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Metabolic profile of cattle receiving "Max Beef" whole grain diet. Perfil metabólico de bovinos recebendo dieta de grão inteiro "Max Beef".

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Abstract

The current study aimed to characterize the effects of diet based on whole corn grain on the dynamics of clinical parameters and biochemical metabolites of confined cattle. Twenty young mixed-breed cattle, with a mean weight of 332 kg, were used, divided into two experimental groups. The experimental group (G1; n=15), composed of animals fed only Max Beef Whole Grain diet and the control group (G2; n=5), composed of animals fed with sugarcane bagasse and the Max Beef Grain Whole grain diet, in the ratio 30:70. Blood samples were taken for analysis of different biomarkers of the energy, protein and enzymatic activity. The data were analyzed using the Statistical Analysis System program, and for all analyzes, the 5% probability level was adopted. The cattle in the G1 group showed characteristic clinical manifestations of ruminal acidosis. Inference from the G1 diet on some analyzed variables occurred, highlighted in the energy profile, without putting the animals' health at risk. However, for the use of this type of diet, careful food planning is recommended, especially during the animals' adaptation period.
Keywords: Ruminants. Feed. Biochemistry. Nutrition of ruminants. Feedlot cattle.

Resumo

Este estudo propôs caracterizar os efeitos da alimentação baseada na dieta do grão de milho inteiro na dinâmica de parâmetros clínicos e metabólitos bioquímicos de bovinos confinados. Foram utilizados 20 bovinos, sem raça definida, jovens, com peso médio de 332 kg, divididos em dois grupos experimentais. O grupo experimental (G1; n=15), composto por animais alimentado somente dieta Max Beef Grão Inteiro e o grupo controle (G2; n=5), composto por animais, alimentado com bagaço de cana e a dieta Max Beef Grão Inteiro, na proporção 30:70. Coletas de sangue foram realizadas para análises de diferentes biomarcadores do perfil energético, proteico e da atividade enzimática. Os dados foram analisados usando o programa Statistical Analysis System, e para todas as análises, foi adotado o nível de 5% de probabilidade. Os bovinos do grupo G1 apresentaram manifestações clínicas características de acidose ruminal Inferências da dieta do G1 sobre algumas variáveis analisadas ocorreram, em destaque no perfil energético, sem colocar em risco a saúde dos animais. Entretanto, para o emprego deste tipo dieta se recomenda um planejamento alimentar criterioso, principalmente no período de adaptação dos animais.
Palavras-chave: Ruminantes. Alimentação. Bioquímica. Nutrição de ruminantes. Confinamento.

Introduction

The intensification of production systems, through improvement in nutritional management, to maximize productivity (ANUALPEC, 2015), has led to the growth of the inclusion of increasing amounts of grains in the feeds of confined cattle, due to the significant national harvest, high costs of supplying fodder, and large areas for planting. In addition, this form of management is appropriate for operational reasons for feeding in large feedlots, the use of which has grown in recent years in the country (MARQUES et al., 2016). Taking into account that the majority of costs come from feed, strategies that can reduce these costs are increasingly recommended. In general, cereal grains represent the main source of energy in feeds for beef cattle finished in feedlots.

Diets with higher grain content provide faster weight gain, better feed conversion, carcasses with better finish and yield, and lower operating costs in confinement, which can make the activity more profitable (ARRIGONI et al., 2013). In this context, diets with whole corn kernels have been used, characterized by the great practicality of providing animals with only two ingredients: whole corn (without crushing) and concentrated protein, vitamin, and mineral pellets (ARRIGONI et al., 2013). Among the characteristics of this diet are rapid weight gain, high feed efficiency, decrease in the time of finishing for slaughter, and lower labor cost (BULLE et al., 2002).

It is known that during fattening, cattle often reach close to physiological limits and may develop organic alterations, which are more pronounced in the organs of the digestive system (SOARES et al., 2006; DOKOVIC et al., 2010), since cattle receiving diets rich in concentrate present considerably different digestive kinetics from those observed in diets rich in bulky feeds (FERNANDES et al., 2011).

High energy density diets can result in the accumulation of organic acids in the rumen and reduced buffering mechanisms (KLEEN et al., 2003). Digestive alterations can occur, through structural changes in organs of the digestive system, such as biochemical alterations in ruminal intake, such as lactic acidosis, which can be ruminal and/or metabolic.

Ruminal acidosis represents a problem owing to the direct effects caused by alterations in ruminal and systemic metabolism, resulting in rumenitis, liver abscesses, and laminitis (NASR et al., 2017), as well as the death of animals, causing great economic losses in the production systems of beef cattle in confinement.

Through the correct evaluation and interpretation of biochemical components of the blood it is possible to diagnose imbalances of metabolic or nutritional origin (SOARES et al., 2006; GONZALEZ, 2000), aiding understanding of the adaptations in the main metabolic pathways as well as the organic functionality (GONZÁLEZ et al., 2000). As it is a relatively new technology, there are few studies on the supply of a whole corn grain diet to finish cattle, with respect to performance and economic viability, as well as the impact on their health. In particular, studies are lacking on the dynamics of biomarkers used in the analysis of energy, protein, mineral, and endocrine profiles, and investigation of the influence of this type of diet on the enzymatic activity of cardiac muscles. In view of the above, the aim of this study was to characterize the effects of a diet based on whole corn grain on the metabolic profile of cattle.

Material and Methods

Twenty adult, male, non-castrated, mixed breed cattle, with an initial average weight of 300kg were used, vaccinated and dewormed. The cattle were reared confined in collective paddocks, with a shade cover in the area of the feeder where there was a concrete trough. The animals were distributed, through probabilistic sampling, into two groups (G1 and G2). The G1 group (MBGI+GM) was composed of 15 animals, which received the Max Beef Whole Grain diet (20% Max Beef Whole Grain and 80% corn whole grain), and the G2 group (Control), composed of five animals, which received sugarcane bagasse and Max Beef Whole Grain, in the ratio 30:70 (Tables 2 and 3). The animals of the G1 group were given an adaptation period to receiving the grain diet, providing a gradual increase of 25% of the expected final quantity every three days, so that at the end of nine days the animals were consuming only the whole corn grain diet, as recommended by the manufacturer (Table 1). The animals of both groups received water at will throughout the experimental period. Feed was provided twice a day, at 7:00 am and 4:00 pm. Clinical observations, blood, and ruminal fluid samples were taken on days 4, 11, 18, 39, 69, and 99 of the experimental period, totaling six collection moments**.**

Table 1 - Adaptation scheme proposed by the manufacturer

Days	**20% Max Beef Whole Grain + 80% Corn Grain**	**VOLUME**
1,2 and 3	0.5% of live weight	2.0% of live weight
4, 5 and 6	1.0% of live weight	1.5% of live weight
7, 8 and 9	1.5% of live weight	1.0% of live weight
10 and after	2.0% of live weight	0.0

Table 2 - Percentage composition of the diet ingredients

Item (%MS)	Diets	
	Control	MBGI+GM
Corn	56	80
Max Beef	14	20
Sugarcane bagasse	30	0

[1] in % of DM. MBGI+GM – Max Beef Whole Grain + Corn Grain

Table 3 - Chemical composition of ingredients used in the experimental diets

Item (%)	**Max Beef**	**Sugarcane Bagasse**	**Corn**
DM	90.55	51.00	89.52
OM[1]	83. 63	96.00	96. 86
MM[1]	16.37	4.00	3.14
CP[1]	28.27	1.60	7.45
NDF[1]	27.94	84.74	23.03
ADF[1]	15.37	55.85	3.88

[1] in % of DM. DM - Dry Matter; OM - Organic Matter; MM - Mineral Matter; CP - Crude Protein; NDF - Neutral Detergent Fiber; ADF - Acid Detergent Fiber

Procedures

Clinical observations

All animals were identified and submitted to evaluation, with verification of posture, behavior, ruminal motility, rectal temperature, and aspects of feces (DIRKSEN et al., 1993). The evaluations were carried out at the time of the respective collections.

Blood collection and analysis

Blood samples were collected on days: 4 (M1), 11 (M2), 18 (M3), 39 (M4), 69 (M5), and 99 (M6) of the experimental period, totaling six collection moments. Blood was obtained by means of jugular venipuncture, in siliconized tubes with a vacuum system (BD - Vacutainer System®), without anticoagulant, to obtain the serum, and with anticoagulant and glucose inhibitor (Oxalate/Sodium Fluoride), to obtain the plasma. The tubes without anticoagulant were left at room temperature to retract the clot and obtain serum. Therefore, both serum and plasma samples were centrifuged at 3600 rpm for 10 minutes; aliquoted in polyethylene Eppendorf type tubes, and stored in an ultra-freezer at -80 ºC, for further analysis.

The biochemical metabolites determined in the blood serum were: urea, creatinine, uric acid, total protein (TP), albumin, fructosamine, lactate, AST, GGT, ALT, AP, and CK. Globulin was obtained by the difference between TP and albumin. The biochemical metabolites determined in the plasma were glucose and lactate. Blood biochemical determinations were performed in an automated biochemical analyzer LABMAX 240 (LABTEST®) using a commercial LABTEST® kit. The analyses were carried out at the Laboratory of Nutritional and Metabolic Diseases of Ruminants, Department of Veterinary Medicine, UFRPE.

The serum concentrations of cardiac enzymes Troponin I and CK-MB were determined by electrochemiluminescence, using a commercial kit and BECKMAN COUNTER - ACCESS 2®mequipment, at the Analytical Chemistry Laboratory of the Research Support Center (CENAPESQ) of the Federal Rural University of Pernambuco.

Statistical analysis

The data are described using means and standard deviations. The parameters were initially tested for normal distribution, using the Kolmogorov-Smirnov test. Those that did not meet the assumptions of normality and homogeneity of variance were subjected to transformation on a logarithmic basis (Log_{X+1}). The data that met the premises of normality or transformed were subsequently subjected to analysis of variance (Test F) which was used to separate, as causes of variation, the effects of groups, collection moments, and their interactions. When there was significance in the F test, the means were compared by the minimum significant difference (m.s.d.)

of the Student–Newman–Keuls test. The data were analyzed using the computer program Statistical Analysis System (SAS, 2009), with the General Linear Model (GLM) procedure of SAS. For all statistical analyzes performed, the significance level (*p*) of 5% was adopted. The project obtained a favorable opinion from the Ethics Committee on the Use of Animals (CEUA), from the Federal Rural University of Pernambuco under license no. 82/2018.

Results and Discussion

Clinical Observations

Throughout the experimental period, the animals presented a temperature within the normal range, with a general mean of 38.8ºC in the MBGI+GM group and 38.3º in the control group. The animals remained standing and active. The rumen dynamics were regular most of the time, with incomplete movements in bowel sounds, some animals presented mild tympania in the MBGI+GM group. The feces were diarrheal in most animals, with corn grains, and two animals in the MBGI+GM group presented blackened feces at one moment. These results corroborate the findings of decreased ruminal contractility and increased fecal consistency reported by Danscher et al. (2015) in cattle with ARDS. Diets rich in energy lead to a considerable drop in ruminal pH after each feeding, and mild ruminal tympanism, loose stools, loss of appetite, decreased ruminal motility, decreased rumination time, and reduced performance have been reported in the literature in cases of ARDS by several authors (ENEMARK et al., 2002; ABDELA, 2016; HERNÁNDEZ et al., 2014).

The alterations observed in the feces are due to the subclinical acidosis framework developed. Considering that the structure and consistency of the feces depend on rumination, microbiota activity, and ruminal passage, the animal will present alterations in color, odor, pH, and consistency and even whole cereal grains can be present (HÉRNANDEZ et al., 2014). In addition, high osmolarity, due to the concentrated diet, could lead to softening of the stool from the passage of fluid to the intestinal lumen (KLEEN et al., 2003). In terms of rumination, bacterial degradation, and passage, impaired ruminal function leads to changes in fecal aspects. Intermittent diarrhea and the presence of undigested particles indicate inadequate digestion and rapid passage of feed (ENEMARK et al., 2002).

Energy Profile

An interaction was observed for plasma glucose concentration (p=0.0063), serum triglyceride concentration (p=0.0220), and fructosamine (p=0.0001), while serum cholesterol and lactate plasma concentrations demonstrated variations between groups and/or collection moments (Tables 4 and 5).

Table 4 - Significance level (Pr>F) of the analysis of variance of the energy profile metabolites as a function of the variation factors (Groups, Moments and Interactions)

Variables	Variation Factors		
	Groups	Moments	Groups x Moments
Glucose	0.6634	0.0003	0.0063
Triglycerides	0.0206	0.0009	0.0220
Cholesterol	0.1031	0.0033	0.7528
Lactate	0.0375	0.0074	0.2957
Fructosamine	0.5300	<.0001	0.0001

The plasma glucose concentration was lower at the initial moment of the experiment for the cattle that received the MBGI+GM diet when compared with the other collections. When evaluating the concentrations between groups, a variation was identified only at the initial moment of the experiment when the highest plasma glucose concentration was observed in the group of cattle in the control group.

According to Kaneko et al. (2008), normal bovine plasma glucose values vary from 45.0mg/dL to 75.0mg/dL. In the current experiment, the glucose values remained within the normal range for the species at all moments in both groups. Analyzing the glycemic response, the animals that were fed only grains (MBGI+GM), presented higher glucose values, due to the higher proportion of propionate absorbed, which is the main precursor of glucose converted at the hepatic level. Starch is an important source of glucose for ruminants, undergoing a fermentation process in the rumen, to produce volatile fatty acids which are absorped through the wall of this organ. Approximately 50% of the circulating glucose in ruminants comes from hepatic gluconeogenesis (GONZÁLEZ; CAMPOS, 2003), mainly from propionate.

Similar values were found by Dokovic et al. (2010) when working with beef cattle, suggesting preserved hepatic capacity for gluconeogenesis in these animals. Within the species, variations occur mainly according to age and physiological conditions (SOARES et al., 2006; GONZÁLEZ et al., 2017). Differences are found when considering dairy cattle, which tend to present alterations in their blood values, due to calving, and the demand for the mammary gland to produce colostrum and milk (SILVA FILHO et al., 2017).

An interaction was observed between groups and moments for the serum concentration of triglycerides, so that a higher concentration was observed at M1 and lower at M3 for cattle that received the MBGI+GM diet. With respect to the control group, there was no variation between the means at different collection moments.

Regarding the comparison of groups at each moment, higher means of serum triglyceride concentration were recorded at moments M1 and M6, while at M3, a greater concentration was identified in the control group when compared with the MBGI+GM group.

Both groups presented values above normal when compared to Kaneko et al. (2008) (0 to 14 mg/dL), at all moments. The highest value was observed in G1 at the initial moment, 31.39±2.13mg/dL. This fact can be explained by the high energy density of starchy foods. Through hormonal regulation, insulin promotes the lipogenesis of excess energy (GONZÁLEZ et al., 2017). Bonilha et al. (2015) when evaluating feed efficiency and blood parameters of Nellore cattle, found

a mean of 27.7mg/dL, values close to those found in the current study, taking into account the fat deposition in the carcass of male cattle. Dokovic et al. (2010) found lower values for this variable in cattle fed corn silage and concentrate. However, when compared with Pogliani and Birgel Junior (2007), the values found in the current study are within the normal range, 16.3 to 36.4 mg/dL for animals up to 48 months of age.

There was no variation between groups for serum cholesterol concentrations, however, in relation to the moments of collection, the highest concentration was recorded at M1 and lowest at M3. The results remained within the reference values in both groups, according to Kaneko et al. (2008), ranging from 80 to 120mg/dL, and there were no differences between groups. The higher value observed at the initial moment may reflect the greater contribution of Acetyl-CoA, a cholesterol precursor, available in cases of feed intake, increased insulin, and increased leptin. Its hepatic synthesis is related to the level ingested in the diet (GONZÁLEZ et al., 2017). In addition, values found within the normal range suggest hepatic metabolism of preserved fat (SOARES et al., 2006; DOKOVIC et al., 2010).

Fructosamine presented an effect of moment and interaction between group and moment (p=0.0001), with a higher value presented by G1 at M3. In the current study, fructosamine presented values that varied slightly below the limit and within normal limits, established by Jensen et al. (1993). As it is a glycated protein, the concentration of fructosamine indicates long-term variations in glucose metabolism, as reported in the literature (SILVA FILHO et al., 2017). In the current study, we observed variations over the period, demonstrating a positive correlation with glucose.

Although no interactions were observed between groups and moments, there were variations in the plasma lactate concentrations between groups (p = 0.0375) and moments (p = 0.0074), in which the highest overall mean of lactate concentration was observed in cattle of the MBGI+GM group compared to the control group. With respect to the moments of collection, the greatest concentration was observed at M1 and lowest at moments M3 and M6.

In relation to plasma lactate, the highest values at the initial moment are the result of the greater supply of rapidly fermentable carbohydrates with an increase in SCFA production and rumen lactate, subsequent to the pH reduction. With the drop in pH, *S. bovis* begins to ferment glucose into lactate, since the lactate pKa is much lower than that of SCFA, favoring its accumulation, further contributing to the drop in pH, death of bacteria that use lactate, and proliferation of lactate producers, such as *Lactobacillus* (OETZEL, 2017), with subsequent absorption through passive diffusion, reflecting the plasma values of the experimental group. Another relevant factor is that lactate, in addition to being directly absorbed by the ruminal wall, can pass along with the ruminal fluid to the abomasum and intestines, for later absorption (MOLLER et al., 1997). The results corroborate Mori et al. (2007), who reported high levels of plasma lactate in beef cattle that experienced acidosis during finishing with concentrated feed, as well as Bevans et al. (2005).

Table 5 - Mean values and standard error of the variables of the energy profile of cattle receiving “max beef” whole grain diet

Variables	Groups	Moments						GA
		M1	M2	M3	M4	M5	M6	
Glucose* (mg/dL)	MBGI+GM	48.01±2.60Bb	61.56±4.96 Aa	66,.58±3.98 Aa	70.97±4.39Aa	75.43±4.98Aa	60.45±2.94Aa	63.83
	Control	71.37±10.00Aa	58.09±3.74 Aa	59.98±3.92 Aa	59.05±2.28Aa	61.63±3.26Aa	57.50±0.80Aa	61.27
	GA	59.69	59.83	63.28	65.01	68.53	58.98	
Triglycerides (mg/dL)	MBGI+GM	31.39±2.13Aa	21.56±1.26Abc	19.64±0.93Bc	25.74±2.11Aabc	25.55±2.05Aabc	26.75±1.47Aab	25.10
	Control	22.75±0.65Ba	22.12±0.90Aa	24.41±1.75Aa	24.58±4.13Aa	19.73±1.55Aa	18.37±1.55Ba	22.00
	GA	27.07	21.84	22.03	25.16	22.64	22.56	
Cholesterol (mg/dL)	MBGI+GM	120.30±6.93	99.91±5.69	81.07±4.98	98.16±5.89	100.51±8.70	106.72±6.77	101.11A
	Control	124.90±11.98	105.18±9.59	99.49±10.82	108.97±12.03	117.69±13.21	99.22±7.78	109.24A
	GA	121.44a	101.23ab	85.67b	100.86ab	104.80ab	104.84ab	
Lactate (mg/dL)	MBGI+GM	25.40±3.45	26.23±4.70	16.21±2.80	23.27±3.48	25.60±4.03	15.21±2.35	21.99A
	Control	27.60±6.46	11.83±3.78	15.06±2.37	16.50±2.89	15.45±2.97	11.03±1.11	16.24B
	GA	25.95a	22.63ab	15.93b	21.57ab	23.06ab	14.16b	
Fructosamine* (µmol/L)	MBGI+GM	188.44±5.77Bde	182.50±4.34Ae	251.04±6.85Aa	225.69±5.08Ab	201.37±6.04Acd	207.31±5.19Ac	209.40
	Control	235.21±9.50Aa	194.18±5.80Ac	226.20±2.89Aab	210.20±6.83Abc	196.47±4.15Ac	205.84±4.56Abc	211.35
	GA	211.83	188.34	238.62	217.95	198.92	206.58	

* Variables that had Groups x Moments interaction. GA - General Average. Different lowercase letters on the same line differ statistically (P<0.05) characterizing the moment effect. Different capital letters in the same column differ statistically (P<0.05) characterizing the group effect. MBGI + GM - Max Beef Whole Grain + Corn Grain.

Protein Profile

Total Protein (TP) presented an effect of moment (P<0.05), in which the lowest means were observed at M2 and M3. Albumin presented a group and moment effect, so that a lower overall mean was observed in the group of cattle in the MBGI+GM group compared to the control. In the time analysis, higher means were recorded at moments M5 and M6. An interaction between groups and moments was observed for the globulin variable, with greater concentration observed in the MBGI+GM group at the M4 moment. With respect to the A:G ratio, an interaction was also observed, with a greater A:G ratio identified at the M6 moment for the MBGI+GM group and at the moments M5 and M6 for the control group (Tables 6 and 7).

Table 6 - Significance level (Pr>F) of the analysis of variance of the metabolites of the protein profile as a function of the variation factors (Groups, Moments and Interactions)

	Variation Factors		
Variables	Groups	Moments	Groups x Moments
PT	0.0700	0.0001	0.1526
Albumin	<.0001	<.0001	0.5880
Globulin	0.6516	<.0001	0.0196
A:G	0.0045	<.0001	0.0075
Creatinine	0.1485	0.0107	0.6469
Urea	0.2860	<.0001	0.0007

An interaction was recorded for the serum urea concentration, in which the highest concentration was observed in the MBGI+GM group, at moment M2 and the reverse occurred at moment M1. Creatinine concentration was higher at the beginning of the experiment and lower at M5. An interaction was observed for the serum concentration of uric acid, with a higher concentration for the animals of the MBGI+GM group. In the same group, a lower concentration was recorded at the beginning compared with the other collection moments, whereas in the control group, lower concentrations were recorded at moments M2 and M3 compared to other collection moments.

The total protein values in this study remained within the normal parameters for adult cattle, with no differences between groups (p>0.05). Protein values remain relatively constant due to the fact that there is a negative correlation between the concentration of albumin and globulins, to maintain adequate blood osmotic pressure (KANEKO et al., 2008). Similar results were found by Fagliari et al (1998) and Soares et al. (2006) when evaluating the blood constituents of cattle.

Low levels of albumin may indicate protein deficiency. Taking into account that a period of one month is necessary to detect significant alterations in serum albumin concentrations, we can infer from the data found that these animals came from periods of food scarcity before entering confinement, in view of the severe drought faced in the months prior to the study. These results

corroborate with González et al. (2010), who found albumin values as low as 2.1 g/dL when the animals experienced less protein availability in the pasture, but differ from the values found by Silva et al. (2008), with Nellore males in confinement, who found an average of 3.01g/dL. According to Kaneko et al. (2008), the elevated globulin values are due to the immune response of the animal organism to infectious challenges, in addition to the fact that the concentration of globulin in animals in tropical conditions is proportionally higher than the concentration of albumin.

The urea concentration is directly related to the protein supply in the diet and the energy: protein ratio. In normal cattle, there is an increase in serum levels between three to nine hours after feeding. In the current study, the animals maintained levels very close to normal. Larger means were found by Borges et al. (2011), ranging from 28.2 to 43.2 mg/dL in adult animals and similar to the means of Fagliari et al. (1998), for Nellore cattle, 12.43 to 23.91 mg/dL. The uric acid values did not signal abnormal conditions in protein metabolism, arising from probable morbid conditions such as liver disorders, inhibitors of the conversion of uric acid to allantoin, and renal failure, endocrine problems and increased nucleic acid recycling (KANEKO et al., 2008).

Table 7 - Average values and standard error of the variables of the protein profile of cattle receiving “max beef” whole grain diet

Variables	Groups	Moments						GA
		M1	M2	M3	M4	M5	M6	
PT (g/dL)	MBGI+GM	7.97±0.09	7.50±0.11	7,59±0.10	8.12±0.11	8.29±0.13	8.16±0.11	7.94A
	Control	8.46±0.24	8.04±0.30	7.93±0.19	8.29±0.42	8.06±0.36	8.00±0.26	8.13A
	GA	8.09a	7.63b	7.67b	8.16a	8.23a	8.12a	
Albumin (g/dL)	MBGI+GM	2.11±0.03	2.03±0.04	1.96±0.03	1.99±0.03	2.58±0.05	2.59±0.05	2.21B
	Control	2.22±0.07	2.13±0.08	2.03±0.08	2.20±0.12	2.85±0.12	2.72±0.10	2.36A
	GA	2.14b	2.06bc	1.98c	2.04bc	2.64a	2.62a	
Globulin* (g/dL)	MBGI+GM	5.85±0.08Aab	5.46±0.10Ab	5.62±0.10Ab	6.13±0.11Aa	5.71±0.11Ab	5.58±0.13Aa	5.73
	Control	6.23±0.25 Aa	5.91±0.25Aa	5.89±0.16Aa	6.09±0.33Aa	5.21±0.26Ba	5.28±0.19Aa	5.77
	GA	6.04	5.69	5.76	6.11	5.46	5.43	
A/G*	MBGI+GM	0.36±0.01Ab	0.37±0.01Ab	0.35±0.01Abc	0.33±0.01Ac	0.45±0.01Ab	0.47±0.02Aa	0.39
	Control	0.36±0.02Ab	0.36±0.01Ab	0.35±0.01Ab	0.36±0.01Ab	0.55±0.02Aa	0.52±0.02Aa	0.42
	GA	0.36	0.37	0.35	0.35	0.50	0.50	
Urea* (mg/dL)	MBGI+GM	22.93±3.12Ba	28.51±2.35Aa	19.83±1.87Aa	19.14±2.19Aa	21.83±1.63Ab	6.43±1.05cA	19.78
	Control	36.00±4.09Aa	13.08±1.43Bc	15.35±3.58Ac	31.51±6.40Abc	17.61±1.12Ad	3.85±0.96dB	19.57
	GA	29.47	20.80	17.59	25.33	19.72	5.14	
Creatinine (mg/dL)	MBGI+GM	1.64±0.08	1.59±0.07	1.39±0.06	1.51±0.06	1.30±0.05	1.55±0.06	1.49A
	Control	1.70±0.19	1.47±0.10	1.50±0.15	1.58±0.16	1.54±0.14	1.68±0.16	1.58A
	GA	1.65a	1.56ab	1.42ab	1.53ab	1.36b	1.58ab	
Uric Acid* (mg/dL)	MBGI+GM	0.64±0.03Ab	0.86±0.05Aa	0.94±0.05Aa	0.85±0.05Aa	1.02±0.05Aa	0.96±0.05Aa	
	Control	0.77±0.06Aa	0.60±0.09Ab	0.69±0.05Ab	0.73±0.06Aa	0.69±0.12Ba	0.83±0.11Aa	
	GA	0.71	0.73	0.82	0.79	0.86	0.89	

Variables that had Groups x Moments interaction. GA - General Average. Different lowercase letters on the same line differ statistically (P<0.05) characterizing the moment effect. Different capital letters in the same column differ statistically (P<0.05) characterizing the group effect. MBGI + GM - Max Beef Whole Grain + Corn Grain.

Enzyme Profile

The enzyme profile data are shown in Tables 8 and 9. The serum ALT activity did not present significant differences ($p>0.05$) between the groups and moments. Although the enzymatic activity of ALT presents low activity in this species, it varied within the normal range for the bovine species (11 to 40 U/L), proposed by Kaneko et al. (2008). For AST, there was a difference between group and moment ($P<0.05$), with greater enzyme activity being evident in the last two experimental moments. With respect to AST activity, which was relatively high in the liver, and skeletal and cardiac muscle, Dokovic et al. (2010) reported an increase in activity in cattle for fattening during high metabolic demands. In our study, despite the fact that the last two moments had higher means, AST activity varied within the normal range, which suggests preserved functional and morphological integrity of the hepatocytes.

Table 8 - Significance level (Pr>F) of the analysis of variance of the enzymatic activity of liver function and of the serum concentration of Troponin I and CK - MB, depending on the variation factors (Groups, Moments and Interactions)

Variables	Variation Factors		
	Groups	Moments	Groups x Moments
AST (U/L)	0.0191	<.0001	0.0743
GGT (U/L)	0.6659	<.0001	0.0016
CK (U/L)	0.0041	<.0001	0.0042
Troponin I (ng/mL)	0.8661	0.2198	0.3731
CK-MB (U/L)	0.5713	0.4586	0.8945

Table 9 - Mean values and standard error of the enzymatic activity variables of liver and muscle function and serum troponin concentration of cattle receiving “max beef” whole grain diet

Variables	Groups	Moments						
		M1	M2	M3	M4	M5	M6	AG
AST (U/L)	MBGI+GM	87.07±3.33	77.36±3.07	85.99±8.20	75.51±5.99	127.20±15.21	130.91±14.50	97.34A
	Control	66.40±8.52	96.65±19.16	62.00±6.17	81.84±12.42	75.30±11.11	125.58±43.44	84.63A
	AG	81.90b	82.19b	80.00b	77.09b	114.23a	119.58a	
GGT* (U/L)	MBGI+GM	17.08±1.59Ac	16.06±1.02Bc	25.41±1.92Ab	35.78±3.68Aa	38.05±5.17Aa	32.81±1.25Aa	27.53
	Control	22.52±1.81Aa	26.95±4.66Aa	27.64±2.75Aa	26.24±2.54Aa	25.61±2.31Aa	29.17±4.46Aa	26,35
	AG	19.80	21.51	26.53	31.01	31.83	30.99	
CK* (U/L)	MBGI+GM	160.45±18.60Aab	135.41±16.73Abc	89.85±4.29Ac	126.94±10.90Abc	227.48±48.91Aa	206.72±24.09Aa	157.81
	Control	129.56±19.16 Ab	99.86±22.87Ab	87.61±15.89Ab	81.26±12.19Bb	80.90±12.91Bb	271.47±20.88Aa	125.11
	AG	145.01	117.64	88.73	104.10	154.19	239.10	
Troponin I (ng/mL)	MBGI+GM	0.09±0.06	0.08±0.03	0,02±0.02	0.02±0.01	0.04±0.01	0.00±0.00	0.05A
	Control	0.00±0.00	0.00±0.00	0.03±0.02	0,.04±0.01	0.01±0.01	0.02±0.01	0.03A
	AG	0.09a	0.08a	0.02a	0.03a	0.03a	0.01a	
CK-MB (U/L)	MBGI+GM	135.40±11.33	109.23±5.76	132.56±7.46	162.82±12.88	164.90±14.87	248.13±22.91	158.84A
	Control	180.35±24.30	121.40±8.56	110.02±8.97	113.55±8.52	110.80±5.12	368.88±54.63	167.50A
	MG	146.64a	112.28a	126.93a	150.50a	151.38a	278.30a	

* Variables that had Groups x Moments interaction. GA - General Average. Different lowercase letters on the same line differ statistically (P<0.05) characterizing the moment effect. Different capital letters in the same column differ statistically (P<0.05) characterizing the group effect. MBGI + GM - Max Beef Whole Grain + Corn Grain.

In G1, AP presented significantly higher values than G2, (P<0.0005), which was more evident at M5 (223.69±19.24 U/L and 141.78±22.12 U/L). When analyzing the effect of moment, a difference (P<0.001) was observed in both groups, in relation to M1, with the highest values verified at M5. Regarding serum AP activity, G1 presented the highest mean, at the last three moments of the experimental period. However, these values varied within normal standards. Fagliari et al., (1998) found mean values of 95.51 U/L for adult male Nellore cattle. Borges et al., (2011) found lower means (86.8 U/L) for Pantanal cattle. High activity of this enzyme is found in young growing cattle, originating from bone tissue (OTTER, 2013). Oliveira Junior et al. (2004) claim that AP activity is mainly determined by bone isoenzyme, therefore, imbalance in their metabolism could result in serum elevation. In animals with stabilized bone development, the majority of the activity of this enzyme comes from the liver (GONZÁLEZ et al., 2017).

Serum GGT activity differed between moments (P<0.05) and a group x moment interaction was observed. At M2, the G1 presented less activity of this variable, in relation to the other moments, and presented increasing values in the last three moments throughout the experiment, behavior similar to AST and AP. The activity of this enzyme is above the normal limits proposed by González et al. (2017), which range from 6.1 to 17.4 U/L. Increased activity of this enzyme is linked to damage to the structure of hepatocytes (DOKOVIC et al., 2010). Although it is found in several tissues, GGT is the best indicator of liver disease in ruminants (RUSSEL; ROUSSEL, 2007). Fagliari et al. (1998) found higher values, with a mean of 17.02 U/L in adult Nellore cattle. Moreira et al. (2012) found a significantly higher mean (P>0.05) in animals with histological lesions (23U/L) compared to animals without hepatic injuries (18U/L). On the other hand, Brown et al. (1999) did not observe effects of a diet with 90% concentrate on the activity of GGT and AST in sheep.

Although used in the routine of detecting liver injuries, Moreira et al. (2012) concluded that the detection of small liver lesions, usually through biochemical tests, is limited. However, the high specificity of GGT allows its use as an indicator of chronic liver damage in cattle herds. The literature reports the occurrence of liver abscesses found after slaughter (NAGARAJA; CHENGAPPA, 1998). For CK there were differences between group, moment, and interaction (P<0.05). The lowest enzyme activity of CK was observed at moments M4 and M5 in G2 and the greatest activity at M8. Considering the normal values of CK enzyme activity proposed by González et al. (2017) (<94U/L), the animals in the current study showed above normal enzyme activity during most of the experimental period. Since it is an indicator of muscle injury, an increase in CK may occur due to exercise, restraint in a cattle crush, sudden or decubitus effort, and activities carried out during collections, justifying its discreet elevation (RUSSEL; ROUSSEL, 2007).

Regarding cardiac markers, CK-MB and Troponin, there were no differences between groups, moments, and their interactions (P>0.05). Troponin I concentrations are sensitive parameters used in the investigation of cardiac injuries in humans and animals. In the current study, there were no differences between groups and moments, and their values remained close to the references cited in healthy cattle by Jesty et al. (2005), ranging from 0.00 to 0.04 ng/mL and within the normal limits cited by Basbugan et al. (2010), from 0 to 0.23 ng/mL. Regarding the serum activity of CK-MB, a less sensitive and specific cardiac marker than Troponin I (SANTOS et al., 2010), there were also no differences between groups and moments.

In the current study, the enzymatic activity was higher than that found by Basbugan et al. (2010), in healthy adult cattle (21.35 UI/L). Fartashvand et al. (2013) found a mean of 249 U/L in

healthy cattle, a comparatively closer mean to our study. These authors state that the enzymatic activity of CK-MB is normally present in skeletal muscle in small amounts and in cases of muscle damage there may be an increase in its activity. This fact could be justified by a slight increase in CK, to indicate muscle injury, since troponin I, which is more sensitive, remained low, excluding myocardial injury.

Conclusions

The cattle of the G1 group showed characteristic clinical manifestations of ruminal acidosis. Inferences of the G1's diet on some of the variables analyzed occurred, prominently, in the energy profile (glucose, triglycerides and Frutosamine), without putting the animals' health at risk. However, for the use of this type of diet, careful food planning is recommended, especially during the animals' adaptation period.

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