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Duodenal histology and carcass quality of feedlot cattle supplemented with calcium butyrate and *Bacillus subtilis*

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ABSTRACT. The experiment was carried out at the Comigo Technology Center, in Rio Verde, State of Goiás, Brazil, with the objective of evaluating the effects of supplementation with calcium butyrate, as a growth promoting agent for the duodenal mucosa and *Bacillus subtilis* as a probiotic performance enhancer in feedlot cattle. Calcium butyrate (5 and 10 g per animal per day) and *Bacillus* (10 g per animal per day) were added to a basal diet. There were used 85 Nelore bulls, with average weight of 315 ± 7 kg. The experiment lasted 118 days, including the adaptation period, until slaughter at 30 months of age. Diets were distributed in a completely randomized design with four treatments, where: T1 = control (basal diet); T2 = basal diet + 5 g calcium butyrate; T3 = basal diet + 10 g calcium butyrate and T4 = basal diet + 10 g calcium butyrate + 10 g probiotic with four replications and five to six animals per replication. It was used a forage: concentrate ratio of 30:70, the roughage used was the corn silage. Height and width measurements of intestinal villi were taken, and carcass and meat quality were evaluated. The supplementation of calcium butyrate and *Bacillus subtilis* positively influenced ($p < 0.05$) the carcass marbling level and calcium butyrate increased the villus height in the small intestine.

Keywords: duodenum, probiotic, ruminants.

Histologia duodenal e características de carcaça de bovinos de corte suplementados com butirato de cálcio e *Bacillus subtilis*

RESUMO. Conduziu-se o experimento no Centro Tecnológico Comigo, localizado no município de Rio Verde, Estado de Goiás, Brasil, com o objetivo de avaliar os efeitos da suplementação com butirato de cálcio, como agente promotor de crescimento da mucosa duodenal e com *Bacillus subtilis* como probiótico melhorador de desempenho em bovinos confinados. Adicionou-se butirato de cálcio (5 e 10 g animal^{-1} dia^{-1}) e *Bacillus* (10 g animal^{-1} dia^{-1}) em uma ração base. Utilizaram-se 85 bovinos machos inteiros, da raça Nelore, com peso vivo médio inicial de 315 ± 7 kg. O experimento transcorreu num período de 118 dias, incluindo o período de adaptação, até o abate aos 30 meses de idade. As dietas foram distribuídas em delineamento inteiramente casualizado sendo quatro tratamentos: T1 = Controle (ração basal); T2 = ração basal + 5 g de butirato de cálcio; T3 = ração basal + 10 g de butirato de cálcio e T4 = ração basal + 10 g de butirato de cálcio + 10 g de *bacillus*. Utilizou-se uma relação volumoso: concentrado de 30:70, o volumoso utilizado foi silagem de milho. Foram realizadas mensurações de altura e largura de vilosidade intestinal, avaliação de carcaça e qualidade de carne. A suplementação de butirato de cálcio e *bacillus subtilis* influenciou positivamente ($p < 0,05$) o grau marmoreio da carcaça e o butirato de cálcio aumentou a altura de vilosidade do intestino delgado.

Palavras-chave: duodeno, probiótico, ruminantes.

Introduction

Nutritional planning, using additives such as organic acids (prebiotics) and probiotics, is an increasingly researched tool in production systems. It is a strategy to improve feed conversion, weight gain or benefit health by preventing the incidence of metabolic diseases, contributing to better performance, particularly in the finishing phase, when feedlot animals are fed with concentrate diets. Some of these additives promote benefits that include reducing the incidence of acidosis and cocciodiosis (Oliveira, Zanine, & Santos, 2005).

Prebiotics are compounds that are not digested by enzymes, acids and salts produced by the gastrointestinal tract but are fermented by microbiota. They stimulate the growth of desirable microorganisms and provide nutrients to these

particular microorganisms, activating the metabolism of a bacterial group that are beneficial to the intestinal tract, acting closely related to probiotics, constituting the "feed" of probiotic bacteria (Névoa et al., 2013). The calcium butyrate has been used to increase animal performance by increasing nutrient digestibility, stimulating the secretion of digestive enzymes, modification of the intestinal microbiota and the improvement of epithelial integrity and protection system (Guilloteau et al, 2010).

Probiotics have a beneficial influence on the microbiota by means of antagonistic and immunological effects or competition with pathogens, resulting in an increased resistance against these. These additives work essentially in competition or inhibition of undesirable microorganisms. Furthermore, they may promote the development of fiber fermenting microorganisms, improving fermentation patterns and the immune system (Massaro Junior et al., 2013).

In order to know the effect of prebiotics associated or not with probiotics in feedlot animals, this study aimed to evaluate the effect of calcium butyrate at different doses and associated with *Bacillus subtilis* on the development of duodenal mucosa, and its effects on carcass of Nellore finished in feedlot.

Material and methods

Animal, Housing and Feeding Management

The experiment was conducted in the Livestock Section at the Comigo Technology Center, located in the Southwestern Goiás State, Central Western Brazil, in Rio Verde, State of Goiás.

The experiment lasted 118 days and there were used 85 Nelore bulls with average weight of 315 ± 7 kg body weight (BW). Animals were vaccinated and dewormed before the beginning of the trial, as well as weighed and separated into uniform groups, with at least five animals in each one, to minimize competition. They were then housed in collective pens of 10.0 meters long and 7.70 meters wide, with feeder and drinker in each stall. Animals were also adapted for a period of 28 days, in which the concentrate was gradually included in diet.

After the adaptation period, animals received a basal diet, formulated according to the requirements of the animal category. The ingredients of the feed were sorghum, soybean hull, soybean meal, urea, salt, molasses, vitamin and mineral premix. The intake was calculated based on 2.5% body weight using a 30:70 forage: concentrate ratio. Diets were isoproteic (180.0 g kg^{-1}) and isocaloric (800.0 g kg^{-1}), formulated containing the additives Butipeal® (calcium butyrate) and Clostat® (*B. subtilis*), and were supplied in two meals per day (9:00 a.m. and 15:30 p.m.). The roughage used was corn silage. Animals were slaughtered at 30 months of age.

The treatments were: T1 = control (basal diet); T2= Basal diet + 5 g calcium butyrate; T3 = Basal diet + 10 g calcium butyrate and T4 = Basal diet + 10 g calcium butyrate + 10 g *B. subtilis*, with four replication and five animals per replicate.

Laboratory analysis

Laboratory analyses were performed at the Animal Nutrition Laboratory of the Animal Science Departament of the Goiano Federal Institute - Rio Verde campus.

Intestinal villi (Duodenal Histology)

At the end of the feedlot, animals were sent to commercial slaughterhouse and slaughtered according to official rules and procedures Ministério da Agricultura (1997).

To determine the histological parameters of the intestinal mucosa in the middle portion of the duodenum, there were collected samples from only 49 animals representing two animals per pen. After slaughtered, it was collected samples of 5 cm length of the small intestine mucosa (duodenum) at 50 cm posterior to the pylorus. Samples were previously washed with distilled water, fixed in formalin (10%) for 48 hours and transferred to solutions with increasing concentrations of alcohol (70, 80 and 90%), followed by two batteries of absolute ethyl alcohol (for 1 hour each), thus concluding the dehydration process. At diafanization, to make the tissue semi-transparent, the alcohol present in the tissues was replaced by xylol and the samples were kept in alcohol: xylol (1:1) for one hour and then immersed in two sequences of xylol for 30 minutes each. During paraffin embedding, xylol was replaced with paraffin in an oven at 60°C.

Once embedded, the tissues were placed in plastic cups at room temperature, obtaining the intestinal mucosa tissue blocks. Blocks were sectioned with a microtome to a thickness of 6 microns. The strands were transferred to a thermostatic equipment at 40°C and then stretched in an aqueous medium to make the slides. For staining, sections were deparaffinized in an oven at 60°C for 30 minutes and placed in two batteries of xylol (two minutes each), then immersed in decreasing alcohol solutions of 90, 80 and 70%. Sections were then stained with aqueous hematoxylin for five minutes, dipped in

hydrochloric acid (HCl) and left in tap water for ten minutes. Subsequently, they were stained with eosin solution for 20 seconds and rehydrated again. A new dehydration was performed with increasing solutions of alcohol (70, 80 and 90%) for two minutes each, and two batteries of ethyl alcohol for three minutes each, starting the diaphanization process with two xylol batteries (for two minutes each).

Slides were mounted with a drop of balm on the slice and covered with cover slips. There were made two slides per animal and each sampled as equivalent to ten intestinal villi to evaluate high (AV) and wide villus (LV) (Jin, Reynolds, Redmer, Caton, & Crenshaw, 1994; Junqueira & Carneiro, 1995), by electron microscopy and an image analyzing system.

Carcass evaluation

The average slaughter weight was 457.75 kg at 118 days of feedlot for all treatments. The same was determined on a mechanical balance, after 12 hours of fasting immediately prior to shipment the animals to be slaughtered under federal inspection.

To evaluate the carcass characteristics, we used the methodology described by Müller (1987). The hot carcass weight was measured at the end of the slaughter, before the entrance to the cooling chamber. The cold carcass weight was measured after 24 hours of cooling in cooling chamber at 0 ± 1°C. The hot and cold carcass yields were obtained by dividing the hot or cold carcass weight by live weight of slaughter x 100.

The weight of primal cuts, hindquarters, forequarter and spare ribs, were obtained in the right half carcass, with percentage values expressed in relation to cold carcass weight. In the right half carcass, we measured the carcass length (measured with tape from the cranial board, in the middle portion of the first rib to the cranial edge of the pubic bone), leg length (from the pubic bone to the tibial-tarsal joint), side thickness (measured with compass, in which one end is fixed on the outer part of the inside and the other on the outer face of the leg), length and arm circumference (measured with tape from the olecranon tuberosity to the distal end of the humerus and its perimeter measured at the mid portion of the humerus, involving muscles that cover the region).

In the half left carcass, a cut between the 12nd and 13rd ribs exposed the Longissimus dorsi (MLD). The thickness of fat covering the MLD was measured at three points with a caliper, whose average provided the subcutaneous fat thickness.

On the exposed face of MLD, its outline was traced on vellum paper, and then scanned in AutoCAD® software, to obtain the loin eye area. On the exposed face of the MLD, subjective evaluations of color, texture and marbling were performed after exposure of the muscle to air for 30 minutes under constant brightness. In this muscle, were further evaluated the color and texture following a 1-5 point scale, in which: 1 = dark color and coarse texture and 5 = very bright red color and fine texture; and marbling, following a 18-point scale, distributed in the standings, corresponding to light traces, small, medium, moderate and abundant, according to Müller (1987). Still considering the methodology of this author, it was evaluated the fat cover thickness (EGS), far above the *Longissimus dorsi* muscle, exposed by a cross-section, performed on the left side of the carcass between the 11st and 12nd ribs at a point in the final third of the distance between the proximal and distal portion of that muscle.

Statistical analysis

The experimental design was completely randomized with four treatments including the control.

Data obtained from measurements of duodenal villi for height and width as well as carcass assessments were analyzed using the statistical software Statistical Amalysis System (SAS, 2000), having as a statistical model:

$$Yij = \mu + Ti + eij$$

where:

Y ij = is the observation of treatment i in repetition j, with i from 1 to 20; μ = is a constant inherent to any parcel; Ti = is the effect of treatment i from 1 to 4; eij = experimental error on the parcel i, j. It was considered significant $p \leq 0.05$ values for all evaluated parameters.

Results and discussion

Intestinal villi (Duodenal Histology)

The functional element of the small intestine is the mucosa, which can be characterized as a nutrient permeable layer and barrier against harmful compounds. The intact mucosa is the possibility of food digestion and nutrients absorption in physiological way (Robles-Huaynate et al., 2013). Competition between host and bacteria for nutrients and the formation of growth depressant metabolites in the intestine can have negative effects on the small intestine mucosa (Oliveira, Marques, Gravena, & Moraes, 2011). Therefore, changes in intestinal microflora can affect the development of the intestinal mucosa.

Table 1 lists the averages of measurements performed in the intestinal villi for height and width, between the different treatments.

Table 1. Effect of calcium butyrate and *Bacillus subtilis* on height and width of duodenal villus of feedlot Nellore cattle.

Parameters[1]	Treatments[2]			
	CON	5BUT	10BUT	10BUT+10CLOST
AV (μm)	2569.551[b]	2783.769[ab]	2829.996[a]	2700.297[a]
LV (μm)	1506.224[a]	1417.272[a]	1543.126[a]	1519.125[a]

Tukey's test at 5% of probability. [1]AV = villus height; LV = villus width. [2]CON = control; 5BUT = 5 g calcium butyrate; 10BUT = 10 g calcium butyrate; 10BUT + 10CLOST = 10 g calcium butyrate + 10 g *Bacillus subtilis*.

Table 1 shows differences ($p < 0.05$) in height of duodenal villus. The highest values were found for 10BUT, followed by 10BUT + 10CLOST and 5BUT. The lowest value was observed for control. This demonstrates the effectiveness, regardless of the additive level in changing one of the villus characteristics, directly related to absorptive capacity. There was no difference ($p > 0.05$) in villus width.

Butyric acid works directly on the pH of the medium where it is found. The stomach acid coating limits the action of the butyric acid on that site, allowing a stronger effect in the gut (Ribeiro, Gaspar, Pinho, Freire, & Falcão-e-Cunha, 2012). The butyric acid decreases the intestinal pH, hindering the adhesion of pathogenic bacteria to the epithelium, thereby increasing gut absorption capacity by generating an epithelium with less damage by bacterial multiplication. According to Pelicano et al. (2003), birds that have higher and wider villus may have a better absorption of nutrients. Therefore, alterations in intestinal villus obtained in this experiment may represent a higher absorptive capacity of animals that received the additives.

Probiotics are microbial supplements thar have maintenance function of intestinal villus, which are, in turn, important to nutrient absorption (Lan Verstegen, Tamminga, & Williams, 2005), directly affecting food digestibility. However, it is observed that there was no difference between treatments 10BUT and 10BUT + 10CLOST (Table 1), that is, the association of prebiotic with probiotic failed to differentiate from prebiotic use only, thus not justifying the combined use of these substances.

Figure 1 illustrates the increase in villus height (VA) caused by calcium butyrate supplementation in this experiment.

Carcass evaluation

Slaughter weight and finishing are considered the main factors to determine meat quality. Mean values for slaughter weight (PA), hot carcass weight (PCQ), yield (RND), forequarter weight (PD), sawcut hindquarters weight (PT), spare rib weight (PPA), cold carcass weight (CCW) and cold carcass pH (pHF) are presented in Table 2.

There was no effect ($p > 0.05$) of treatments on body weight at slaughter (PA) of evaluated animals and the mean value found between treatments was 479.03 kg, close to those observed by Pacheco et al. (2013), around 486 kg. Likewise Rigobelo, Machado and Cardozo (2014) verified no effects of different probiotics on weight gain, efficiency, conversion and carcass characteristics of cattle, similar to the observed in herein (Table 2).

There was no effect of the treatments ($p > 0.05$) on carcass parameters (Table 2). Values close to hot carcass weight (PCQ), Yield (RND) and cold carcass weight (PCF) were found by Vaz, Restle, Pádua, Fonseca, and Pacheco (2013) for male adult Nellore in feedlot. Comparin et al., (2013) also registered no effect of the additive *Lithothamnium calcareum* on animal performance.

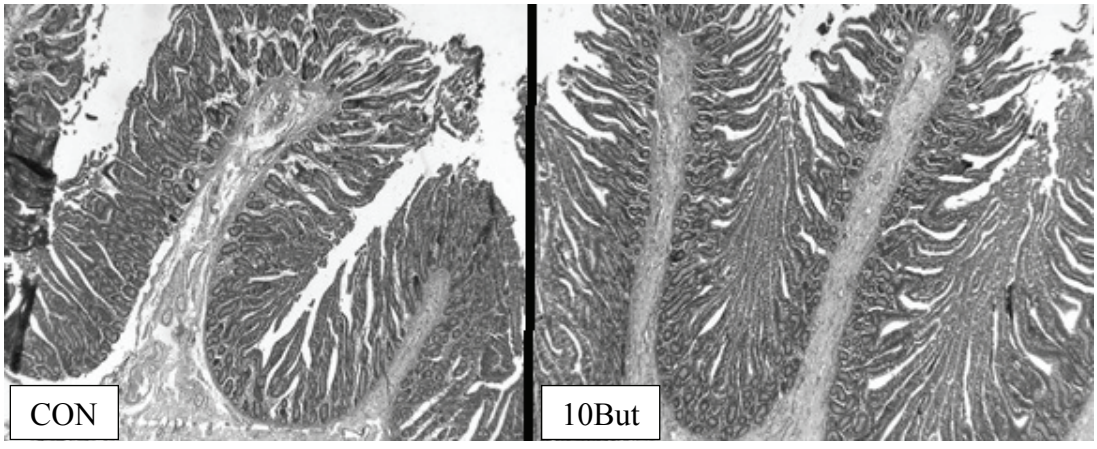

Figure 1. Effect of calcium butyrate on the height of duodenal villus of feedlot Nellore cattle. CON = control; 10BUT = 10 g calcium butyrate.

Table 2. Effect of the use of calcium butyrate and *B. subtilis* on the carcass evaluation of feedlot Nellore cattle.

Parameters[1]	Treatments[2]			
	CON	5BUT	10BUT	10BUT+10CLOST
PA (kg)	476.65	487.08	473.66	478.75
HCW (kg)	267.47	271.75	265.91	267.74
RND (%)	56.12	55.79	56.05	55.93
PD (kg)	106.27	108.90	105.63	106.48
PT (kg)	126.88	128.51	125.61	126.74
PPA (kg)	27.75	27.78	27.25	27.88
PCF (kg)	260.92	265.20	258.50	261.10
PHF	5.71	5.72	5.71	5.75

Tukey's test at 5% of probability. [1]PA = weight at slaughter; HCW = hot carcass weight; RND = Yield; PD = forequarter weight; PT = sawcut hindquarter weight; PPA = spare rib weight; PCF = cold carcass weight; PHF = cold carcass pH. [2]CON = control; 5BUT = 5 g calcium butyrate; 10BUT = 10 g calcium butyrate; 10BUT + 10CLOST = 10 g calcium butyrate + 10 g *B. subtilis*.

Moreover, body weight determines carcass yield, because as weight increases, there is higher deposition of muscle and fat, contributing to the increase in carcass weight in relation to slaughter weight (Missio et al., 2013).

In relation to the yield of carcass commercial cuts, its estimation is important to complement the evaluation of animal performance during its development (Cabral Neto et al., 2013). Carcass is mainly composed of the muscular portion, bones and fat, and the fat is the most variable of the three components with the highest influence on yield.

The cuts considered the most important of beef carcass in the Brazilian market are the special hindquarters (sawcut), forequarters with the first five ribs and the spareribs (ribs) (Cabral Neto et al., 2013). There was no effect ($p > 0.05$) of treatments on the weight of the forequarters (PD), hindquarters (PT) and spareribs (PPA). However, PD and PT, amounted, on average, to 38.9 and 59.14%, respectively, of the hot carcass weight. Hirai et al. (2014) reported values of 36.42 and 63.58%, for the forequarter and hindquarter yields, respectively.

There was no effect ($p < 0.05$) of the treatments on cold carcass weight (PCF) and on the cold carcass pH (PHF). In the period comprising the first 24 hours after slaughter, there is a strong impact of pH on the tenderness and color of meat, which begin the cooling process, resulting in negative or positive effects on the final product quality (Savell Mueller, & Baird 2005). Cooling losses may be due to factors, such as moisture loss and chemical reactions that occurs in the muscle tissue. Therefore, the lower this percentage, the greater the probability of proper storage and handling of the carcass. Cooling losses were of the order of 2.57%.

Control of pH is important because it is related to meat color and texture. Furthermore, the time required to reach the final pH varies depending on the animal species, cooling temperature and cooling rate and level of activity prior to slaughter (Li et al, 2006; Rodbotten, Kubberød, Lea, & Ueland, 2004). Lopes et al. (2012) obtained similar values for pH in feedlot Nellore carcasses.

Table 3 presents the effect of additives on the quality of meat from experimental animals.

There was no effect ($p > 0.05$) of treatments for conformation (CF), subcutaneous fat thickness (EGS), color (COR), texture (TEX) and rib eye area (AOL).

On the other hand, it was detected effect ($p < 0.05$) of treatments for marbling (MAR), and the highest value was observed for the treatment 10BUT + 10CLOST, followed by CON, 10BUT and 5BUT.

Table 3. Effect of calcium butyrate and *B. subtilis* on meat quality of feedlot Nelore cattle.

Parameters[1]	Treatments[2]			
	COM	5BUT	10BUT	10BUT+10CLOST
CF (score)	10.53	10.41	10.00	10.00
EGS (mm)	4.17	4.57	4.13	4.26
COR (score)	3.56	3.52	3.52	3.59
TEX (score)	3.58	3.40	3.52	3.57
MAR (score)	1.69^{ab}	1.16^{b}	1.58^{ab}	1.83^{a}
AOL (cm^2)	71.90	82.82	69.95	69.15

Tukey's test at 5% of probability. [1]CF = Conformation; EGS = subcutaneous fat thickness; COR = color; TEX = texture; MAR = Marbling; AOL = rib eye area. [2]CON = control; 5BUT = 5 g calcium butyrate; 10BUT = 10 g calcium butyrate; 10BUT + 10CLOST = 10 g calcium butyrate + 10 g *B. subtilis*.

Cattelam et al. (2013) observed similar values for conformation in feedlot steers without using additives in the diet; in our experiment, the use of additives did not influence this parameter.

The finishing degree of carcasses, evaluated by the subcutaneous fat thickness (EGS), Table 3, was considered appropriate, regardless of the treatment. The results obtained for fat thickness were higher than those found by Vaz et al. (2013), who evaluated animals finished with different weights. This subcutaneous fat difference may be due to the diet used and even the genetic group assessed.

According to Müller (1987) texture is evaluated by the granulating that the muscle surface features when cut, since it is composed of a group of muscle fibers. Similar values of TEX and COR were found by Hirai et al. (2014), who evaluated steers finished on pasture. Lower values for color were found, regardless of the treatments, with a mean value of 3.54. Lower values may indicate that animals were subjected to stress, which results in high loss of carcass cooling, however the value for cooling loss was only 2.57%.

Vaz & Restle (2005) worked with two groups of young bulls and registered a coloured mean value of 4.67. These same authors also reported that the texture and marbling were also similar between the two groups of steers.

Meat marbling is related to the deposition of intramuscular fat, which, in turn, affects the meat sensory characteristics, directly influencing the flavor (Faturi et al., 2002). In this sense, the treatment 10BUT + 10CLOST was more effective in producing a meat that tends to be tastier.

Some researches have suggested that the increase of concentrate in the diet improves the finishing quality (Costa et al., 2005) and the conformation (Vaz et al., 2005). Nevertheless, there was no difference ($p > 0.05$) between AOL, either for EGS.

Lopes et al. (2012) reported similar values for all characteristics of carcass quality and meat quality in Nellore steers finished in feedlots.

Conclusion

Supplementation with calcium butyrate influenced the intestinal villus height, by increasing villus height values, whereas the combination of calcium butyrate with *B. subtilis*, improved the meat marbling level.

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