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ROUGHAGES AND CRUDE GLYCERIN ON BEEF CATTLE DIETS: EFFECT ON HEMOGRAM AND SERUM BIOCHEMISTRY

VOLUMOSOS E GLICERINA BRUTA NA DIETA DE BOVINOS DE CORTE: EFEITO SOBRE O HEMOGRAMA E BIOQUÍMICA SÉRICA

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SUMMARY

The objective of this study was to evaluate the blood parameters of cattle fed different roughages and crude glycerin. Six castrated Nellore cannulated in the rumen, approximately 30 months old, 400 kg body weight were used for blood sampling. The animals housed in individual pens were fed six experimental diets that consisted of corn silage, hydrolyzed sugarcane or bulky hay and concentrate composed of ground corn, soybean hulls, sunflower meal, glycerin and mineral supplement, at 50:50 roughage: concentrate ratio. The experiment lasted 150 days, divided into six sub-periods of 25 days each. Blood samples were taken on the last day of each experimental sub-period. The experimental design was a 6 × 6 Latin Square, in 3 × 2 factorial (three roughages × presence or absence of glycerin). The treatments did not affect (P> 0.05) any of the hemogram parameters. However, the treatments affected the AST enzyme activity (P = 0.023) and cholesterol levels (P = 0.021). There was no interaction effect between different roughages and the inclusion of 10% glycerin in any of the studied parameters, except between treatment containing hydrolyzed sugarcane (86.01 mg/dL) and treatment with hydrolyzed sugarcane with 10% glycerin added (60.78 mg/dL) on glucose levels (P = 0.0486). Different roughages with or without glycerin added in the diets fed to beef cattle, promote changes in the blood parameters of the animals, except on hemogram parameters.

KEY-WORDS: Biodiesel. Blood. Ruminants.

RESUMO

Objetivou-se com este estudo avaliar os parâmetros sanguíneos de bovinos alimentados com diferentes volumosos associados à glicerina. Seis bovinos Nelore, castrados, canulados no rúmen, com aproximadamente 30 meses de idade e 400 kg de peso corporal foram utilizados para colheitas de sangue. Os animais foram mantidos em baias individuais recebendo seis dietas experimentais, sendo a silagem de milho, a cana-de-açúcar hidrolisada ou o feno os volumosos e o concentrado composto por milho moído, casca de soja, farelo de girassol, suplemento mineral e glicerina, formuladas na proporção volumoso:concentrado de 50:50. O período experimental foi de 150 dias, dividido em seis subperíodos de 25 dias cada. As colheitas de sangue foram realizadas no último dia de cada subperíodo experimental. O delineamento utilizado foi o quadrado latino 6×6 em esquema fatorial 3×2 (três volumosos × presença ou ausência de glicerina). Os tratamentos estudados não alteraram (P>0,05) nenhum dos parâmetros do hemograma. Houve efeito dos tratamentos sobre a atividade da enzima AST (P = 0,023) e sobre a concentração de colesterol (P = 0,021). Não houve efeito da interação entre os diferentes volumosos e a inclusão ou não de 10% de glicerina associada a estes tratamentos em nenhum dos parâmetros estudados, exceto entre o tratamento com cana-de-açúcar hidrolisada (86,01 mg/dL) e o tratamento com cana-de-açúcar hidrolisada adicionada de 10% de glicerina (60,78 mg/dL) sobre a concentração de glicose (P = 0,0486). Diferentes volumosos associados ou não à glicerina em dietas para bovinos de corte, promovem alterações nos parâmetros sanguíneos dos animais, exceto nos parâmetros do hemograma.

PALAVRAS-CHAVE: Biodiesel. Ruminantes. Sangue.

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INTRODUCTION

Conserved forage use is a common practice in the diet of ruminants, in both intensive and semi-intensive systems, during periods of the year when pasture is not able to provide all the nutrients required by the livestock. The main conservation practices are silage and hay (CAVALCANTE et al., 2004), for example, corn silage and Tifton hay. Other commonly supplied roughage, in the dry season, is sugarcane *in natura,* which presents high lignocellulosic content. Therefore, in order to make the sugarcane feasible it is necessary to develop methods that are able to break the structure of the fiber to increase its digestibility. Therefore, sugarcane hydrolysis becomes an alternative, as it improves digestibility, increases voluntary intake, and water consumption, and prevents acidosis (EZEKIEL et al., 2005a).

Biofuel production has expanded dramatically in the last decade, and the ramifications of this growth are becoming increasingly evident in the cattle industry. The conversion of grains into biofuels has resulted in large amounts of byproducts (DROUILLARD, 2011) with great potential for animal feed such as glycerin (DONKIN, 2007; PARSONS, 2009; MACH, 2009). Therefore, it becomes necessary to study the association of glycerin with different roughages since there is no data about its productive efficiency and effects on animal metabolism. It is known that the methanol residues and other contaminants, usually present in glycerin, can cause metabolic changes in the animals, thus hindering their performance and causing losses to the producers.

Silva et al. (2012) evaluated glycerin added up to 30% to diets of feedlot cattle and reported changes in the hematological parameters of animals, suggesting that high intake of concentrate with added glycerin in the diet led to increased hepatic metabolism due to higher activity of energy metabolism. Liver damage due to infiltration of fat derived from lipomobilization increases the possibility of disorders such as ketosis, displaced abomasum and hypocalcemia (AMETAJ, 2005), which may hamper animal performance and even lead to death. These conditions may trigger an inflammatory process; attract defense cells (polymorphonuclear and mononuclear cells) into the bloodstream and change the hematological parameters of animals.

The metabolic profiles are used as a routine procedure to monitor metabolic disorders, detect nutritional deficiencies, prevent subclinical disorders, as well as investigate health issues and herd performance (DUFFIELD & LEBLANC, 2009). The biochemical composition of the blood reliably reflects the balance between the ingress, egress and metabolism of nutrients in animal tissue. This balance is called homeostasis and its breakdown leads to reduced growth performance and, depending on the degree of imbalance, to disease (GONZALEZ, 2000).

Therefore, the aim of this study was to assess possible changes in serum biochemical values and blood count parameters of beef cattle fed different forages associated with glycerin.

MATERIAL AND METHODS

The experiment was conducted at the Animal Unit of Digestive and Metabolic Studies of the Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias of the Universidade Estadual Paulista "Julio de Mesquita Filho", Jaboticabal (Unesp/FCAV).

Six diets (Table 1) were formulated to test three types of roughage with and without the inclusion of 10% glycerin in the diet dry matter (DM): C - hydrolyzed sugarcane without glycerin; CG - hydrolyzed sugarcane with 10% glycerin based on DM; S - Corn silage without glycerin; SG - Corn silage with 10% glycerin based on DM; F - Tifton 85 hay without glycerin; and FG - Tifton 85 hay with 10% glycerin based on DM. The roughage concentrate ratio was 50:50; and consisted of corn silage, hydrolyzed sugarcane or Tifton 85 hay. Feed was offered twice daily, at 8h and 16h.

The glycerin used was obtained from soybean oil and had 83% glycerol, 11% water, 6% salt (of which 99% is NaCl) and 0.01% methanol, known commercially as "blond glycerin."

The sugarcane used was the IAC 862480 approximately 18 months of growth that was mechanically chopped into 8 to 10 mm pieces. The lime used in the hydrolysis was calcitic hydrated lime with the following chemical composition: total calcium oxide (CaO) = 72% minimum; total magnesium oxide (MgO) = 2% max; calcium hydroxide ($Ca(OH)_2$) = 95% min; maximum moisture = 1%, according to analysis provided by the manufacturer. The mixture for the hydrolysis consisted of 0.5 kg of lime in 2 liters of water for 100kg of sugarcane *in natura.* The obtained suspension facilitates the hydrolysis process since more sugarcane particles come into contact with lime. The mixture was homogenized on a concrete floor inside a shed, twelve hours before animal feeding.

Six castrated Nellore cattle, approximately 30 months old and with 400 kg body weight, fitted with permanent rumen cannulas, were used for blood sampling. The blood was drawn by puncture of the coccygeal vein using Vacutainer ® tubes, with the animals contained by the trunk and before the treatments started. The blood samples were used for complete blood count (CBC) and to determine the activity of AST (aspartate aminotransferase), GGT (gamma-glutamyltransferase) and AP (alkaline phosphatase) enzymes; levels of cholesterol and triglycerides, and plasma glucose concentration using sets of commercial reagents from Labtest Diagnostica. The blood samples used for CBC were placed in Vacutainer® tubes containing the anticoagulant tripotassium ethylenediaminetetraacetic acid (EDTA) while the samples used for biochemical analyzes did not have the anticoagulant, except those used for plasma glucose analysis, which contained fluoride. These were centrifuged at 2054.33 g for 15 minutes to separate the serum. The blood samples with EDTA were used to determine leukocytes and erythrocytes, the hemoglobin and hematocrit concentrations using automatic analyzer (Poch - iV 100 Diff), on the same day of sampling. The readings of the biochemical parameters were performed in semi-automatic spectrophotometer (LabQuest), with specific wavelength for each constituent, also on the same day of sampling.

The experimental design was a 6 × 6 Latin Square, in 3× 2 factorial (three roughages × with or without glycerin). The experiment lasted 150 days, divided into six sub-periods of 25 days each. Blood samples were drawn on the last day (day 24) of each experimental sub-period. The contrast analysis was performed for the effects Silage *vs* (Hydrolyzed Cane + Tifton 85 Hay) and Hydrolyzed Cane *vs* Tifton 85 hay, besides the glycerin inclusion effect associated with each roughage, using the MIXED procedure of the Statistical Analysis System (SAS) software version 9.1. The significance level was set at 5%.

RESULTS AND DISCUSSION

The inclusion of 10% glycerin in the diets associated or not with different roughages did not change (P> 0.05) any of the CBC parameters (Tables 2 and 3).

Mean values for blood and platelet counts, hemoglobin and hematocrit levels are within the ranges suggested in the literature (AENGWANICH et al., 2009; KANEKO et al., 2008, OLIVER et al., 2000).

Ezekiel et al. (2010b, c) studied the inclusion of 30% glycerin in the diet fed to 30 beef cattle, and also found no differences in hematocrit concentrations and platelet counts while the parameters remained within the normal range, which corroborates the present study.

The leukocyte count did not change significantly with the inclusion of 10% glycerin in different forages. However, Ezekiel et al. (2010b) evaluated the inclusion of 30% glycerin based on diet DM and reported changes in the leukocyte counts, probably due to metabolic challenges that arise when the inclusion percentage increases, especially for the hematopoietic organs such as the spleen and liver, to metabolize and use the nutrients (EZEKIEL et al., 2010b, c). The same authors found significant effects on total leukocytes, monocytes and segmented neutrophils, which did not occur in this study; therefore, it is suggested that up to 10% glycerin inclusion in the roughages change neither erythrocyte nor WBC of the animals.

Table 1 - Percent and chemical composition of experimental diets.

Ingredients	Experimental diets					
	SM	SMG	CH	CHG	F	FG
	Percent composition (% DM)					
Corn silage	50	50				
Hydrolyzed sugarcane			50	50		
TIFTON 85 Hay					50	50
Corn	24.50	13.50	13.40	7.40	33.30	18.30
Soybean hulls	10.10	6.80	11.50	3.00	8.00	10.00
Sunflower meal	13.10	17.65	24.00	28.75	7.20	10.70
Limestone	1.00	1.20	0.00	0.00	0.00	0.00
Salt	0.50	0.00	0.50	0.00	0.50	0.00
Urea	0.30	0.35	0.10	0.35	0.50	0.50
Glycerin	0.00	10.00	0.00	10.00	0.00	10.00
Mineral supplement[1]	0.50	0.50	0.50	0.50	0.50	0.50
	Chemical composition of the experimental diets					
DM	59.96	60.32	58.75	59.03	86.85	87.26
	% DM					
CP	12.45	12.63	12.00	12.67	12.15	12.12
EE	3.21	2.77	1.34	1.03	2.30	1.78
NDF	41.02	39.16	47.50	42.86	52.05	52.80
ADF	26.08	25.56	31.69	28.81	29.03	30.65
STARCH	16.82	15.92	28.05	27.91	23.01	19.20
CNF	37.37	36.40	30.80	33,25	27.32	22.59
ME (Mcal/kg) [2]	2.68	2.67	2.42	2.43	2.56	2.54

SM = Corn silage; SMG = Corn Silage with glycerin, CH = hydrolyzed sugarcane; CHG = hydrolyzed sugarcane with glycerin; F = TIFTON 85 Hay and FG = TIFTON 85 with glycerin; DM = dry matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, ME = metabolizable energy; [1]Mineral Supplement for Cattle, levels guaranteed by 1000g Product: Phosphorus 40g, Calcium 80g, Sodium 195g, Chlorine 300g, Magnesium 5g, Sulfur 26g, Zinc 2000mg, Copper 1000mg, Manganese 500mg, Cobalt 100mg, Iodine 100mg, Selenium 5mg, Fluor (max.) 400mg, vehicle q.s.p. 1000g; [2] metabolizable energy (Mcal / kg MS) estimated by the NRC (1996).

Table 2 - Red blood cell and platelet counts, hemoglobin and hematocrit levels determined for the different treatments.

Item	Treatments						Average	Contrasts		
	C	C10	S	S10	F	F10		1[a]	2 [b]	CV[c]
HE[d]	8.47	8.54	7.92	8.04	8.48	7.93	8.22	0.1818	0.2621	8.851
HG[e]	12.50	12.52	11.94	12.08	12.17	11.72	12.15	0.3661	0.2710	7.826
HT[f]	37.24	37.48	35.30	35.76	36.75	35.00	36.23	0.2250	0.3697	8.122
PLA [g]	391.2	386.8	398.2	373.2	379.7	404.0	389.1	0.9802	0.6483	24.05

[a] silage *vs* (Hydrolyzed sugarcane + Hay), [b] Hydrolyzed sugarcane *vs* Hay, [c] Coefficient of variation (%), [d] RBCs ($x10^6/\mu L$), [e] hemoglobin (g/dL), [f]Hematocrit (%), [g] Platelets ($x10^3/\mu L$).

Table 3 - Total leukocytes, segmented and rod neutrophils, monocytes, lymphocytes, basophils and eosinophils for the different treatments.

Item [d]	Treatments						Average	Contrasts		
	C	C10	S	S10	F	F10		1 [a]	2 [b]	CV[c]
LT [e]	10.80	10.04	10.56	10.88	9.025	9.640	10.19	0.140	0.117	13.00
NS[f],	1.911	1.745	1.886	2.003	1.804	2.357	1.956	0.987	0.541	34.53
NB[g],	0.000	0.000	0.000	0.000	0.000	0.000	-	-	-	-
MON[h]	0.084	0.096	0.114	0.082	0.065	0.047	0.082	0.154	0.587	72.35
LINF[i]	7.494	7.766	7.804	7.715	6.655	6.636	7.369	0.267	0,066	13.73
BAS [j]	0.000	0.000	0.000	0.000	0.000	0.000	-	-	-	-
EOS [k]	1.310	0.431	0.755	1.034	0.371	0.598	7.632	0.144	0,204	72.27

[a] silage *vs* (Hydrolyzed sugarcane + Hay), [b] Hydrolyzed sugarcane *vs* Hay, [c] Coefficient of variation (%), [d] $x10^3/\mu L$, [e] total leukocytes, [f] segmented and [g]rod neutrophils, [h]monocytes, [i]lymphocytes, [j] basophils and [k]eosinophils.

Regarding serum activity of liver enzymes analyzed (Table 4), the treatments affected only the activity of AST (P = 0.023) enzyme, whose values were higher in the treatments with corn silage. However, the treatment containing hydrolyzed sugarcane was expected to increase this enzyme activity since the partial solubilization of the fiber cell wall that occurs during sugarcane hydrolysis increases its digestibility (OLIVEIRA et al., 2008) and the metabolism, suggesting an increase in hepatic activity.

The increased production of the AST enzyme is due to increased hepatic energy intake provided by the intake of corn silage compared to both hay and sugarcane, which have lower lipid levels and higher fiber in their compositions, hindering their utilization by ruminal microorganisms. Thus, corn silage treatment increased propionic acid production, which when metabolized in the liver may have increased the AST enzyme activity compared to other treatments.

The AST and GGT values are within the ranges suggested in the literature (27.2 to 44 U/L and 0 to 139.8 U/L, respectively) by Barros Filho (1995). However, these enzyme concentrations were below the values of 65.7 U/L AST and 28.5 U/L GGT reported by Silva et al. (2010), who included 30% glycerin in the diet fed to 30 beef cattle. These results show that different forages associated or not with glycerin can modify hepatic metabolism, but high glycerin concentrations (30%) associated with high levels of concentrate (70%) can produce major changes in the metabolism, especially the hepatic.

The FA enzyme concentration remained within the range suggested by Kaneko et al. (2008). This enzyme has diagnostic value when associated with changes of the GGT enzyme activity, suggesting that alterations in bile canaliculi may cause cholestasis, which was not observed in the present study.

The treatments with corn silage also influenced (P = 0.021) cholesterol concentration (Table 5) while

Table 4 - Concentrations of alkaline phosphatase, gamma-glutamyltransferase and aspartate aminotransferase for the different treatments.

Item [**]	Treatments						Average	Contrasts		
	C	C10	S	S10	F	F10		1 [a]	2 [b]	CV[c]
AP [d]	154.2	136.0	162.5	140.9	134.3	140.9	144.8	0.561	0.712	16.70
GGT[e]	26.01	26.01	24.48	22.95	26.01	22.95	24.79	0.650	0.613	19.96
AST [f]	36.66	35.61	41.90	37.71	35.62	34.57	37.01	0.023 [*]	0.919	12.43

[e] silage *vs* (Hydrolyzed sugarcane + Hay), [b] Hydrolyzed sugarcane *vs* Hay, [c] Coefficient of variation (%), [d] alkaline phosphatase, [e] gamma-glutamyltransferase, [f] aspartate aminotransferase, [*] P <0.05, [**] U/L.

Table 5 - Levels of Cholesterol, Triglycerides and Glucose of beef cattle, for different treatments.

Item [**]	Treatments						Average	Contrasts		
	C	C10	S	S10	F	F10		1 [a]	2 [b]	CV[c]
COL [d]	106.4	109.0	133.6	135.8	111.9	106.1	117.1	0.021 [*]	0.625	22.87
TRIG[e]	22.52	29.02	17.14	18.76	20.21	22.37	21.83	0.056	0.059	27.86
GLI [f]	86.01	60.78	63.19	79.96	95.55	74.48	76.01	0.742	0.181	25.44

[a]silage *vs* (Hydrolyzed sugarcane + Hay), [b]Hydrolyzed sugarcane *vs* Hay, [c]Coefficient of variation (%), [d]Cholesterol, [e]Triglycerides, [f]Glucose, [*] P <0.05, [**] mg/dL.

the highest value was found for 10% glycerin treatment (S10). This difference compared to other treatments highlights the high hepatic activity of animals fed corn silage probably due to the higher lipid concentration of these diets (Table 2). Corroborating this result, Nunes et al. (2010) suggest that the increase of lipids in the diet raises plasma cholesterol levels. The largest amount of cholesterol in the diet with 10% glycerin and corn silage is probably due to the replacement of corn with glycerin (Table 2), probably due to increased rumen propionate production caused by glycerin and consequent increase of cholesterol production by the liver.

There was no significant effect between treatments with and without 10% glycerin on any of the studied parameters. Except, however, between hydrolyzed sugarcane (86.01 mg/dL) and hydrolyzed sugarcane with 10% glycerin (60.78 mg/dL) on glucose levels, which were significantly affected (P = 0.0486). Although it appears that not only the inclusion of glycerin may have influenced this parameter since glycerin added on hay and corn silage did not change any blood parameter.

The blood glucose levels in this study are abo ve the range suggested by Kaneko et al. (2008) of 45 to 75 mg/dL, except for the treatment with corn silage and hydrolyzed sugarcane with 10% glycerin. It is noteworthy that the treatment with 10% glycerin associated with sugarcane hydrolysate resulted in the lowest glucose value compared to other treatments, so it can be inferred that this treatment caused less liver activity.

Glycerol is directly absorbed by the rumen epithelium, metabolized in the liver to gluconeogenesis by the action of glycerol kinase enzyme, which converts it into glucose. Part of glycerol can be fermented to propionate in the rumen, which in turn is metabolized to oxaloacetate via the Krebs cycle in the liver and can be used to form glucose by the gluconeogenic pathway. Thus, crude glycerin has potential application as gluconeogenic substrate for ruminants (KREHBIEL, 2008). Therefore, it is suggested that the addition of glycerol to the experimental treatments can alter the blood glucose levels and therefore the energy metabolism of the animals, resulting in better or worse use of nutrients provided in the diet according to the roughage used with the glycerin.

The different forages with or without glycerin changed the parameters evaluated, suggesting energy metabolism changes; however, treatments with hydrolyzed sugarcane resulted in lower hepatic activity compared to other treatments. Corn silage increased liver activity, evidenced by the difference (P = 0.021) between corn silage and other treatments regarding cholesterol levels, and these treatments (corn silage and corn silage with 10% glycerin) yielded the highest values, providing better energy and possibly better weight gain performance.

CONCLUSION

The inclusion of up to 10% glycerin associated with different forages in diets for beef cattle promotes changes in serum biochemical profile of these animals, indicating metabolic changes, especially in the liver, and corn silage displayed the highest hepatic effect.

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