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Characterization of ruminal acidosis and initial phase of laminitis inducted by oligofructose in crossbred calves[1]

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ABSTRACT.- Noronha Filho A.D.F, Freitas S.L.R., Rodrigues D.F., Mendes F.F., Miguel M.P., Cunha P.H.J., Fioravanti M.C.S. & Silva L.A.F. 2019. **Characterization of ruminal acidosis and initial phase of lamnitis inducted by oligofructose in crossbred calves.** *Pesquisa Veterinária Brasileira 39(2):99-106.* Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Campus Samambaia, Avenida Esperança s/n, Goiânia, GO 74690-900, Brazil. E-mail: dionisiofnf@hotmail.com

One of the ways to study cattle laminitis is its experimental induction by supplying a large amount of high fermentation carbohydrate. The most effective protocol until now has been the use of oligofructose. The objective of this study was to evaluate clinical and histological aspects of the hoof in experimental induction of ruminal acidosis and laminitis in calves using oligofructose. Six crossbred (*Bos taurus* x *Bos indicus*) yearling calves divided into Group I (GI) and Group II (GII) were used. Animals in GI and GII received intraruminal oligofructose in doses of 13 and 17g/kg, respectively. During 28 hours the calves were clinically evaluated and 30 hours after induction, samples were taken from coronary and abaxial wall of the hoof for histologic evaluation. Were noticed signs of ruminal and metabolic acidosis like rumen distension with fluid, diarrhea, ruminal pH reduction and, at blood gas analysis, pH and bicarbonate below reference range. Lameness was not observed however, some animals had a slower gait and apathy, possibly due to metabolic acidosis, though. Histologically, typical lesions of laminitis like circulatory changes and inflammatory infiltrate in the dermis, irregularities and areas of detachment at basement membrane and morphologic changes in cells from basal epidermis were found. The protocol induced, in the first 30 hours, clinical signs of ruminal and metabolic acidosis and low grade histologic lesions in the digits. Lameness and digit pain were not observed, characterizing the prodromic phase of the disease.

INDEX TERMS: Ruminal acidosis, lamnitis inducted, oligofructose, crossbred calves, metabolic acidosis, bovine, lameness, hoof histology, cattle.

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RESUMO.- [**Caracterização da acidose ruminal e da fase inicial da laminite induzidas por oligofrutose em bezerros mestiços.**] Uma das formas de se estudar a laminite bovina é sua indução experimental por meio do fornecimento de grande quantidade de carboidrato de alta fermentação. O protocolo mais eficaz até o momento foi o uso de oligofrutose. Objetivou-se avaliar aspectos clínicos e histológicos dos dígitos de bovinos na indução experimental de acidose ruminal e laminite usando oligofrutose. Utilizaram-se seis bezerros mestiços (*Bos taurus* x *Bos indicus*) de um ano, divididos em Grupo I (GI) e Grupo II (GII). Os animais em GI e GII receberam oligofrutose por via intrarruminal nas doses de 13 e 17g/kg respectivamente. Os bovinos foram avaliados clinicamente por 28 horas e fragmentos de coroa e muralha abaxial dos dígitos foram

colhidos para histologia 30 horas após a indução. Foram identificados sinais de acidose ruminal e metabólica como distensão ruminal com líquido, diarreia e baixo pH ruminal. Os resultados de hemogasometria indicaram baixos pH e nível plasmático de bicarbonato. Os animais não apresentaram claudicação, entretanto, observaram-se apatia e marcha mais lenta, atribuídas à acidose metabólica. Histologicamente foram observadas lesões indicativas de laminite como alterações circulatórias e infiltrado inflamatório na derme, irregularidades e áreas de destacamento da membrana basal e alterações morfológicas de células da epiderme basal. O protocolo induziu, nas primeiras 30 horas, sinais de acidose ruminal e metabólica e lesões histológicas de baixa intensidade nos dígitos. Não foi observada claudicação ou sensibilidade nos dígitos, caracterizando a fase prodrômica da enfermidade.

TERMOS DE INDEXAÇÃO: Acidose ruminal, laminite induzidas, oligofrutose, bezerros mestiços, acidose metabólica, bovinos, claudicação, histologia dos dígitos.

INTRODUCTION

The growing productivity of bovine over the last decades has been accompanied by the greater occurrence of diseases associated with the production system such as indigestions and foot diseases (Greenough 2007, Radostits et al. 2007). Among the indigestions, ruminal acidosis is a fermentative imbalance caused by the ingestion of excessive amounts of rapidly fermentable carbohydrate (Nagaraja & Lechtenberg 2007). As for foot diseases, laminitis is considered one of the main diseases affecting bovine hooves. It is characterized by inflammation of the digital dermis and has ruminal acidosis as an important element of its pathogenesis (Nocek 1997, Thoefner et al. 2004). In the clinical form of laminitis, the animal shows marked lameness in all limbs (Thoefner et al. 2004, Danscher et al. 2009). In chronic cases, the hoof is elongated and with a marked concavity in the wall, defined as "slipper foot" (Greenough 2007). In the subclinical form, changes in living tissues of the hoof precede painful lesions such as sole ulcers and white-line disease by two to three months (Mendes et al. 2013).

The fermentative changes that characterize ruminal acidosis result in the production and absorption of substances that can act locally in the dermis and epidermis of the digits (Nocek 1997, Greenough 2007). Some elements generated in ruminal acidosis are indicated as the cause of laminitis such as histamine, lactic acid and endotoxins. Isolated or together, they would cause vascular lesions and degradation of the suspensory apparatus of the third phalanx within the digit (Singh et al. 1994, Nocek 1997, Concha et al. 2014). The period between the triggering factor and the appearance of the clinical signs of laminitis is called the prodromal phase, or development stage, and is studied in the equine species (Martins Filho et al. 2008). In the bovine species, the prodromal period has a varied duration (Thoefner et al. 2004, Danscher et al. 2009) because the experimental induction protocols used do not always result in specific clinical signs of clinical laminitis, such as lameness and digital sensitivity (Boosman et al. 1991a, Singh et al. 1994, Momcilovic et al. 2000). The knowledge of the changes in bovine laminitis in the development phase is important, since in addition to allowing early diagnosis, before more evident signs such as lameness, allows the adoption of more effective therapeutic measures, as occurs in the equine species, including the use of anti-inflammatory and cryotherapy (Van Eps 2010).

An important and unexplored aspect of the studies on rumen acidosis and laminitis are breed differences, with the exception of studies on experimental induction of acute acidosis (Ortolani et al. 2010). Zebu and their crosses account for a large part of the national herd, and laminitis is cited as a health problem in these animals, both for milk aptitude (Mendes et al. 2013) as for meat production (Oliveira & Millen 2014). It is known that zebu and taurine can respond differently to experimental induction of ruminal acidosis (Ortolani et al. 2010) and that they have microscopic differences in the structure of the hooves (Mendonça et al. 2003, Rabelo et al. 2015). It is assumed that these differences could also be reflected in laminitis.

As a method of study of laminitis, its induction allows the control of different variables that influence the appearance of the lesions as well as the evaluation of different aspects of the etiopathogeny (Thoefner et al. 2004, Danscher et al. 2010, Concha et al. 2014). One of the ways to try to induce laminitis is by inducing ruminal acidosis. Some protocols were not effective, others were, but failed to be replicated in other studies (Thoefner et al. 2004, Greenough 2007). A protocol that has shown more consistent results is the induction of ruminal acidosis with the use of oligofructose (Thoefner et al. 2004, Danscher et al. 2009). Oligofructose is a fructose polymer with up to ten sugar subunits and is present, among other plants, in grasses of temperate climate (Thoefner et al. 2004). In the consulted literature (Thoefner et al. 2004, Danscher et al. 2009, Concha et al. 2014), data about experimental induction of laminitis in zebu cattle, or even crosses (*Bos taurus x Bos indicus*), were not found. A study option that could help to clarify the disease at the developmental stage would be in weaned crossbred calves. One of the advantages of the study in young animals is the ease of manipulation and clinical follow-up.

The aim of the present study was to evaluate the protocol for the induction of ruminal acidosis and laminitis by employing oligofructose and to study its effects on the laminar corium in the prodromal stage of the disease using one year old crossbred bovine as an experimental model.

MATERIALS AND METHODS

The project was evaluated and approved by the Ethics Committee on the Use of Animals of the UFG (CEUA/UFG), having received protocol number 26/2013. Six crossbreed calves were used (*Bos taurus* x *Bos indicus*) with an approximate age of 12 months and an average weight of 175±22.6 kg. Three months before the start of the study, the animals were submitted to ruminostomy with implantation of ruminal cannula with 8.89cm of internal diameter (KEHL®, São Carlos, Brazil). The calves were kept in pastures of *Brachiaria decumbens* and were supplemented with Tifton 85 grass hay. In the composition of the diet, there was a mineral supplement and water supplied at will.

The crossbreed calves were divided in Group I (GI), with three animals that received oligofructose (Oligofrutose®, Viafarma®, Sao Paulo, Brazil) by intraruminal way at 13g/kg and Group II (GII), with three animals receiving oligofructose at 17g/kg in the same way. The protocol and doses adopted for administration of oligofructose were based on the literature consulted (Thoefner et al. 2004). The study, for both Group I and Group II, was divided into three phases. In stage I, during three days the calves received 10% of the calculated dose of

oligofructose per day, divided in two moments with 5% each, totaling 30%. In this period, according to the literature, clinical signs were not expected (Thoefner et al. 2004, Danscher et al. 2009), therefore, doses were considered for adaptation of the ruminal microbiota (Thoefner et al. 2004). On the fourth day, beginning of phase II, the calves received the remaining 70% at once, being considered the zero mark for the beginning of clinical alterations (Thoefner et al. 2004, Danscher et al. 2009). In both phase I and phase II oligofructose was diluted at 80% concentration in warm water. Phase III began 30 hours after phase II and consisted of harvesting hoof fragments for histological examination.

The calves were evaluated before starting phase I and during 28 hours from the beginning of phase II, when they received the oligofructose overload, 70% of the dose. Prior to phase I, physical examination and ruminal fluid were performed. The evaluations during phase II occurred every four hours, the first evaluation being titled T0, composed of general physical examination, gait evaluation and digital sensitivity, and laboratory tests, including examination of the ruminal fluid, evaluation of the globular volume and hemogasometry. In all samples, ruminal fluid samples were obtained directly from the rumen after opening of the ruminal cannula, when organoleptic and pH characteristics were evaluated (Dirksen et al. 1993).

Then, lameness and digital sensitivity analysis were performed according to methodologies cited in the literature (Sprecher et al. 1997, Thoefner et al. 2004). In the evaluation of the locomotion, calves were analyzed as they walked in a straight line on concrete floor. The evaluator was positioned laterally on the course where he assigned a score according to the severity of lameness that the animal could present (Table 1). For digital sensitivity assessment, the thoracic limb was lifted by an auxiliary and the examiner pressed the soles of both digits at different points using a hoof tester. If there was no reaction, score 1 was assigned. A discrete reaction was attributed score 2 and a marked reaction was assigned score 3 (Thoefner et al. 2004). Both the locomotion score and the digital sensitivity score were always evaluated by the same examiner. The samples destined to evaluate the globular volume were of venous blood collected in tube containing ethylenediaminetetracetic acid (EDTA). After homogenization and centrifugation, the globular volume was measured in a measuring table. The hemogasometry was done with venous blood samples collected in heparinized syringe and evaluated in bench hemogasometer (COBAS B 121®, Roche Diagnóstica, São Paulo/SP, Brasil).

After 28 hours of the start of phase II, the calves received supportive treatment with ruminal content removal and intravenous fluid therapy with lactated Ringer's solution (Lactated Ringer's, Equiplex, Aparecida de Goiânia/GO, Brazil) and sodium bicarbonate solution to 6% (Sodium Bicarbonate Solution 6%®, Prado S.A. Labboratory, Curitiba/PR, Brazil) for the treatment of metabolic acidosis. The amount of bicarbonate solution administered was calculated according to the bicarbonate deficit, a parameter evaluated in hemogasometry. After two hours with this supportive treatment phase II was closed and phase III was started, in which the animals were sedated and submitted to hoof biopsy for histological evaluation. In order to collect the samples, the bovine were sedated with 2% xylazine hydrochloride (Anasedan®, Ceva Brasil, Paulínia/SP, Brazil) at a dose of 0.2mg/kg and placed in dorsal decubitus position. To collect samples, the lateral digit of the right pelvic limb and the medial digit of the right thoracic limb were selected. The distal extremities of the locomotor limbs evaluated were initially washed with soap and water. Then, both the coronary band and the hoof wall regions were surgically prepared with topical iodopovidone and alcohol. The anesthetic block consisted of the infusion of 10mL of 2% lidocaine hydrochloride in the dorsal digital vein (Lidovet®, Bravet, Engenho Novo/RJ, Brazil).

Fragments were collected from the coronary region and the abaxial wall. For biopsy of the coronary region, a site was selected, approximately 3cm lateral to the dorsal margin of the hoof, dorsal transition region between axial to abaxial walls. Hair clipping was performed near the withdrawal site as well as application of 3mL of 2% lidocaine hydrochloride in the subcutaneous tissue around the biopsy site. Using a scalpel, forceps and scissors, a rectangular fragment of approximately 10x5mm was removed. For the biopsy of the wall region, the horn case was gradually worn with a grinder, and the wall was often complied with forceps as well as the coloring of the wall. When it became soft, by sinking lightly under pressure, the biopsy was performed by initially delimiting the scalpel fragment and then withdrawing it as a use of the Falcão-Faleiros' lamellotome (Mendes 2015).

The process began with the delimitation of three sides of a rectangle, using a scalpel blade that was introduced until reaching the phalanx. On the fourth side, not sectioned, the Falcão-Faleiros' lamelotome (Mendes 2015) was inserted perpendicularly until it reached the bone, and then directed towards the opposite cut. In this way the fragment was removed with all layers of the dermis, transition between dermis and epidermis, living layer of the epidermis and part of the worn horn layer. After removal of the fragments, iron perchloride (Friezol Estankasangue®, Pinus, Jundiaí/SP, Brazil) was applied, powdered oxytetracycline hydrochloride was sprinkled on the wounds (Terramicina Pó, Pfizer), and orthopedic cotton and bandages were applied around the hoof. The dressings continued to be changed every two days until complete healing.

The samples collected were fixed in 10% buffered formalin, routinely processed and stained by hematoxylin and eosin (HE) and periodic acid from Schiff (PAS). A same evaluator performed the histological analysis blind using a common optical microscope in the 10x objective. In HE staining, they were evaluated in the dermis, hyperemia, hemorrhage, edema and inflammatory infiltrate (Thoefner et al. 2005, Mendes et al. 2013). For these parameters, the scores 0 = absent, 1 = rare, 2 = discrete, 3 = moderate, 4 = accentuated

Table 1. Lameness score in cattle

Score	Name	Description
1	Normal	Straight back when standing in quadrupedal position and walking. Normal step.
2	Mild lameness	Straight back quadrupedal and arched when walking. Normal step.
3	Moderate lameness	Arched back when standing and walking. Shortened step of one or more members.
4	Evident lameness	Arched back when standing and walking. Locomotion changed with one step at a time or avoiding the support of a limb.
5	Severe lameness	In addition to previous signs, the calves are reluctant or have difficulty supporting one or more limbs even when standing.

Adapted from Sprecher et al. (1997).

were assigned (Mendes et al. 2013). In the epidermis cell necrosis and morphological changes were evaluated in the cell nuclei. For cell necrosis the same scores were considered as those used in the evaluation of the dermis. For the morphological alteration score 1 = approximately 50% of cells with oval nucleus perpendicular to the basement membrane and 50% of cells with round nucleus were attributed; 2 = predominance of epidermal cells with round nucleus; 3 = predominance of cells with elongated and flattened nucleus or absence of nucleus. In the PAS-stained samples, areas of irregularities and detachment of the basement membrane represented by separation between basal cells and basement membrane (Thoefner et al. 2005, Mendes et al. 2013). Scores were similarly assessed in the evaluation of the dermis, with values from 0 to 4 (Mendes et al. 2013).

For each parameter, the average of all samples of each calf was considered. In all evaluations, physical, laboratory and histological exams, the data were evaluated by descriptive statistics (Sampaio 2010).

RESULTS

Heart rate and rectal temperature did not show significant changes in none of the groups. Respiratory rate increased in both groups eight hours after induction, but decreased gradually in the following moments. Ruminal motricity was reduced in both groups reaching close to zero in the Group GII 20 hours after induction (Table 2). During the 28 hours of clinical evaluation, the calves of both groups did not present lameness or digital sensitivity. Despite this, slower gait associated with apathy was observed in some animals.

The ruminal content presented changes in all evaluated parameters (Table 3). The odor and color of the contents have changed. At first, the color was olive green and the aromatic odor. During the evaluations, the odor became acid and the coloration became milky or yellowish. The accumulation of fluid in the rumen was more pronounced in GII. In the GI the average minimum ruminal pH was 5.32 and in GII the average minimum ruminal pH was 4.6.Hemogasometry showed a reduction in blood pH, pCO2 and bicarbonate values in both groups along evaluation (Table 4). In both groups, there was a gradual increase in the values of globular volume (Table 4). It was observed that the calves of the Group GII already started the first evaluation, beginning of phase II, with values indicative of metabolic acidosis.

Regarding the biopsy of the hoof, the technique used was adequate and allowed to obtain viable samples for histological evaluations. At the collection sites there was a slight bleeding,

Table 2. Mean and standard deviation of heart rate in beats per minute (HR), respiratory rate in motions per minute (RP), ruminal movements in five minutes (MR) and rectal temperature in degrees Celsius (T°C) for crossbred calves (*Bos taurus* x *Bos indicus*) receiving 13g/kg oligofructose (GI) and 17g/kg oligofructose (GII)

		Phase I	Phase II							
			T0	T4	T8	T12	T16	T20	T24	T28
FC	GI	45.00 ± 3.00	51.00 ± 6.08	60.00 ± 13.86	58.00 ± 7.00	54.00 ± 8.89	52.00 ± 5.00	51.00 ± 9.54	51.67 ± 3.21	49.00 ± 4.58
	GII	43.00 ± 2.65	42.67 ± 7.57	51.67 ± 11.68	53.00 ± 4.36	46.00 ± 3.46	50.67 ± 13.61	60.67 ± 1.15	62.00 ± 25.53	56.00 ± 7.21
FR	GI	24.33 ± 4.73	14.33 ± 4.73	23.33 ± 7.02	30.00 ± 3.61	18.67 ± 3.51	14.67 ± 3.06	18.00 ± 5.29	11.33 ± 4.16	17.00 ± 2.65
	GII	25.30 ± 4.16	10.33 ± 2.31	23.00 ± 10.44	26.67 ± 5.77	18.00 ± 2.65	12.33 ± 5.77	11.67 ± 3.21	13.33 ± 3.06	16.33 ± 7.51
MR	GI	4.70 ± 1.53	5.33 ± 0.58	4.33 ± 0.58	3.67 ± 1.53	4.00 ± 1.73	5.00 ± 1.00	4.00 ± 1.73	4.50 ± 0.71	3.00 ± 1.73
	GII	4.00 ± 1.73	5.33 ± 0.58	2.00 ± 1.00	4.00 ± 0.00	3.33 ± 3.06	1.33 ± 0.58	0.33 ± 0.58	2.33 ± 1.15	1.33 ± 2.31
T°C	GI	38.30 ± 0.77	36.77 ± 1.04	38.37 ± 0.15	39.17 ± 0.5	38.83 ± 0.51	37.53 ± 0.7	36.97 ± 0.95	36.77 ± 1.63	38.43 ± 0.38
	GII	38.20 ± 0.26	36.47 ± 0.51	37.67 ± 0.87	38.30 ± 0.14	38.20 ± 0.2	36.80 ± 1.82	36.43 ± 0.81	35.77 ± 1.10	37.43 ± 0.51

Table 3. Means and standard deviation of ruminal pH for crossbred calves (*Bos taurus* x *Bos indicus*) receiving oligofructose (GI) 13g/kg and oligofructose (GII)

	Phase I	Phase II							
		T0	T4	T8	T12	T16	T20	T24	T28
GI	6.71 ± 0.29	6.89 ± 0.11	5.72 ± 0.49	5.32 ± 0.57	5.38 ± 1.43	5.57 ± 1.47	5.97 ± 1.41	5.88 ± 1.95	6.78 ± 0.68
GII	6.92 ± 0.02	6.69 ± 0.10	5.50 ± 0.06	5.14 ± 0.43	4.83 ± 0.36	4.70 ± 0.35	4.6 ± 0.27	5.24 ± 1.31	5.62 ± 0.86

Table 4. Means and standard deviation of blood pH values, CO2 pressure in mmHg (pCO2), bicarbonate in mmol/l (HCO3) and globular volume in% (VG) for crossbred calves (*Bos taurus* x *Bos indicus*) receiving 13g/kg oligofructose (GI) and 17g/kg oligofructose (GII)

		T0	T4	T8	T12	T16	T20	T24
pH	GI	7.38 ± 0.12	7.39 ± 0.10	7.35 ± 0.14	7.32 ± 0.19	7.26 ± 0.16	7.23 ± 0.21	7.24 ± 0.22
	GII	7.29 ± 0.08	7.32 ± 0.07	7.28 ± 0.05	7.21 ± 0.04	7.12 ± 0.03	7.08 ± 0.01	7.07 ± 0.03
PCO2	GI	41.00 ± 5.01	41.53 ± 6.27	36.57 ± 9.16	36.00 ± 7.39	37.80 ± 16.84	34.90 ± 10.7	35.23 ± 10.65
	GII	29.03 ± 9.86	28.63 ± 7.07	36.57 ± 9.83	21.50 ± 2.77	19.57 ± 3.31	18.33 ± 3.48	20.07 ± 1.68
HCO3	GI	24.83 ± 8.54	25.80 ± 8.87	21.63 ± 10.45	20.10 ± 10.14	19.23 ± 12.16	16.53 ± 9.65	17.43 ± 10.45
	GII	14.50 ± 7.81	14.90 ± 6.39	13.80 ± 6.46	8.47 ± 1.50	6.23 ± 0.65	5.33 ± 0.90	5.73 ± 0.76
VG	GI	30.33 ± 2.31	29.67 ± 2.08	30.00 ± 1.73	33.33 ± 2.89	33.33 ± 4.16	35.67 ± 5.51	36.67 ± 7.02
	GII	32.67 ± 3.51	33.00 ± 2.00	33.33 ± 3.06	33.00 ± 2.65	40.33 ± 5.69	45.00 ± 4.25	46.00 ± 3.75

which stoped by compression at the site with gauze and using haemostatic agent. On the days following the biopsy the animals did not present lameness.

In the evaluation of the dermis, alterations such as edema and inflammatory infiltrate were detected in both groups (Table 5). The edema was predominantly perivascular (Fig.1). The inflammatory infiltrate, besides also perivascular, occurred especially close to the dermis-epidermal junction (Fig.2). Hemorrhage and hyperemia were observed few times in both groups. In the epidermis, morphological changes were observed in the nuclei of the basal epidermal cells. Cellular necrosis was observed only in GII. Irregularities and detachment of the basement membrane were observed in all groups (Fig.3).

DISCUSSION

In the 28-hour phase II evaluation period, no specific signs of laminitis were observed such as lameness or digital sensitivity. There were clinical signs of ruminal acidosis and microscopic changes in the digits indicative of laminitis, characterizing this period as the prodromal phase of the disease. Other studies in which oligofructose was used indicated that the first signs of lameness started 39 hours after induction (Thoefner et al. 2004) and within 30 to 48 hours after carbohydrate administration (Danscher et al. 2009), compatible with the present study where lameness did not occur on the first day after the carbohydrate overload.

Regarding the absence of lameness on the days following the induction, there was probably a relation with the removal of all altered ruminal contents 28 hours after induction. With the emptying of the rumen, the absorption of substances potentially harmful to the digital thorium ceased. In the other studies in which the animals did not have the ruminal content removed, the permanence of the intake may have benefited the longer absorption of these substances, favoring the lameness associated with laminitis (Thoefner et al. 2004, Danscher et al. 2009). Although no lameness or digital sensitivity was observed in the evaluation period, some calves presented a slower gait associated with apathy. This effect is a result of the reduced state of consciousness that can occur in metabolic acidosis due to D-lactate observed in ruminal acidosis (Radostits et al. 2007). Probably the neurological signs associated with D-lactate acidosis are due to interference in the energetic metabolism of the brain tissue. D-lactate would impair the metabolism of pyruvate and L-lactate (Ling et al. 2012, Lorenz & Gentile 2014), important energetic substrates in neurons (Adeva-Andany et al. 2014).

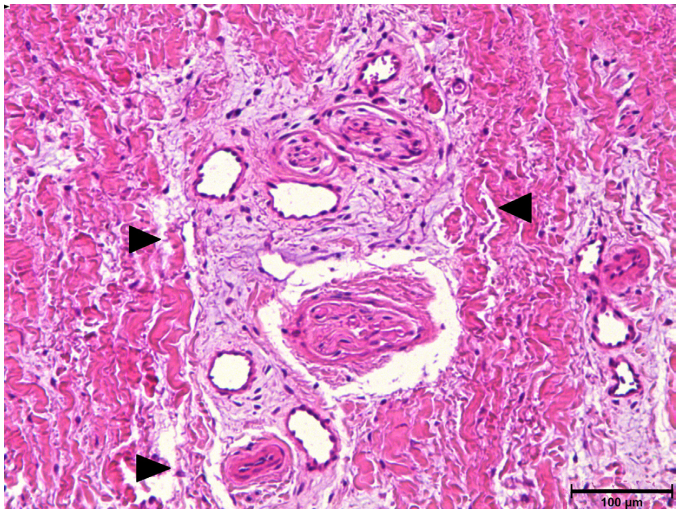

Fig.1. Fragment of the wall region of crossbred calf from group GII showing perivascular edema (arrowhead). HE, obj.10x.

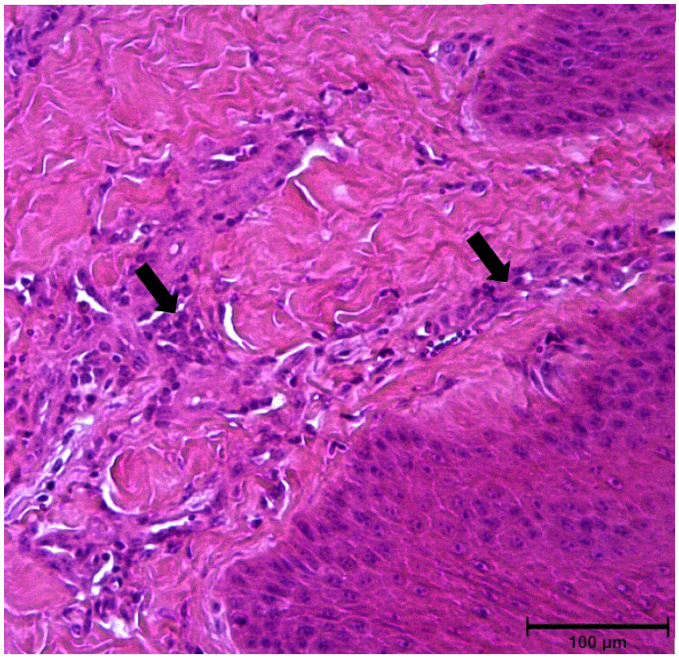

Fig.2. Fragment of the coronary region region of crossbred calf from group GI showing subepidermal inflammatory infiltrate (arrows). HE, obj.10x.

Table 5. Histological changes in samples of crown and dorsal hull wall of crossbred (*Bos taurus* x *Bos indicus*) calves receiving 13g/kg oligofructose (GI) and 17g/kg oligofructose (GII)

Histological alteration	Groups	
	GI	GII
Hyperemia	0.14 ±0.38	0.17 ±0.39
hemorrhage	0	0.33 ±0.78
Edema	1.86 ±0.38	1.75 ±0.75
Inflammatory infiltrate	1.14 ±1.07	1.25 ±0.96
Death of basal epidermal cells	0	0.5 ±0.67
Morphological alteration of basal epidermal cells	1.14±0.39	2.08 ±0.9
Morphological alteration of basement membrane	0.57 ±1.13	1.17 ±0.94

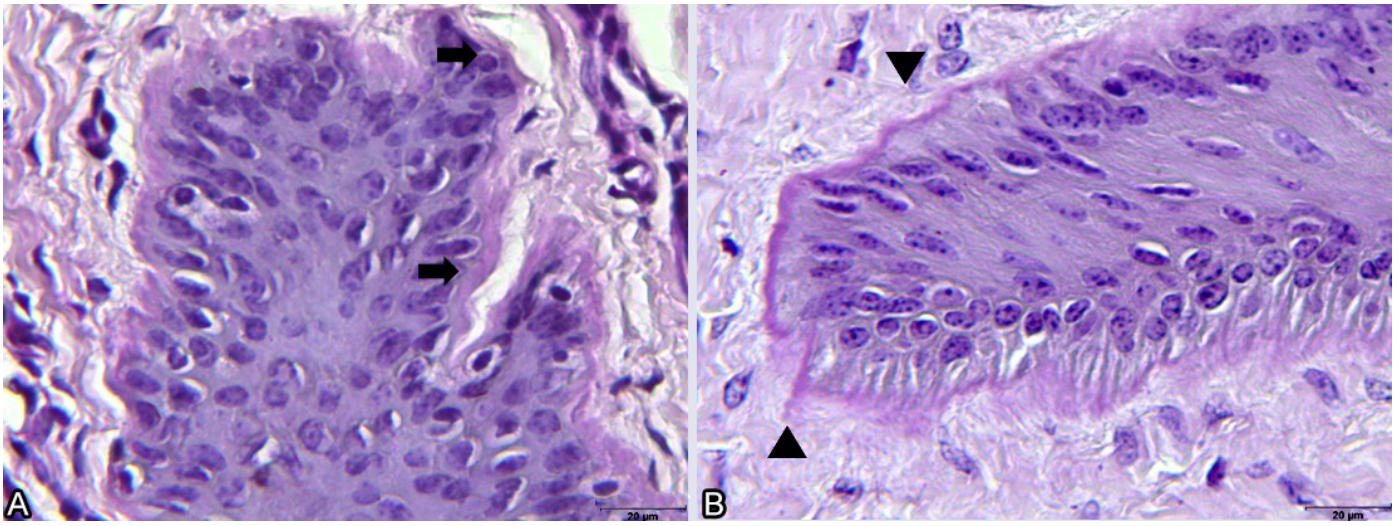

Fig.3. Photomicrographs of bovine digit fragments. (**A**) Fragment of the coronary region of calf from group GII showing a discrete multifocal detachment of the basement membrane (arrow). (**B**) Fragment of wall of calf from group GI showing irregularities of the basal membrane (arrowhead). PAS, obj.40x.

As expected (Thoefner et al. 2004, Danscher et al. 2009), after the administration of oligofructose overload, there was a marked reduction in ruminal pH, reaching levels compatible with acute acidosis in Group II and subacute acidosis in Group I. Lower values for ruminal pH in relation to the present study were reported in other studies that used oligofructose to induce ruminal acidosis. When the carbohydrate dose was 13g/kg, pH 5.0 (Thoefner et al. 2004) and 4.2 (Concha et al. 2014) were observed, while at the 17g/kg dose, pH 4.5 (Thoefner et al. 2004) and 4.3 (Danscher et al. 2009) were registered. It is believed that the highest values for pH observed were related to the average mean weight of the animals, since in other studies the mean weight of bovine was 408 kg (Thoefner et al. 2004), 375 kg (Danscher et al. 2009), and between 280 and 310kg (Concha et al. 2014). The influence of weight on the effects of ruminal acidosis was already observed in another study using oligofructose (Danscher et al. 2009) and a protocol for the induction of acidosis using sucrose (Ortolani 1995).

In addition to the reduced ruminal pH, another change observed was the decrease in ruminal motility, almost absent in GII. The reduction of ruminal motility is an expected effect of ruminal acidosis (Nagaraja & Lechtenberg 2007, Ortolani et al. 2010), even when this is established by administration of oligofructose (Thoefner et al. 2004, Danscher et al. 2009).

The group receiving the highest dose of oligofructose, 17g/kg, developed metabolic acidosis with only 30% of the total dose. It has already been demonstrated that the addition of fructose in the diet considerably increases the production of lactic acid in the rumen, when compared to the starch (Golder et al. 2012). In addition to contributing to the reduction of ruminal pH, part of this lactate is absorbed causing metabolic acidosis (Thoefner et al. 2004, Danscher et al. 2009, Concha et al. 2014). In another protocol using oligofructose, a reduction in baseline excess was observed during the three days in which the calves received 30% of the dose. However, values compatible with metabolic acidosis were not observed (Thoefner et al. 2004).

Although the calves in GII presented metabolic acidosis at the time they received most of the oligofructose dose, ruminal pH was within the reference value. It is inferred that the fermentative disorder was regularized faster than the systemic one. The same behavior was observed after administration of the remaining 70% oligofructose, elevation of ruminal pH and persistence of metabolic acidosis at the end of the evaluation period. The different responses among the studies may reflect breed aspects of the studied animals - crossbred-, since taurine and zebu may react differently to metabolic acidosis after ruminal acidosis (Ortolani et al. 2010).

The reduction of pCO2, observed in both groups, favored elevation of the blood pH to the physiological limits, being considered a compensatory mechanism (Dirksen et al. 1993, Reece 2004). Regarding respiratory frequency and pCO2, it was observed that the highest value of RF occurred eight hours after oligofructose overload, whereas the lowest values of pCO2 occurred only 20 hours later. It is believed, in this case, that the increase in respiratory rate was related to the ambient temperature. The maximum value occurred at 2:00 p.m., a warmer period on the day. The reduction of pCO_2, with minimum values 20 hours after oligofructose overload, indicated that the respiratory mechanism acted by increasing the range of respiratory movements and not necessarily by increased respiratory rate. Respiratory rate values below the reference range (Dirksen et al. 1993) were observed at various times in both groups. Decreasing values of respiratory rate over 24 hours were also observed in other research on the induction of ruminal acidosis in bovine (Ortolani et al. 2010). It is possible that this reduction in respiratory rate is due to the depression of the respiratory center due to the severe acidosis installed (Huber 1976).

In the crossbred calves of the present study, we opted for the treatment of support at a predetermined time. The time of treatment in animals submitted to experimental induction of ruminal acidosis may be based on predetermined moments or changes in hemogasometry parameters (Momcilovic et al. 2000, Thoefner et al. 2004, Danscher et al. 2009). The emptying of

the ruminal contents ceased the absorption of substances produced in greater quantity in ruminal acidosis like lactic acid, endotoxins and histamine, that could be harmful to the organism. In addition to the use of intravenous fluid therapy containing bicarbonate to correct metabolic acidosis (Radostits et al. 2007), deterioration of the clinical picture and the appearance of possible complications such as death, as reported by other authors (Thoefner et al. 2004, Danscher et al. 2009). On subsequent days, calves resumed to normal appetite and behavior, confirming the efficacy of the supportive treatment.

Regarding the hoof biopsy, the integrity of the samples obtained confirmed the efficacy of the technique used to obtain the clinical specimens (Mendes 2015). The hoof biopsy technique as performed here allowed the evaluation of changes in all layers without the need for euthanasia of the animals, which has already been done in studies on equine laminitis (Visser & Pollitt 2011). The circulatory and morphological alterations observed as in the digits are compatible with the inflammatory process triggered by the acidosis caused by the carbohydrate. Similar findings have been reported in experimental induction protocols with oligofructose (Thoefner et al. 2005), endotoxins (Boosman et al. 1991a, Singh et al. 1994) and cases of natural occurrence (Boosman et al. 1991b, Mendes et al. 2013). The predominant monocytic inflammatory infiltrate has been described in other studies (Boosman et al. 1991a, Thoefner et al. 2005, Danscher et al. 2010), which is a common finding in bovine digits after administration of oligofructose.

The histological changes scores indicated, in general, intensities from rare to discrete. In one of the studies that also performed histological evaluation of bovine receiving oligofructose (Thoefner et al. 2005), the changes were recorded only in terms of present or absent, without indication of intensity, which makes it difficult to compare with the results presented. In another study (Danscher et al. 2009), the authors used scores and compared them with control animals, which did not receive oligofructose, and the samples were collected in the phases without and with clinical signs of the disease, 24 and 72 hours post induction, respectively. In the initial phase, the only parameter where the induced calves presented significant difference in relation to the control was presence of leukocytes. In contrast, at 72 hours after induction, with evident clinical signs, a difference was observed in almost all parameters evaluated, indicating that in the initial phase of oligofructose induced laminitis the findings are still very discrete, accentuating with the appearance of the clinical signs. In the present study, it is likely that if induction were continued and a new harvest was performed after the appearance of clinical signs, higher scores for histological lesions would be observed.

The histological findings in the cases of laminitis are similar among the different studies, even considering differences of breeds, age of the animals, protocol of induction used and time of harvest (Boosman et al. 1991b, Singh et al. 1994, Thoefner et al. 2005, Danscher et al. 2010, Mendes et al. 2013). In the present study, crossbred animals (*Bos taurus* x *Bos indicus*) were used. It is possible that the same protocol of induction in zebu animals reveals a different result considering histological differences of the hoof (Mendonça et al. 2003, Rabelo et al. 2015) and metabolic differences in relation to ruminal acidosis (Ortolani et al. 2010).

The histological changes observed in the digital dermis, circulatory and inflammatory alterations, may be related to some triggers of ruminal acidosis, including histamine (Nocek 1997), lactic acid (Concha et al. 2014) and lipopolysaccharides (Boosman et al. 1991a, Zebeli & Metzler-Zebeli 2012). These components, when deposited more markedly, are responsible for changes in the microcirculation of the digit, such as vasodilation, opening of arteriovenous anastomosis, edema and thrombosis, and interfere with the migration and function of defense cells such as neutrophils (Boosman et al. 1991a, Nocek 1997, Greenough 2007, Zebeli & Metzler-Zebeli 2012). The observed basement membrane changes, areas of irregularity and detachment of the epidermis were possibly due to the action of proteases acting on components of the basal membrane, such as collagen fibers. Basal membrane alterations with activation of metalloproteinases were observed 12 hours after treatment with oligofructose in horses (Visser & Pollitt 2011, Visser & Pollitt 2012), being one of the probable mechanisms for the occurrence of the observed lesions. The morphological changes observed in the epidermal basal cells are probably due to failure of oxygen and nutrients, secondary to circulatory changes in the digits (Greenough 2007). Similar changes were observed in spontaneous and experimental cases of laminitis (Boosman et al. 1991a, Danscher et al. 2009, Mendes et al. 2013).

In parallel to the ruminal and metabolic alterations, microscopic changes were observed in the digits compatible with laminitis. The lack of lameness or digital sensitivity characterizes the period of evaluation as the prodromal stage of the disease. The results are similar to other protocols for induction of ruminal acidosis and laminitis, but have been studied only in taurine. In the prevention of laminitis associated with rumen acidosis in crossbred bovine (*Bos taurus* x *Bos indicus*), animals should be monitored not only for lameness, but also for signs of indigestion such as abdominal distension, diarrhea and apathy.

CONCLUSIONS

The intrarruminal administration of oligofructose in crossbred calves of one year as an experimental model for induction of ruminal acidosis and acute laminitis results, in the first 30 hours, in ruminal and metabolic acidosis and histological lesions with low degree of intensity in the digits.

During this period clinical signs such as lameness or digit sensitivity were not observed, characterizing the prodromal phase of laminitis.

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