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BIOCHEMICAL ENERGY AND HEMATOLOGICAL PROFILE OF SHEEP SUPPLEMENTED WITH SOY MOLASSES

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ABSTRACT

Soy molasses is a by-product of the soy industry, with low commercial cost and rich in carbohydrates and proteins. Despite the great variety of by-products and being potentially beneficial, it is not fully understood how the interaction between the nutrients in this product and the physiological changes in sheep occurs. Thus, the objective was to evaluate the effects of supplementation levels with soy molasses on the energy metabolites and hematological parameters of sheep. The treatments consisted of 0, 3, 6, 9, and 12% of inclusion of soy molasses in dry matter basis for a basal diet of corn silage, which met the nutritional needs of the sheep in maintenance, distributed in a 5 × 5 Latin square design. The energy metabolites evaluated were: cholesterol, triglycerides, very-low-density lipoproteins (VLDL), and fructosamine. The hematological parameters were: red blood cells, hemoglobin, hematocrits, and platelets, in addition to leukocytes, rods, neutrophils, eosinophils, monocytes, and lymphocytes. Supplementation with soy molasses did not significantly alter (P> 0.05) the serum concentrations of cholesterol, triglycerides, VLDL, and fructosamine. There was no significant difference in the blood cell, hemoglobin, hematocrit, and platelet concentrations in sheep receiving soy molasses supplementation (P> 0.05), which may indicate that the level of inclusion was sufficient to meet the nutritional needs of the animals, without damage. Leukocyte, rod, neutrophil, eosinophil, monocyte, and lymphocyte concentrations did not change

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(P> 0.05) with the inclusion of soy molasses in the sheep's diet, indicating the absence of intoxication, diseases, and infections. It can be concluded that supplementation with soy molasses does not alter the energy metabolites and hematological parameters of sheep in maintenance.

Keywords: agro-industrial by-products, metabolites, sheep.

INTRODUCTION

The by-products are agro-industrial residues from the processing of plant products, such as flour, seeds, pulp and husks generated on a large scale with potential for use in animal feed (OLIVO et al., 2017). Among the main interests of the use by-products of food industry in the animal food, there is a reduction in the cost of diets, through the use of regional products, reductioning the inclusion of commodities. Among the by-products used in animal feed, soy molasses comes from the soy industry, which has a low commercial cost and implies environmental problems when disposed of incorrectly (RODRIGUES et al., 2017). Several forms of use are proposed for molasses, such as in fermentation processes due to its high sugar content (SIQUEIRA et al., 2008) and in animal nutrition, to improve the quality of the final product (MILETIĆ et al., 2017; RODRIGUES et al., 2020).

Soybean molasses is rich in carbohydrates, produced through the alcoholic extraction of the sugars present in the defatted bran, such as glucose, fructose, sucrose, raffinose and stachyose. In addition, it has a glutinous texture, brownish color and a bittersweet flavor, which can influence the palatability when used in animal feed (SQUEIRA et al., 2008). Despite a wide variety of by-products, its benefits are obtained through the interaction between nutrients and animal physiology, in other words, digestion, absorption and use of metabolites (MARTINS et al., 2000), which makes prediction difficult results when offered as a constituent of the diet.

According to Silveira et al. (2012), through energy metabolites it is possible to predict the energy balance of the animal as a form of nutritional assessment, determining their presence in the bloodstream in animals. In this way, the energy metabolite indicators are adequate to understand the use of soybean molasses in sheep diets. In addition, energy parameters aim to assess energy balance and indicate the degree of deposition and mobilization of energy reserves, associated with lipid metabolism (FERNANDES et al., 2012). The cholesterol concentrations affect energy metabolism in the liver, especially about the export of lipids in the form of very low-density lipoproteins (VLDL) (NDLOVU et al., 2007). As with fructosamines, which are proteins glycosylated by a non-enzymatic mechanism and markers of the average blood glucose concentration, the concentrations of energy metabolites are also not influenced by momentary changes in the glycemic profile (ARMBRUSTER, 1987; FILIPOVIC et al., 2011; LIMA et al., 2016).

The inclusion of certain alternative foods may be potential causes of metabolic disorders, due to interferences in the hematological composition, and clinical hematological monitoring being able to indicate possible imbalances in nutrient homeostasis (BEZERRA et al., 2013). Thus, the tool can be used to assess the feasibility of use and level of inclusion of by-products in the diet for both humans and animals (VIANA et

al., 2002). Therefore, we hypothesize that soy molasses supplementation alters the energy metabolites and hematological parameters of sheep in maintenance. Thus, the objective was to evaluate the effects of levels of soy molasses supplementation on energy metabolites and hematological parameters of sheep.

MATERIAL E METHODS

All procedures were carried out in accordance with the Ethics Committee for the Use of Animals for Research at the Federal University of Uberlândia, under license number 069/14. The experiment was carried out in the goat and sheep sector of the Federal University of Uberlândia, in the city of Uberlândia, Minas Gerais, Brazil.

Five crossbred ewes in maintenance ½ Dorper x ½ Santa Inês with mean body mass of 45 ± 3.5 kg and 12 ± 2 months of age, distributed in a 5 × 5 Latin square design, were used. The ewes were housed in individual metabolic cages of 2m2, provided with feeders and drinkers, following the recommendations of the National Institute of Science and Technology (INCT).

The treatments consisted of 0, 3, 6, 9 and 12% inclusion of soybean molasses on a dry matter basis for a basal corn silage diet, which met the nutritional needs of sheep in maintenance (NRC, 2007). The composition and energy content of the diet ingredients is presented in Table 1. Food samples were collected daily and subsequently analyzed to determine the concentrations of ash, crude protein, dry matter and crude energy (DETMANN, 2012). Neutral detergent fiber concentration was performed according to Van Soest et al. (1991).

Table 1. Composition and energy concentration of diet ingredients

Chemical composition	Soy molasses	Corn silage
Dry matter (DM) (g/kg)	650	260,1
Crude Protein (g/kg de DM)	110,0	70,4
Neutral detergent fiber (g/kg de DM)	0	350,4
Mineral Matter (g/kg de DM)	160,8	60,2
Gross energy (kcal/kg)	4,1	3,9
Ca (g/kg de DM)	3,9	-
Cu (mg/L)	19,6	-
Fe (mg/L)	55,3	-
K (K_2O) (g/kg de DM)	20,2	-
Mg (g/kg de DM)	2,1	-
Na (mg/L)	160,6	-
P (P_2O_5) (g/kg de DM)	80,5	-
Zn (mg/L)	52,2	-

The diets were offered twice a day (08:00 and 16:00). The amount of feed provided was corrected daily to provide 10% leftovers based on natural matter.

The experimental period lasted 60 days and was divided into five periods of 12 days. From the 1st to the 5th day, the adaptation period took place, based on the stabilization of consumption. Blood samples were collected from the 8th to the 12th day of each experimental period, before the first feeding, by jugular vein puncture, in tubes without anticoagulant. After collection, blood samples were immediately centrifuged at 2,700 × g for

20 min, and plasma samples were frozen at -18 °C for further analysis. Each sample was used as a replicate, calculating the average per experimental period.

Plasma samples for the metabolites were performed with the specific semi-automatic Bio-2000 equipment with commercial Labtest kits. The energetic metabolites analyzed were cholesterol, triglycerides, VLDL and fructosamine. The analyzes of the hematological profile were performed on an automatic analyzer Cobra Integra, Roche Diagnostics – Basel, Swizertland. The hematological profile considered the concentrations of red blood cells, hemoglobins, hematocrits, platelets, leukocytes, and the percentage of rods, neutrophils, eosinophils, monocytes and lymphocytes.

The data obtained were analyzed following the mixed statistical model:

$$Y_{ijkl} = \mu + \tau_i + P_j + (\tau_i * P_j) + A_k + \varepsilon_{ijkl}$$

Where: Y_{ijkl} = variable ijkl; μ = general mean; τ_i = treatment effect i; P_j = efeito fixo de period effect j; $(\tau_i * P_j)$ and interaction A_k = animal random effect k; ε_{ijkl} = random error. Analysis of variance was performed considering 5% significance (P<0.05). When relevant for comparison between means, the Tukey test was used at 5% significance (P<0.05). All analyzes were performed using the SAS software.

RESULTS AND DISCUSSION

Energy metabolites

Since soy molasses is rich mainly in carbohydrates, such as the monosaccharides glucose and sucrose (GRIJÓ and MELO, 2017), its use is aimed at energy supplementation to replace grains (Rodrigues et al., 2020). However, soy molasses supplementation did not significantly alter (P>0.05) the serum concentrations of cholesterol, triglycerides, VLDL and fructosamine (Table 2), with the analyzed parameters remaining within the reference values for the species, according to with Silva et al. (2020).

Although soy molasses is an energy alternative, ewes in maintenance have lower nutritional requirements compared to growing ewes (NRC, 2007). Due to the control between requirement, consumption and homeorhetic mechanisms, the energy metabolism for gain does not change, and consequently, the energy biochemical profile is maintained even with the increasing inclusion of soy molasses in the diet.

Serum concentrations of different metabolites are widely used to assess the nutritional status of the animal. As an example, there is cholesterol, which changes its concentration when animals consume amounts of nutrients below their nutritional requirements (NDLOVU et al., 2007). Therefore, the lack of difference in cholesterol concentrations between treatments imply the same nutritional status of ewes consuming up to 12% of soy molasses in the diet.

Elevated serum triglyceride levels are associated with a positive energy balance when the metabolic pathway of lipogenesis is activated (FERNANDES et al., 2012). Values within the reference values indicate that the

energy balance was the same for all treatments and met the nutritional requirements.

VLDL are lipoproteins that carry the function of transporting triglycerides and cholesterol to storage and use sites (SANTOS et al., 2017). The ability of the liver to synthesize VLDL is stimulated when eating a diet rich in non-fibrous carbohydrates (NFC). The basic diet of the sheep was corn silage, a roughage characterized by a low concentration of NFC (33.66%) (VALADARES FILHO et al., 2017). For this reason, the inclusion of up to 12% of soy molasses did not change the VLDL concentration.

Table 2. Energetic parameters of sheep fed with increasing levels of soy molasses

Treatment	Cholesterol (mg dL-1)	Triglycerides (mg dL-1)	VLDL (mg dL-1)	Fructosamine (mg dL-1)
0%	51,72	9,12	1,82	196,50
3%	58,52	8,60	1,72	201,40
6%	57,88	8,68	1,73	180,40
9%	59,24	9,00	1,80	189,80
12%	61	8,56	1,71	201,20
RV*	14 -126	5 -71	1 – 16,4	119-451
p-valor	0,33	0,97	0,004	0,005

* RV: Reference values. Source: Silva et al. (2020). VLDL: very low-density lipoproteins.

The maintenance of energy metabolism between treatments was also observed through the concentration of fructosamine. According to Kaneko (2008), fructosamine is a metabolite positively correlated with glucose concentrations.

Hematological profile

There was no significant difference in the concentrations of red blood cells, hemoglobins, hematocrits and platelets in sheep with soy molasses supplementation (P>0.05) (Table 3).

Hematological parameters are still poorly explored in sheep production. Studies such as de LIMA et al. (2015) emphasize the importance of these parameters in the evaluation of the hematological profile and its relationship with hormone production. MADUREIRA et al. (2013) report that the hematological profile can vary between age, management, food and environment.

The concentration of red blood cells, hematocrit and platelets are within the reference values for the species (Table 3). The mean hemoglobin concentration of 7.28 was below the minimum recommended by KRAMER (2006).

Red blood cells have the essential function of transporting oxygen, and hemoglobins are its main components. Hematocrit and platelets act in the blood clotting process and their values help in the interpretation of homeostasis (GOMES, FODRA and MASSABNI, 2021).

Hematological parameters, such as red blood cell and hematocrit concentrations, are affected by failure to meet the nutritional requirements of animals (BEZERRA et al., 2013), in the same way that the reduction in hemoglobin concentration is linked to energy deficiency (PEREIRA et al., 2015).

Therefore, according to the results found for the hematological parameters (Table 2), it is suggested that the ewes supplemented with increasing levels of soy molasses did not present an energy deficit, which may be linked to a lag between the reference values used as a base with those observed in the study.

As the concentrations of leukocytes, rods, neutrophils, eosinophils, monocytes and lymphocytes did not show significant changes (P>0.05) (Table 4), the inclusion of up to 12% of soy molasses in the diet of the sheep, in this aspect, may be recommended.

Table 3. Hematological parameters of sheep supplemented with soy molasses

treatments	Red Cells (100 µ L^{-1})	Hemoglobins (g dL^{-1})	Hematocrit (%)	Platelets (100 µ L^{-1})
0%	8,90	6,90	25,60	290.700,00
3%	9,20	7,20	26,90	276.300,00
6%	9,50	7,40	26,50	405.300,00
9%	9,50	7,50	27,30	335.200,00
12%	9,60	7,40	26,90	342.100,00
RV*	8 - 180	8 - 12	22 - 38	300.000 - 800.000
p-valor	0,33	0,33	0,49	0,21

* RV: Reference values (Kramer, 2006)

The leukogram is the blood test that aims to evaluate and quantify the leukocytes, which are the cells responsible for activating the animal's immune system against infections and diseases, being part of the animal's immunity (LEPHERD et al., 2009) and are divided according to their structure in rods, neutrophils, eosinophils, monocytes and lymphocytes. The leukocyte concentration was within the reference values for all treatments, not indicating the presence of diseases and infections (Table 4).

Table 4. Blood parameters of sheep supplemented with soy molasses

Treatments	Leukocytes (10^3 UL^{-1} mm^3)	Rods %	Neutrophils %	Eosinophils %	Monocytes %	Lymphocytes %
0%	7340	1,80	45,20	8,80	4,00	41,20
3%	6830	1,40	54,60	7,00	2,20	35,60
6%	6500	1,40	47,60	9,60	2,80	39,40
9%	6600	0,80	46,00	12,20	2,80	39,00
12%	6050	1,00	47,20	8,00	2,40	42,40
RV*	4000 - 12000	0 - 2	10 - 50	1 - 10	1 - 6	40 - 75
p-valor	-	0,62	0,45	0,13	0,35	0,40

* RV: Reference values (Weiss e Wardrop, 2010)

Neutrophils showed values above the reference for the species for treatment with 3% soy molasses supplementation and lymphocytes showed values below the ideal for treatments with 3, 6 and 9% molasses inclusion in the diet.

Normally, neutrophils are increased when there is the presence of a bacterial or fungal infection, acting on the immune system. According to VIEIRA et al. (2012), the increase in neutrophils may be related to inflammation of the rumen mucosa, caused by the high concentration of lactic acid in the rumen content, triggering the process of ruminitis, which may be caused by excess carbohydrate in the diet and/or lack of adaptation of the animals.

Regarding the values below lymphocytes, which act in the recognition and destruction of infectious microorganisms, it has not yet been described in the literature whether supplementation with soy molasses has an immunostimulating action that can cause its decrease, which may be related to the possible presence of verminosis. VIEIRA et al. (2010) reports that traditional methods of controlling gastrointestinal worms are ineffective for control, especially of helminths. During the study, no methods were carried out to identify possible parasitic resistance, thus, even if the sheep have been dewormed, some parasites may have resistance and continue to cause some degree of anemia in the animals, even without apparent clinical signs.

CONCLUSION

Supplementation with soy molasses up to 12% on a dry matter basis does not alter the energy metabolites and hematological parameters of sheep in maintenance.

PERFIL BIOQUÍMICO ENERGÉTICO E HEMATOLÓGICO DE OVINOS SUPLEMENTADOS COM MELAÇO DE SOJA

RESUMO

O melaço de soja é um subproduto da indústria da soja, com baixo custo comercial e rico em carboidratos e proteínas. Apesar da grande variedade de subprodutos e de serem potencialmente benéficos, ainda não está totalmente elucidado como ocorre a interação entre os nutrientes deste produto e as mudanças fisiológicas em ovinos. Dessa forma, objetivou-se avaliar os efeitos de níveis de suplementação com melaço de soja sob os metabólitos energéticos e parâmetros hematológicos de ovinos. Os tratamentos consistiram em 0, 3, 6, 9 e 12% de inclusão de melaço de soja na base da matéria seca para uma dieta basal de silagem de milho, que atendia as necessidades nutricionais das ovelhas em mantença, distribuídas em delineamento em quadrado latino 5 × 5. Foram avaliados os metabolitos energéticos: colesterol, triglicérides, lipoproteínas de muito baixa densidade (VLDL) e frutosamina. Já os parâmetros hematológicos foram: hemácias, hemoglobinas, hematócritos e plaquetas, além de leucócitos, bastonetes, neutrófilos, eosinófilos, monócitos e linfócitos. A suplementação com melaço de soja não alterou significativamente (P>0,05) as concentrações séricas de colesterol, triglicérides, VLDL e frutosamina. Não houve diferença significativa nas concentrações

de hemácias, hemoglobinas, hematócritos e plaquetas das ovelhas com suplementação de melaço de soja (P>0,05), podendo ser indício que o nível de inclusão foi suficiente para atender as necessidades nutricionais dos animais, sem causar balanço energético positivo. As concentrações de leucócitos, bastonetes, neutrófilos, eosinófilos, monócitos e linfócitos não alteraram (P>0,05) com a inclusão de melaço de soja na dieta das ovelhas, não indicando a presença de intoxicação, doenças e infecções. Conclui-se que a suplementação com melaço de soja não altera os metabólitos energéticos e parâmetros hematológicos de ovinos em mantença.

Palavras chave: metabólitos, ovelhas, subprodutos agroindustriais.

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