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Mucous Membrane of the Rumen of Ovines, Fed With Spineless, Forrage Cactus or Palm (Barbary Fig) (Opuntia ficus indica Mil): Hystochemical 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


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). 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).
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 fore stomachs 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: 

- \( D_1: 60.0\% \) forrage palm + 40.0% concentrated ration;
- \( D_2: 47.5\% \) forrage palm + 12.5% elephant,
- napier grass + 40.0% concentrated ration and
- \( D_3: 34.8\% \) forrage palm + 25.2% elephant, napier grass + 40.0% concentrated ration and
- \( D_4: 60.0\% \) elephant, napier grass + 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 6 \( \mu \)m 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 enzimatic 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 cylindric cells disposed in an only layer; the stratum spinosum, formed by poliedric cells, slightly larger than the basal cells, stratum granulosum, with flattened cells and granules of queratohyaline, present in the citoplasma, 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 cylindric 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, \( (D_1, D_2 e D_3) \), 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 \( D_4 \) (control) and \( D_4 \) (forrage palm + concentrated ration) the stratum granulosum of the papillar epithelium was slightly more developed and had a less eroded aspect compared to diets \( D_1 \) and \( D_3 \) (Figs. 1 and 4). In the animals in diets \( D_1 \) and \( D_4 \) (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.

<table>
<thead>
<tr>
<th></th>
<th>Fundic region</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
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<tr>
<td>PAS</td>
<td>++</td>
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<td>++</td>
<td></td>
<td>+</td>
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<tr>
<td>Amylase + PAS</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Acetylation + PAS</td>
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<tr>
<td>Acetylation + saponification + PAS</td>
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<tr>
<td>A.B. pH = 2.5</td>
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<tr>
<td>A.B. pH = 0.5</td>
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<tr>
<td>Methylion + A.B. pH = 2.5</td>
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<tr>
<td>Methylion + saponification + A.B. pH = 2.5</td>
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D1 - 60.0% forrage palm + 40.0% concentrated ration. D2 - 47.5% forrage palm + 12.5% elephant gras + 40.0% concentrated ration. D3 - 34.8% forrage 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.

<table>
<thead>
<tr>
<th></th>
<th>Piloric region</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
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<tbody>
<tr>
<td>PAS</td>
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<td>Amylase + PAS</td>
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<td>Acetylation + PAS</td>
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<tr>
<td>Acetylation + saponification + PAS</td>
<td>++</td>
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<tr>
<td>A.B. pH = 2.5</td>
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<tr>
<td>A.B. pH = 0.5</td>
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<tr>
<td>Methylion + A.B. pH = 2.5</td>
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<tr>
<td>Methylion + saponification + A.B. pH = 2.5</td>
<td>+</td>
<td>+</td>
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**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 papillary bodies and para-nuclear valcuoles (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” (Smithison, 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...
the animals in diet D1 was much less eroded than the one of the animals in diet D4. This probably happened due to the silicon 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 D1, we could expect the incidence of parakeratosis in the animals that consumed this diet, as it has been reported in animals receiving concentrated diets.
Resumen: La mucosa del romen actúa en la absorción, transporte, metabolismo y protección, debido a la presencia de microorganismos en este compartimieto 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 glicoconjungados de la mucosa del estómago de las ovejas que fueron alimentadas con forraje de cactus o palmeras (Giant Palm - Opuntia ficus indica Mil). Fueron utilizadas 12 ovejas, con aproximadamente 14 meses de edad, las cuales fueron distribuidas en cuatro dietas: D1 - 60.0% forraje de palmera + 40.0% racimo, y D3 - 34.8% forraje de palmera + 25.2% hierba alta africana + 40.0% racimo, y D4 - 60.0% hierba alta africana + 40.0% racimo concentrada. La experiencia duró 90 días y al final, los animales fueron sacrificados y fragmentos de diferentes compartimentos del romen, cuando fueron comparados con los animales sometidos a las dietas D2, D3 y D4, que: 1. Forraje palm en el racion de ovine triggered a greater development of the papillas. 2. The association of forraje 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.

Referencias Clave: Membrana mucosa; Histología; Histoquímica; Forraje de cactus; Forraje de palmera; Ovino.


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