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Effects of high concentrations of crude glycerin on blood biochemical profile of feedlot finishing lambs

[Efeitos de altas concentrações de glicerina bruta no perfil bioquímico sanguíneo de cordeiros terminados em confinamento]

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ABSTRACT

The objective of this study was to evaluate changes in the biochemical parameters of feedlot lambs fed with high concentrations of crude glycerin (GC). Forty crossbred lambs (Dorper × Santa Inês), non-castrated, weighing 21.7±2.7kg and 90 days old, were randomly assigned to four treatments (0, 10, 20, and 30% GC in the diet). To evaluate the biochemical parameters, blood samples (pre-prandial and 4 h after morning feeding) were collected on the first day and the day before slaughter, in each treatment. The high-density lipoprotein was linearly decreased (P<0.0001) when GC concentrations were increased in the diet. Alkaline phosphatase was quadratically affected by treatments (P=0.05), with greater values observed in blood from animals fed intermediate concentrations of CG, and gamma-glutamyl transferase was linearly increased (P=0.008). The blood urea was quadratically affected by treatments with lower values observed in blood from animals fed intermediate concentrations of CG. In conclusion, the replacement of up to 30% (based on dry matter) of corn grain by crude glycerin, in diets for feedlot finishing lambs, does not compromise the health status of these animals.

Keywords: Blood, by-product, glycerol, sheep

RESUMO

O objetivo deste estudo foi avaliar as alterações nos parâmetros bioquímicos de cordeiros confinados, alimentados com elevadas concentrações de glicerina bruta (GC). Quarenta cordeiros mestiços (Dorper × Santa Inês), não castrados, com 21,7±2,7kg de peso e 90 dias de idade, foram distribuídos aleatoriamente em quatro tratamentos (0, 10, 20 e 30% de GC na dieta). Para avaliar os parâmetros bioquímicos, amostras de sangue (pré-prandial e 4h após a alimentação matinal) foram coletadas no primeiro dia e no dia anterior ao abate, em cada tratamento. A lipoproteína de alta densidade no sangue diminuiu linearmente (P<0,0001) quando as concentrações de GC foram aumentadas. A fosfatase alcalina foi afetada quadraticamente pelos tratamentos (P=0,05), com valores maiores observados no sangue de animais alimentados com concentrações intermediárias de GC, e a gamaglutamiltransferase aumentou linearmente (P=0,008). A ureia sanguínea foi afetada de forma quadrática pelos tratamentos, com valores menores observados no sangue de animais alimentados com concentrações intermediárias de GC. Em conclusão, a substituição de até 30% (com base na matéria seca) do grão de milho por

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glicerina bruta, em dietas para cordeiros em terminação em confinamento, não compromete o estado de saúde desses animais.

Palavras-chave: coprodutos, glicerol, ovinos, sangue

INTRODUCTION

The expansion of the biodiesel industry worldwide leads to a large surplus of crude glycerin (CG) in the market, which is its major by-product. Approximately 10% of all products from the biodiesel industry become CG (Dasari *et al*., 2005). This by-product has been studied in animal feeding due to its energy value, similar to corn grain (Donkin, 2008). This fact, coupled with the increasing cost of corn grain, especially owing to the progressive use of corn in ethanol production, makes crude glycerin a valuable alternative feedstuff for livestock. Despite taking advantage of CG as an alternative feed for feedlot lambs, contaminants, and impurities, such as methanol (Thompson and He, 2006) may contribute to metabolic changes in animals.

The influence of crude glycerin on the biochemical parameters of feedlot cattle (Van Cleef *et al*., 2014) and sheep (Ribeiro *et al*., 2018), has already been reported. However, to our knowledge, the inclusion of high levels of CG and its effects on the biochemical parameters of blood from ruminants has not yet been demonstrated. In this sense, biochemical profiles help the comprehension of metabolic changes (Mojtahedi and Mesgaran., 2011) and the diagnosis of metabolic diseases (Jaramillo-López *et al*., 2017). Moreover, they bring information on the nutrition and health status of animals (Silva *et al*., 2018).

We hypothesized that elevated concentrations of crude glycerin fed to lambs could cause significant changes in the animals' metabolism, which would be translated into changes in blood biochemical parameters. Therefore, the objective of this study was to evaluate the changes in biochemical parameters of feedlot lambs fed elevated concentrations of crude glycerin.

MATERIAL AND METHODS

The trial was carried out at the Department of Animal Science, São Paulo State University, Jaboticabal, Brazil (FCAV/Unesp). All procedures were evaluated and approved by the FCAV/Unesp Institutional Animal Care and Use Committee (Protocol 06329/14).

Forty crossbred (Dorper × Santa Inês), uncastrated male lambs, weighting 21.7±2.7kg and 90 days old, were blocked (initial weight) and randomly assigned to four treatments, containing increasing concentrations of CG (0, 10, 20, and 30%). The experimental diets were labeled as CON (no crude glycerin), G10 (addition of 10% crude glycerin, in DM basis), G20 (addition of 20% crude glycerin, in DM basis), and G30 (addition of 30% crude glycerin, in DM basis), as shown in Table 1.

In treatment G30, all corn was replaced by crude glycerin. Diets were formulated according to NRC (Nutrient…, 2007), to have approximately 17.7% CP and 2700 kcal ME/kg DM and to achieve average daily weight gains of 250g. The crude glycerin used in the present experiment was donated from a commercial oil and meal industry, and its composition was: 83% glycerol, 95% DM, 1.1% CP, 6% salts, and less than 0.1% methanol.

The animals were housed in individual pens ($1.2m^2$), which were equipped with feed bunks and waterers, under a hoofed area. Upon arrival, lambs were vaccinated and dewormed and were fed *ad libitum* exclusively corn silage, for 7 days. After this period, animals were adapted to experimental diets for 14 days, using three step-up diets, increasing the concentrate level (20, 40, and 60%). Lambs were fed experimental diets for 42, 41, 44, and 51 days (CON, G10, G20, and G30, respectively) until they reached approximately 35kg BW, when they were slaughtered.

Animals were submitted to blood sampling on the first day of the experimental period and on the day before the slaughter, two times (pre-prandial and 4h after morning feeding). Blood samples were obtained by jugular venipuncture, using vacuum tubes (BD Vacutainer, Becton Dickinson Ind. Cirúrgicas Ltda., Juiz de Fora, Minas Gerais, Brazil). All analyses were performed according to the manufacturer's instructions for each kit (Labtest Diagnóstica, Belo Horizonte, Minas Gerais, Brazil) and analyzed using an automatic spectrophotometer (Labmax Plenno, Labtest, Belo Horizonte, Minas Gerais, Brazil).

Table 1. Ingredient and chemical composition of diets containing 0 (G0), 10 (G10), 20 (G20), or 30% (G30) of crude glycerin

	Treatments			
Item	G0	G10	G20	G30
Ingredient composition (%)				
Corn silage	40.0	40.0	40.0	40.0
Corn cracked grain	30.0	20.0	10.0	0.0
Soybean hulls	7.8	7.2	6.3	4.5
Soybean meal	20.6	21.0	21.6	23.1
Urea	0.6	0.9	1.1	1.3
Crude glycerin	0.0	10.0	20.0	30.0
Mineral/vitamin premix[a]	0.5	0.5	0.5	0.5
Limestone	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.0	0.0	0.0	0.2
Nutrient calculated composition				
DM, %	65.8	66.1	66.4	66.6
CP, %	17.7	17.7	17.7	17.7
ME, Mcal/kg	2.8	2.8	2.7	2.7
EE, %	3.0	2.7	2.3	2.0
aNDF, %	34.8	33.0	31.1	28.7
ADF, %	19.2	18.5	17.7	16.5
Ca, %	0.5	0.5	0.5	0.5
P, %	0.3	0.3	0.3	0.3

[a] Composition per kg: P (75g), Ca (223g), S (10g), Zn (3g), Na (60g), Co (20mg), I (40mg), Se (24mg), F (750mg), Mg (5g), Mn (1.8g), Fe (402mg), Vit A (312,500UI), Vit D (50,000UI), Vit E (437UI).

Samples from tubes without anticoagulant were used for analyses of serum total protein (PROT), albumin (ALB), urea, uric acid (URAC), total creatinine (CREAT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), creatine kinase (CK), cholesterol (CHOL), triglycerides (TRIG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL). Samples from tubes containing fluoride were used for the evaluation of blood plasmatic glucose (GLUC). The globulin (GLOB) was estimated as the difference between total protein and albumin, and the G:A as the ratio between globulin and albumin.

Data were analyzed as a randomized complete block design using the R® software (R Foundation for Statistical Computing, Vienna, Austria). The models included effects of treatments (G0, G10, G20, and G30), sampling time (pre-prandial, post-prandial), and the treatment × sampling time interaction. Orthogonal contrasts were used to evaluate the linear and quadratic effects of the inclusion of crude glycerin in the diet on the blood parameters of the lambs. The data from day 1 was used as a covariate for all blood parameters evaluated. Treatment means were computed as least square means and significance was defined as P<0.05.

RESULTS

Increasing concentrations of crude glycerin (0, 10, 20, and 30%, for 42, 41, 44, and 51 days, respectively) in a 40:60 roughage:concentrate ratio, on a dry matter basis, were included in diets for feedlot lambs. However, no differences were observed in any of the blood parameters between sampling times on the same day (pre-prandial and post-prandial, P>0.05). Furthermore, no interaction between the time of sampling and treatments was observed (P>0.05).

Blood parameters of energy metabolism GLU, TRIG, CHOL, LDL, VLDL were not altered (P>0.05), averaging 86.58, 11.44, 43.14, 27.68, and 2.32g/dL, respectively (Table 2), neither blood enzymes CK and AST were affected by experimental treatments (P>0.05), presenting averages of 125.30 and 91.88U/L, respectively (Table 3).

On the other hand, HDL showed a linear reduction (P<0.0001) from 14.63 (G0) to 11.84g/dL (G30) as shown on Table 2, and the ALP was quadratically affected by treatments, with greater value observed in animals fed G10, and GGT was linearly increased from 81.76 (G0) to 95.26U/L (G30), or 16.5% increase (P=0.008).

The blood parameters of protein metabolism PROT, ALB, GLOB, G:A, CREAT, and URAC were not affected by the increasing inclusions of GC in the diets, presenting averages of 6.79g/dL, 2.83g/dL, 3.95g/dL, 0.72, 0.60mg/dL, and 3.02mg/dL, respectively (Table 4). Nonetheless, the urea level was quadratically affected by the CG inclusion, with greater values observed in animals fed G0 and G30 (P = 0.03).

Table 2. Blood parameters of energy metabolism from feedlot lambs fed 0, 10, 20, or 30% crude glycerin

Item, g/dL	Treatment				SEM	P-value	
	G0	G10	G20	G30		L	Q
GLU	85.68	85.51	83.74	91.38	0.75	ND	ND
TRIG	12.63	12.00	10.07	11.07	0.96	ND	ND
CHOL	45.95	46.03	39.28	41.31	0.77	ND	ND
HDL	14.63	14.05	12.07	11.84	0.22	< 0.0001	0.90
LDL	28.79	29.59	25.20	27.13	0.64	ND	ND
VLDL	2.52	2.40	2.01	2.35	0.19	ND	ND

G0 = Diet without crude glycerin, G10 = 10% crude glycerin in diet's DM. G20 = 20% crude glycerin in diet's DM, G30 = 30% crude glycerin in diet's DM, GLU = Glucose; HDL = High-density lipoprotein; CHOL = Total cholesterol; LDL = Low-density lipoprotein; VLDL = Very-low-density lipoprotein; TRIG = Triglycerides; ND = no treatment difference.

Table 3. Blood enzymes from feedlot lambs fed 0, 10, 20, or 30% crude glycerin

Item	Treatment				SEM	P-value	
	G0	G10	G20	G30		L	Q
CK	143.89	118.57	116.21	122.52	15.82	ND	ND
AST	91.21	91.76	80.64	103.89	1.92	ND	ND
ALP	329.00	409.43	328.07	323.95	8.09	0.37	0.05
GGT	81.76	84.57	83.50	95.26	0.88	0.008	0.25

G0 = Diet without crude glycerin, G10 = 10% crude glycerin in diet's DM. G20 = 20% crude glycerin in diet's DM, G30 = 30% crude glycerin in diet's DM, CK = Creatine kinase (U/L); AST = Aspartate aminotransferase (U/L); ALP = alkaline phosphatase (U/L); GGT = Gama glutamyl transferase (U/L); ND = no treatment difference.

Table 4. Blood parameters of protein metabolism from feedlot lambs fed 0, 10, 20, or 30% crude glycerin

Item	Treatment				SEM	P-value	
	G0	G10	G20	G30		L	Q
PROT	6.93	6.75	6.65	6.81	0.05	ND	ND
ALB	2.92	2.86	2.79	2.76	0.03	ND	ND
GLOB	4.00	3.89	3.87	4.05	0.04	ND	ND
G:A	0.72	0.74	0.72	0.70	0.01	ND	ND
CREAT	0.61	0.58	0.59	0.62	0.006	ND	ND
UREA	44.68	44.33	46.43	53.90	0.63	0.0001	0.03
URAC	3.02	3.02	3.01	3.03	0.09	ND	ND

G0=Diet without crude glycerin, G10 = 10% crude glycerin in diet's DM. G20 = 20% crude glycerin in diet's DM, G30=30% crude glycerin in diet's DM, PROT = Total protein (g/dL); ALB = Albumin (g/dL); GLOB = Globulin (g/dL); G:A = Globulin/albumin ratio; CREAT = Creatinine (mg/dL); UREA = Urea (mg/dL); URAC = Uric acid (mg/dL); ND = no treatment difference

DISCUSSION

To date, there are only a few studies reporting information on the energy metabolism of lambs fed CG (Gunn *et al*., 2010) or a more comprehensive approach to animal health [5]. Moreover, glycerin levels in these studies were more conservative than the current study (18 and 21% on a dry matter basis, respectively), but because of its cost-benefit, crude glycerin has been proven as a suitable ingredient in diets for lambs (Van Cleef *et al*., 2018). Therefore, higher levels of inclusion of this by-product are safe and can improve the profitability of the production system, but a better understanding of its influence on the metabolism of feedlot lambs is needed.

The intake of glycerol activates the fermentation in the rumen leading to a larger production of propionate, acetate, and butyrate acids (Trabue *et al*., 2007). Propionate is, then, converted to glucose in the liver through gluconeogenesis (Werner *et al*., 2015). Therefore, higher concentrations of crude glycerin would increase the concentration of blood glucose. Indeed, the total replacement of corn with crude glycerin (G30) led to the greatest concentration of blood glucose (91.38mg/dL) in the present study. Moreover, the values found are greater than the range suggested as normal for sheep, from 50 to 80mg/dL (Kaneko, 2008).

The concentration of blood glucose was not affected by the increasing concentrations of CG in the current study. On the other hand, studies demonstrated a linear decrease of blood glucose in lambs also fed CG up to 21% DM basis, which was explained by the authors as an outcome related to an early satiety mechanism, hence lower dry matter intake (Ribeiro *et al*., 2018).

The energy metabolism of ruminants might also be evaluated through the evaluation of the blood concentration of CHOL (Kessler *et al*., 2014). In the present study, the increase in liver activity led to greater concentrations of CHOL and lipoproteins. However, an increase in CHOL was also observed in the diet with no glycerin (G0), which, therefore, indicates that not only the CG was responsible for changes in the concentrations of these blood parameters.

The high-density lipoproteins, which were linearly reduced from 14.63 to 11.84mg/dL herein, are responsible for the reverse CHOL transport from the extrahepatic tissues to the liver. On the other hand, low-density lipoproteins are responsible for the transport of CHOL in blood and its delivery from the liver to the peripheral tissues, and their concentrations were not influenced by the inclusion of CG (Kessler *et al*., 2014). Although concentrations of total CHOL were not altered, they were lower than the suggested normal for sheep (Kaneko, 2008), as well as those concentrations reported in lambs fed CG.

Changes in the nutrient composition of diets for ruminants due to the addition of CG were reported (Gomes *et al*., 2015). The present study showed decreasing percentages of fiber fractions and ether extract, as corn grain was replaced by CG in the diets (Table 1). Therefore, the lowest intake of lipids when corn grain was replaced by 30% of CG, led to lower synthesis of CHOL by the liver, and its transport through the blood by lipoproteins. It explains the lowest concentrations of blood CHOL and lipoproteins in the blood of lambs fed higher concentrations of CG. Similar results were reported when corn grain was replaced by CG in diets for cattle (Van Cleef *et al*., 2014) and when different lipid sources were evaluated in diets for lambs (Silva *et al*., 2014).

The activity of the liver has also been evaluated by the concentration of hepatic enzymes of ruminants fed CG, such as AST, ALP, and GGT (Ribeiro *et al*., 2018; Ezequiel *et al*., 2015). The inclusion of increasing concentrations of CG has linearly increased the activity of GGT in the present study. Furthermore, the serum concentrations of GGT were greater than the interval suggested as normal for sheep, from 20 to 50mg/dL (Nutrient…, 2007) and greater than the values recently reported for sheep also fed CG (Kaneko, 2008).

Increased concentrations of serum GGT indicate cholestasis and hepatobiliary disease (Braun *et al*., 2010). Despite high concentrations of GGT reported in the present study, liver damage may not be related to the inclusion of CG, because other signs of hepatic failure were not observed in the animals. Moreover, the concentration of GGT in the diet without CG (G0) was also greater than the interval suggested as normal for sheep (81.76mg/dL).

The activity of the enzyme alkaline phosphatase (ALP) might be influenced by the animal growing process. This enzyme has great activity in the bone tissue of lambs, mainly in the first month of life (Sousa *et al*., 2014). Greater ALP activity was also reported in goats up to 6 months old (Silva *et al*., 2004). Even though the concentration of serum ALP has increased over the interval suggested as normal for sheep (Kaneko, 2008), when 10% of CG was included in the diet (G10), lower serum concentrations (323.95U/L) were found when 30% of crude glycerin was included (G30). Therefore, the inclusion of increasing concentrations of CG was not the cause of the slight changes in ALP activity.

To detect hepatic abnormalities, the measurement of aspartate aminotransferase (AST) activity is also an important tool. This enzyme has key roles in gluconeogenesis and the formation of urea (Tennant and Center, 2008). Indeed, the greatest concentrations of AST (103.89U/L) and urea (53.90mg/dL) were observed when 30% (G30) of CG was included in the diet, highlighting the increasing activity of the liver. However, the concentrations of AST and urea are within the range suggested as normal for sheep (Kessler *et al*., 2014).

The blood concentration of urea is highly influenced by crude protein intake (Braun *et al*., 2010; Wallace *et al*., 2006). Although the diets were isonitrogenously formulated, the greatest concentration of urea in the G30 diet (1.3%) might lead to a greater concentration of blood urea in the animals fed this diet. Additionally, blood creatinine concentrations, which are expected to increase under kidney abnormalities (Braun *et al*., 2010), were neither increased nor influenced by the diets. Therefore, kidney function was considered normal and healthy.

CONCLUSIONS

The present study demonstrated the suitability of the substitution of up to 30% (dry matter basis) of corn grain by crude glycerin, in diets for feedlot finishing lambs, without compromising the health of these animals.

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