentrada y D₄ - 60.0% hierba alta africana + 40.0% ración concentrada. La experiencia duró 90 días y al final, los animales fueron sacrificados y fragmentos de diferentes compartimientos de sus estómagos fueron directamente recogidos. Los animales que habían consumido dietas D₁, D₂ y D₃ presentaron papilas más desarrolladas, mientras que los alimentados con dieta D₄ presentaron papilas menos desarrolladas. El estrato córneo del epitelio del rumen de los animales de la dieta D₁ presentaron pequeña erosión, cuando fueron comparados con los animales sometidos a las dietas D₂, D₃ y D₄.

PALABRAS CLAVE: Membrana mucosa; Histología; Histoquímica; Forraje de palmera; Forraje de cactus; Ovino.

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