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Soybean meal from damaged grains replacing standard soybean meal in diets of feedlot lambs

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ABSTRACT - Two studies were carried out to evaluate the effects of levels of soybean meal produced from damaged soybean grains replacing normal soybean meal on *in vitro* and *in situ* digestibility of ruminant diets as well as on intake, digestibility, and animal performance of growing/finishing lambs. In trial 1, we evaluated the *in vitro* digestibility of each soybean meals (normal and damaged), as well as diets containing levels of the damaged replacing the normal (0, 333, 667 and 1,000 g.kg^{-1}) soybean meal on kinetic parameters of *in vitro* cumulative gas production, *in vitro* dry matter and crude protein digestibility, *in situ* rumen-degradable protein, rumen-undegradable protein, and *in vitro* intestinal digestibility of rumen-undegradable protein. In trial 2, we used 48 growing/finishing lambs to evaluate the effects of damaged soybean meal levels (0, 333, 667, and 1,000 g.kg^{-1}) replacing normal soybean meal in feedlot diets (20:80 roughage:concentrate ratio) on intake and digestibility of nutrients and on animal performance. The damaged soybean meal presented lower values for total gas production and *in vitro* dry matter digestibility than normal soybean meal. Higher rumen-undegradable protein was estimated for damaged soybean meal than for the normal and consequently lower rumen-degradable protein for damaged compared to normal. Because of the lower rumen-degradable protein, damaged soybean meal promoted lower *in vitro* ammonium nitrogen (NH_3-N) concentrations than the normal in feedlot diets. In the *in vivo* trial, there were no effects of damaged soybean meal levels in the diets on intake and digestibility of nutrients (dry matter, organic matter, crude protein, and fiber) as well as on total weight gain, average daily gain, carcass yield, or feeding efficiency. Thus, damaged soybean meal can fully replace the normal one in lamb feedlot diets (in up to of 1,000 g.kg^{-1} of the normal soybean meal) without causing adverse effects on intake and digestibility of nutrients and on animal performance.

Keywords: digestibility, degradable protein, gas production, intake, ruminal degradability, sheep

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1. Introduction

Soybean grains and their byproducts, especially soybean meal (SBM), are the major protein sources worldwide for livestock, including monogastric and ruminant animals. For high-performance animals

such as dairy cows, replacing SBM for other protein sources causes a decrease in animal performance, because SBM presents simultaneously a high rumen-degradable protein, good amino acids (AA) profile, as well as high intestinal digestibility of its crude protein (CP; Santos et al., 1988).

However, the chemical composition and quality of soybean grains and their byproducts is reduced by damage, which may be caused by many factors, including attack by insects and other causes occurring during planting, harvesting, or the drying process (Lehmkuhl, 2011). Soybean grains are classified by Brasil (2007), according to the main types of damage, as heat-damaged, moldy, fermented, germinated, and immature, in which the maximum tolerance limit for these types of damaged grains is 8%, above which the soybean producer is penalized, receiving a lower payment for soybean grains in the industry.

Although every country where soybean is produced has its own rules for grain classification, it must be highlighted that all these rules try to ensure the production of high-quality grains, which includes aspects associated to appearance, composition, and safety of the grains (Brasil, 2007).

According to companies which produce soybean oil and meal, this regulation is necessary because poor-quality soybean grain presents problems during its processing that affect its nutritive value for use in animal nutrition. However, Lehmkuhl (2011) and Andrade (2016) did not find any effect of damage in soybean grains on its oil and CP amount, which allowed us to create the hypothesis that damaged grains do not negatively affect the nutritive value of soybean and its byproducts for use in animal nutrition, such as for ruminant diets.

Thus, the objective of this study was to determine the chemical composition and *in vitro* and *in situ* degradability of dry matter (DM) and CP of damaged soybean meal (79.7% damaged grains of which 60.1% were fermented), its effects on feed intake and nutrient digestibility, as well as on animal performance, by replacing regular soybean meal in feedlot lamb diets.

2. Material and Methods

This research was approved by the local Ethics Committee on the Use of Animals (case number 23108.193858/2017-62).

The two types of soybean grains were obtained by the Associação dos Produtores de Soja e Milho do Estado de Mato Grosso (APROSOJA) and were classified at an oil and soybean production company, located in Cuiabá, as standard (normal), which contained less than 8% of damages (NSBG), while the second one was classified as damaged soybean grain (DSBG), presenting 79.7% of damages (60.1% fermented, 18.2% total of burnt and completely fermented, 1.4% moldy). Both soybean grains (normal and damaged) were processed to produce oil and soybean meal. These soybean meals (normal and damaged) were used in this study and are described as normal soybean meal (NSBM) and damaged soybean meal (DSBM).

2.1. Trial 1: *In vitro* digestibility

To determine the kinetic parameters of *in vitro* cumulative gas production as well as *in vitro* dry matter (IVDMD) and crude protein (IVCPD) digestibility, by the gravimetric method, both for experimental diets and for the two qualities of SBM, these two analyses were carried out simultaneously in two *in vitro* incubation runs; furthermore, *in vitro* assays of both experimental diets and two qualities of SBM (NSBM and DSBM) were carried out together. The four experimental diets were formulated, being one without adding DSBM and other three with levels (0, 333, 667, 1,000 $g.kg^{-1}$) of DSBM replacing NSBM (Table 1). The samples of the concentrate (Table 1) and corn silage were collected, and after that, approximately 0.5 g of mass composed of equivalent to 20% of corn silage and 80% of the concentrate were used, both previously pre-dried (55 °C in forced-air oven) and ground at 1 mm. All incubations were performed in amber glass flasks of 120 mL and water bath with automatic elliptical movement.

Regarding the gravimetric assays of *in vitro* digestibility with SBM, NSBM, and DSBM, the incubations were carried out with nine replicates for each SBM and three pre-established observation times: 24, 48, and 72 h throughout incubation. Six blanks for corrections were used in both assays (IVDMD and IVCPD). According to the recommendations of Cortés et al. (2009), each mass of incubated sample should contain at least 0.15 g of CP. Therefore, following this recommendation, 0.39 g of samples were used in the gravimetric incubations of the two tested soybeans grains. For the experimental diets, sixteen flasks were used, and 0.5 g of sample was weighed into each flask.

For incubation, 40 mL of McDougall's buffer was added into each flask (McDougall, 1948), which had a pH adjusted to 6.8 by CO_2 flushing, and 10 mL of rumen fluid, which was collected from two rumen-cannulated sheep fed a diet based on Bermudagrass (*Cynodon spp*), hay (200 g.kg^{-1} of DM), concentrate (800 g.kg^{-1} of DM), and a mineral mixture. After inoculation, the flasks were sealed with rubber stoppers and aluminum seals and kept in a water bath at 39 °C.

Simultaneously with gravimetric *in vitro* digestibility assays, gas production was recorded at 6, 12, 18, 24, 30, 36, and 48 h over the 72 h of incubation. From these records, the *in vitro* cumulative gas production profiles were obtained to estimate the kinetic parameters of *in vitro* cumulative gas production.

The quantification of *in vitro* gas production was carried out through systematic recording of pressure, in psi (pressure per square inch), using a pressure transducer device (Datalogger Pressure®, Press Data 800, LANA-CENA/USP, 116 Piracicaba-SP).

Conversion from psi to mL was carried out using the regression equation $y = a + bx$, in which the coefficient b of the equation allowed the correction and transformation of pressure (psi) into gas volume (mL) corrected for the barometric pressure of the day and ambient temperature. For this, a known gas volume was injected into a 120-mL flask and kept under the same conditions as the incubated samples. The pressure values corresponding to the known volumes of atmospheric air injected into the flasks, using graduated syringe, were used to obtain the first-order regression equation between gas pressure and volume.

The IVDMD and IVCPD were measured at 24 and 48 h of incubation for two qualities of SBM and only at 48 h for experimental diets with four levels of DSBM replacing NSBM, after inoculum

Table 1 - Ingredients and chemical composition of the experimental diets (dry matter (DM) basis) containing damaged soybean meal (DSBM) replacing normal (NSBM) soybean meal

Item	DSBM level replacing NSBM (g.kg^{-1} of NSBM)			
	0	333	667	1,000
Ingredient (g.kg^{-1} DM in the diet)				
Corn silage	200.0	200.0	200.0	200.0
Ground corn	630.0	630.0	630.0	630.0
NSBM	140.0	93.8	47.6	0.0
DSBM	0.0	46.2	92.4	140.0
Mineral mixture[1]	30.0	30.0	30.0	30.0
Chemical composition (g.kg^{-1})				
Dry matter (DM)	946.4	951.3	950.2	962.8
Crude protein (CP)	157.7	151.7	155.9	156.6
Neutral detergent fiber (NDF)	116.1	127.7	126.2	126.1
Acid detergent fiber (ADF)	44.9	45.7	46.2	43.4
Indigestible NDF (iNDF)	61.1	64.9	63.5	69.8

[1] Mineral mixture commercial mix for sheep, guaranteed levels/kg: Ca, max 160 g, min 125 g; P, 33.5 g; Mg, 31 g; S, 33 g; Co, 122 mg; Fe, 2,550 mg; I, 123 mg; Mn, 1,020 mg; Se, 15 mg; Zn, 6,121 mg; salinomycin, 112 mg; Na, 76 g; F, 335 mg; 506.7 g.kg^{-1} DM as CP from urea.

addition. For these purposes, three, four, and two replicates (flasks) were taken, respectively, for *in vitro* degradability assay of the two qualities of SBM, experimental diets, and the blanks, and after ceasing the fermentation in ice bath, the residual content of each flask were filtered through filter crucibles and dried for 12 h at 105 °C to measure the DM and CP of the undigested residue by rumen microorganisms. After filtering the residues, 15 mL of filtrate from each flask was collected for NH_3-N measurements, which were determined by the micro-Kjeldhal method after distillation with 50% NaOH solution and titration with 0.005 N HCl. Subsequently, these DM and nitrogen (CP) residues were quantified by the analytical methods described in subsection 2.3. Chemical analyses. After 48 h of incubation, three and four flasks for each quality of SBM were incubated for an additional 24 h (i.e., 72 h of incubation) with 6 mL of HCl solution (6.21N) and 2 mL of pepsin solution (50 $g.L^{-1}$) to estimate total degradation of DM and CP according to Tilley and Terry (1963) with adaptations proposed by Cortés et al. (2009). Therefore, the residues were filtered into crucibles, dried, and weighed as previously described.

The two qualities of soybean meal (NSBM and DSBM) were evaluated for an estimation of the intestinal digestibility of the rumen-undegradable protein (RUP) by the three-stage *in vitro* technique, according to Gargallo et al. (2006). Therefore, sample mass of approximately 5.0±0.005 g of each SBM ground with 2 mm sieves were weighed into non-woven textile bags and incubated in the rumen of two rumen-cannulated bulls for 27 h (assuming a fractional passage rate of 0.037 h for bulls on tropical pasture); the animas were kept grazing in paddocks of Marandu grass (*Urochloa brizantha*) pasture, with daily supplementation of concentrate at 1% of body mass. This concentrate supplementation was intended to maximize the diversification and quantity of the rumen microbiota.

After ruminal incubation, the bags were washed in tap water until the water became clear, and then they were pre-dried in a forced-air oven for 48 h at 55 °C and dried at 105 °C for 4 h to obtain the residues. The residual mass, approximately 0.250±0.050 g from each bag, were weighed for analysis of residual protein and 0.5±0.005 g was weighed into non-woven textile bags and incubated in a bottle in a Daisy incubator Ankom with a preheated 0.1 N HCl solution (pH 1.9) containing 1 $g.L^{-1}$ of pepsin (P-7000, Sigma, St. Louis, MO) at 39 °C for 1 h. Subsequently, the bags were incubated with preheated pancreatin solution (0.5 M KH_2PO_4 buffer, pH 7.75, containing 50 ppm of thymol and 3 $g.L^{-1}$ of pancreatin; Sigma P-7545) for 24 h at 39 °C, and the N in the residues was measured. These analyses made it possible to estimate rumen-digestible protein (RDP) and RUP, both in $g.kg^{-1}$ CP. From the sequential incubations in pepsin-HCl and pancreatin, the digestible RUP was estimated in relation to RUP (ID; $g.kg^{-1}$ RUP) and digestible and indigestible RUP based on CP (RUP_{CP}; $g.kg^{-1}$ CP) and DM (RUP_{DM}; $g.kg^{-1}$ DM) incubated.

2.2. Trial 2: Intake and performance

The *in vivo* field trial was carried out from August to October 2017 in Santo Antônio de Leverger, MT, Brazil, located 30 km from the state Capital, Cuiabá. During the study, the average temperature was 27 °C, and average humidity and precipitation were 67% and 2.8 mm, respectively.

The experimental diets were composed of corn silage (20% DM basis) and concentrate (80% DM basis), the latter composed of ground corn, mineral mixture, and levels of DSBM replacing NSBM. The replacement levels applied were 0, 333, 667, and 1,000 $g.kg^{-1}$ NSBM (Table 1).

Forty-eight three-month-old crossbreed lambs (Santa Inês × Dorper), non-castrated males, with an initial body weight of 25.20±2.46 kg, were distributed in 24 pens (two lambs per pen) of 4.1 m^2 and concrete floor, containing a feed bunk and water fountains. Initially, the animals were vaccinated against clostridium, weighed, identified by ear tags, and treated against worms. The experiment was carried out using a completely randomized block design with the initial body weight as block criteria; hence, two blocks were formed. The experimental diets were formulated with a 20:80 roughage:concentrate ratio (DM basis) using corn silage as roughage source. Such experimental diets were formulated to contain 14% CP considering the nutrient requirements for growing crossbreed

lambs with a potential of average daily gain (ADG) of 250 g.day^{-1}, according to equations suggested by Cabral et al. (2008a).

The study lasted 90 days, with the initial 30 days used to adapt the animals to the experimental diets (20:80 roughage:concentrate ratio) and pens, and the remaining 60 days to evaluate feed intake, digestibility, and animal performance. The meals were offered twice per day (08.00 and 15.00 h) as total mixed diet. Furthermore, daily intake was monitored and regulated daily to contain between 10 to 15% of orts during all experimental phases. Thus, DM intake (DMI) was obtained by the difference between the amount of DM offered and respective orts.

The measurements of nutrient intake and digestibility were performed in two sampling periods. The components of the total mixed diet (silage corn, concentrates), orts, and feces were sampled. The first sampling period was done between the 32nd and 34th days, and the second was between the 55th and 57th days of the experiment phase. Throughout the sampling period, samples of orts were collected daily from each pen, prior to the morning meals, while fecal samples were obtained individually from each animal directly from the rectum at 09.00 and 16.00 h. To estimate the digestibility of nutrients, indigestible neutral detergent fiber (iNDF) in feces was used as an internal indicator to estimate fecal excretion according to Cabral et al. (2008b).

All samples collected were kept at −20 °C until analysis. Samples of corn silage, orts, and feces were pre-dried in a forced-air oven at 55±5 °C for 72 h and then ground in a Wiley mill using a 1-mm sieve. Fecal samples were analyzed individually per animal and per period.

Samples (ingredients, orts, and feces) were collected and placed in plastic bags. In the case of SBM, a grain sampler was used at different points in the bag to obtain a composite sample from each SBM, then they were homogenized, identified, and sent for analysis.

To evaluate the animal performance in terms of total body weight gain (TBWG) and ADG, all lambs were weighed at the beginning (initial body weight; IBW) and the end (final body weigh; FBW) of the experiment (60-day duration), after fasting from solid feed for 16 h. In addition, to monitor the animal growth, the animals were weighed every 30 days but without subjecting them to fasting. The TBWG was calculated as the difference between the IBW and FBW, while ADG was calculated by dividing the TBWG by the duration of the experiment (60 days). Feed efficiency (FE) was calculated as the ratio between TBWG and DMI, considering the data collected during the last 60 days of the experimental period.

At the end of the experimental phase, all lambs were slaughtered, and the carcass weight was used to calculate carcass yield (CY), which was calculated considering the ratio between the hot carcass weight (HCW) and FBW.

2.3. Chemical analyses

The AA profiles of the NSBM and DSBM were assayed using Near Infrared Reflectance Spectrophotometer (NIRS) (Table 2). The quantitative analyses of aflatoxins B1, B2, G1, G2, and M1 were carried out by a specialized laboratory (Samitec – Soluções Analíticas Microbiológicas e Tecnológicas Ltda).

The contents of DM (method 967.03; AOAC, 1990) and ash (method 942.05; AOAC, 1990) were determined. Organic matter (OM) was determined by the difference between DM and ash. In CP content determination, some adaptations were adopted: the sample mass (0.25 g) was digested with 5 mL of H_2SO_4 and 1 g of a 56:1 mixture of Na_2SO_4 and $Cu_2SO_4.5H_2O$ in micro-Kjeldhal tubes using aluminum digestion blocks according to the guidelines outlined in method 984.13 (AOAC, 1990). The evaluations of neutral detergent fiber (NDF) were performed according to procedure “B” suggested by Van Soest et al. (1991) (Table 3). The iNDF was calculated according to Valente et al. (2011).

Table 2 - Amino acid profile of normal (NSBM) and damaged (DSBM) soybean meal

Amino acid	NSBM (%DM)	DSBM (%DM)	NSBM (%CP)	DSBM (%CP)	Difference (%DM)
Methionine	0.668	0.659	1.279	1.272	−1.347
Cystine	0.712	0.690	1.363	1.332	−3.089
Met+Cys[1]	1.356	1.324	2.597	2.557	−2.359
Lysine	2.990	2.951	5.726	5.700	−1.304
Threonine	1.986	1.950	3.803	3.766	−1.812
Tryptophan	0.657	0.654	1.258	1.263	−0.456
Arginine	3.690	3.694	7.067	7.135	0.108
Isoleucine	2.388	2.360	4.573	4.558	−1.173
Leucine	3.959	3.898	7.582	7.529	−1.540
Valine	2.457	2.427	4.705	4.688	−1.221
Histidine	1.327	1.303	2.541	2.516	−1.808
Phenylalanine	2.688	2.660	5.148	5.138	−1.041
Glycine	2.191	2.160	4.196	4.172	−1.414
Serine	2.595	2.565	4.970	4.954	−1.156
Proline	2.579	2.552	4.939	4.929	−1.046
Alanine	2.229	2.216	4.269	4.280	−0.583
Aspartic acid	5.949	5.889	11.39	11.375	−1.008

[1] Estimated by specific calibration equation. NIRS Calibration equation.

Table 3 - Chemical composition of normal (NSBM) and damaged (DSBM) soybean meal, ground corn (GC), and corn silage (CS)

Item	NSBM	DSBM	GC	CS
Dry matter (DM; g.kg^{-1} as fed)	932.3	937.1	944.3	309.0
Ash (g.kg^{-1} DM)	75.0	77.0	13.0	69.0
Organic matter (g.kg^{-1} DM)	857.2	859.6	987.0	931.0
Crude protein (g.kg^{-1} DM)	501.6	506.7	76.9	77.1
Crude fat (g.kg^{-1} DM)	8.6	9.3	86.97	82.56
NDF (g.kg^{-1} DM)	143.8	144.1	131.4	450.1
iNDF (g.kg^{-1} DM)	27.4	24.02	66.7	359.7
NDIN (g.kg^{-1} CP)	57.70	75.70	85.40	157.7

NDF - neutral detergent fiber; iNDF - indigestible NDF; NDIN - neutral detergent insoluble nitrogen.

2.4. Statistical analysis

The gas production profiles were analyzed using the NLIN procedure of SAS (Statistical Analysis System, version 9.2), in which we used the GOMPERTZ (Gompertz, 1825; Schofield et al., 1994) nonlinear model to estimate the kinetic parameters of each soybean meal (DSBM and NSBM), and for the diets in function of the DSBM levels included in the diet replacing NSBM.

The *in vitro* incubation data of cumulative gas production and gravimetric technique (IVDMD and IVCPD) were analyzed using the PROC MIXED of SAS. The *in vitro* cumulative gas production assay was the second step of a two-step analytical procedure (PROC NLIN and MIXED, respectively), which is described in Eq. 1, while the *in vitro* gravimetric analysis was considered as a repeated measure in Eq. 2. The treatments (NSBM and DSBM) were considered fixed effects and incubations random effects (Eqns. 1 and 2). The significance level of $P<0.05$ was considered to assume a difference between the treatments.

$$\theta_{ij} = \mu + \tau_i + r_j + e_{ij}, \quad (1)$$

$$y_{ijk} = \mu + \tau_i + r_j + \delta_{ij} + t_k + (\tau \times t)_{ik} + e_{ijk}, \quad (2)$$

in which θ_{ij} and y_{ijk} correspond to the estimated values for each parameter of the *in vitro* cumulative gas production model (V_f in mL and k in h^{-1}) and *in vitro* digestibility by gravimetric technique (IVDMD and IVCPD; $g.kg^{-1}$), respectively. The Greek letters μ and τ_i represent the fixed parameters such that the mean for the i-th treatment was $\mu_i = \mu + \tau_i$, in which i = 1 to 2, and δ_{ij} is the random error between experimental units (incubation) within treatment or the covariance between repeated measurements within incubation; hence, we tested the first-order autoregressive covariance structure (AR (1)), compound symmetry (CS), unstructured (UN), and variance components (VC) as candidate structures and variance components (VC) as candidate structure, and the AICc values were adopted as selection method of the most likelihood covariance structure. The Latin letters r_j, t_k, e_{ij}, and e_{ijk} are random effects, the first being associated with the j-th (j = 1 to 2) incubation run, the second letter assigned to the effect of the k-th incubation times (h), and the third and fourth letters were considered random errors associated with the flasks in the j-th run (experimental unit) that received the i-th treatment between measurements within experimental units.

The statistical model for estimation of RDP and RUP using the three-step technique was:

$$y_{ij} = \mu + \tau_i + a_j + e_{ij}, \quad (3)$$

in which y_{ij} denotes an observation in treatment i and animal j; the Greek letters μ and τ_i represent the fixed effects such that the first letter is the constant inherent in the model and the second is the treatment, in which i = 1 to 2 (following the explanation given in Eq. 1); the two rumen-cannulated animals (a_j) were considered random effects (j = 1 and 2), and e_{ij} is the random error.

The data on TBWG, ADG, HCW, CY, DMI, and FE were analyzed using the PROC MIXED of the SAS, considering a randomized block design (Eq. 4). In contrast, diet responses were determined by linear, quadratic, and cubic effects. Because no cubic effects were presented among the evaluated variables, this effect was not shown in the results. Statistical effects were declared at P<0.05.

$$y_{ijk} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + e_{ijk}, \quad (4)$$

in which y_{ijk} is assigned as the performance and intake variables in the k-th animal (ADG, HCW, and CY, in this case k = 1, ..., 48) or pen (DMI, for this variable, k = 1, ..., 24) in the i-th treatment (τ_i; i = 1 to 2) and j-th block (β_j; j = 1 to 2). The interaction between treatment × block was assigned by the expression $\tau\beta_{ij}$. The letters μ and e_{ijk} represent the constant inherent in the model and the random error assigned to each observation, respectively.

3. Results

For the AA profile, similar values were obtained for the two types of SBM (Table 2); however, some numerical differences were observed; thus, the DSBM presented a lower percentage for 16 AA out of the 17 that were measured compared with NSBM, which ranged from −0.456 to −3.089% for tryptophan and cystine, respectively. None of the aflatoxins B1, B2, G1, G2, and M2 were detected in either the normal and damaged soybean meals. In addition, the nutritional compositions of the NSBM and DSBM (DM, ash, OM, CP, CF, NDF, iNDF, and NDIN) were similar (Table 3).

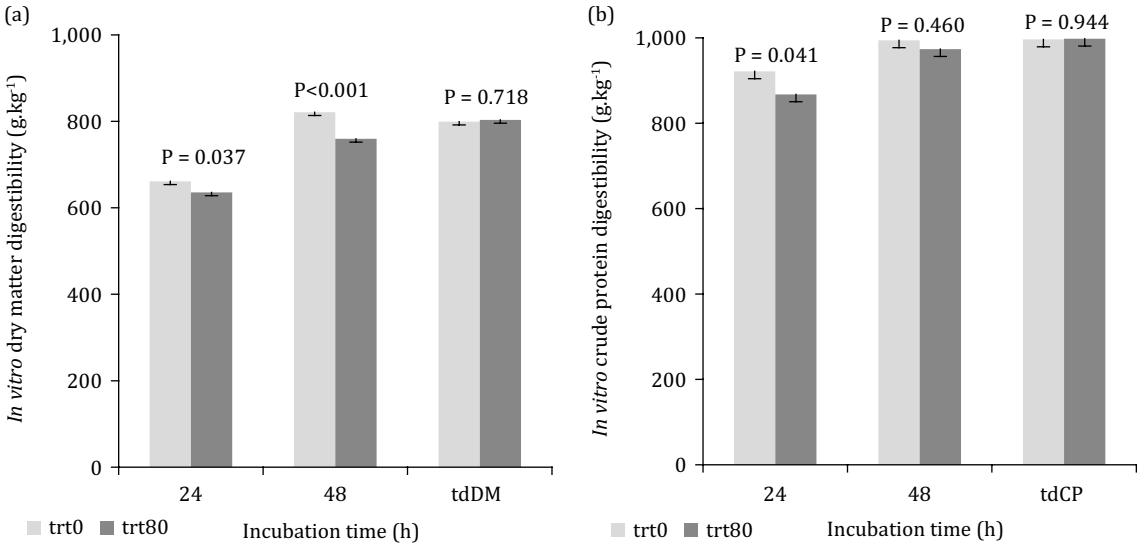
The total *in vitro* gas production and the *in vitro* cumulative gas production at 12, 24, 36, and 48 h of incubation estimated for NSBM was higher (P<0.05) than those estimated for DSBM. The ammoniacal nitrogen content (NH_3-N) at 48 h of *in vitro* incubation was not different between NSBM and DSBM, since the P-value was ≥0.05 (Table 4). Furthermore, we must emphasize that the standard error value for NH_3-N represented 30.56% of the mean (13.71 $mg.dL^{-1}$) taking into account both soybean meals.

Statistical analyses of IVDMD and IVCPD assigned as repeated measures over time had the variance components among the other tested candidate structures (AR (1), CS, and UN) as the most likelihood covariance structure, using the lowest AICc value as the selection method. Henceforth, the DSBM showed lower IVDMD than NSBM, which was about 5% lower at 24 and 48 h (P>0.05), while the IVCPD

showed difference only at 24 h (P<0.05). After addition of pepsin-HCl into the flasks, to simulate the digestion occurring in the abomasum, there was no difference between the two types of SBM related to IVDMD or IVCPD (Figures 1a and 1b).

The DSBM presented higher RUP (P<0.05) and RUP_{CP} RUP_{DM} (P<0.05) when compared with NSBM that presented higher content of RDP (P<0.05). The digestible (ID) and indigestible (RUPu) fractions in the intestine were not different for the two qualities of SBM (P>0.05) (Table 5).

The replacement levels of NSBM by DSBM in the diet caused a quadratic effect on the digestion rate as well as on *in vitro* cumulative gas production at each incubated time, except at 48 h (Table 6). The variables that had a quadratic effect showed $\beta_2 > 0$, i.e., data behavior with upward-facing concavity. On the other hand, the DSBM levels did not affect total gas production or latency (Table 6).



Downward bars represent standard errors.

Figure 1 - Means for dry matter and crude protein digestibility at 24 and 48 h of *in vitro* incubation by rumen microbial population; total *in vitro* of dry matter (tdDM - a) and crude protein (tdCP - b) digestibility after digestion by rumen microbial during 48 h, following 24 h with pepsin-HCl incubation for both qualities of soybean meal: normal (NSBM; trt0) and damaged (DSBM; trt80).

Table 4 - Kinetic parameters and *in vitro* cumulative gas production at specific incubation times for normal (NSBM) and damaged (DSBM) soybean meal

Item	Type of soybean meal		SE	P-value
	NSBM	DSBM		
TGP (mL)	106.0	103.2	11.8	0.008
K ($mL.h^{-1}$)	0.0812	0.0800	0.0023	0.708
Gas 6 h (mL)[1]	22.7	22.5	1.4	0.827
Gas 12 h (mL)[1]	41.9	39.8	4.2	0.048
Gas 24 h (mL)[1]	69.9	66.5	6.0	0.046
Gas 36 h (mL)[1]	89.4	85.6	9.4	0.002
Gas 48 h (mL)[1]	98.0	94.2	10.6	0.001
NH_3-N 48 h (mg. dL^{-1})[2]	17.38	10.04	4.19	0.050

TGP - total gas production; k - gas production rate; SE - standard error.
[1] *In vitro* gas production at times 6, 12, 24, 36, and 48 h.
[2] Ammoniacal-nitrogen content at 48 h of *in vitro* incubation.

Table 5 - *In situ* and *in vitro* digestibility technique results for contents of crude protein (CP; g.kg^{-1} DM); rumen-degradable protein (*in situ* step, RDP; g.kg^{-1} CP); rumen-undegraded protein (*in situ* step, RUP; g.kg^{-1} CP); intestinal digestibility of RUP (ID; g.kg^{-1} RUP), i.e., digestibility of the RUP that are subjected to treatment with pepsin-HCl and then pancreatin (*in vitro* steps); digestible RUP in *in vitro* steps as a function of dry matter (RUP_{DM} g.kg^{-1} DM) and of CP (RUP_{CP} g.kg^{-1} CP); and undigestible RUP as a function CP (RUP_{u} g.kg^{-1} CP) with CP for normal (NSBM) and damaged (DSBM) soybean meal

SBM	CP	RDP	RUP	ID	RUP_{CP}	RUP_{u}	RUP_{DM}
NSBM	501.6	502.5	487.4	735.6	367.3	130.1	184.2
DSBM	501.7	428.3	571.6	761.4	435.3	136.4	220.5
P-value	0.183	<0.001	<0.001	0.497	0.017	0.760	0.012

Table 6 - Kinetic parameters of *in vitro* cumulative gas production from diets containing damaged soybean meal (DSBM) replacing normal (NSBM) soybean meal in diets of feedlot lambs

Item	DSBM level replacing NSBM (g.kg^{-1} of NSBM)				SE	P-value	
	0	333	667	1,000		L	Q
TGP (mL)	182.5	174.9	177.8	175.1	6.8	0.446	0.660
k (mL.h^{-1})	0.100	0.079	0.088	0.090	0.003	0.016	<0.001
Lag (h)[1]	0.92	1.63	1.07	1.16	0.35	0.872	0.111
Gas 6 h (mL)[2]	39.0	28.8	32.8	32.7	1.391	0.266	0.001
Gas 12 h (mL)[2]	77.4	56.3	64.5	64.7	2.4	0.010	0.002
Gas 24 h (mL)[2]	136.9	108.4	122.0	123.6	3.6	0.115	0.004
Gas 36 h (mL)[2]	161.0	138.2	144.0	147.5	5.3	0.158	0.021
Gas 48 h (mL)[2]	178.8	163.3	168.6	167.8	5.3	0.254	0.177
NH_3-N 48 h (mg. dL^{-1})[3]	7.43	5.47	5.55	4.73	0.529	<0.001	0.128

TGP - total gas production; k - gas production rate; L - linear effect; Q - quadratic effect.
[1] Latency time.
[2] *In vitro* gas production at times 6, 12, 24, 36, and 48 h.
[3] Ammoniacal-nitrogen content at 48 h of *in vitro* incubation.

Table 7 - Intake and digestibility of nutrients of growing-finishing lambs fed diets containing damaged soybean meal (DSBM) replacing normal (NSBM) soybean meal

Item	DSBM level replacing NSBM (g.kg^{-1} of NSBM)				SE	P-value	
	0	333	667	1,000		L	Q
Intake (kg.day^{-1})							
DMI	1.35	1.31	1.36	1.43	0.042	0.120	0.188
DMI_{BW}	3.82	3.73	3.71	3.81	0.081	0.898	0.245
OMI	1.27	1.23	1.28	1.35	0.039	0.120	0.187
CPI	0.181	0.177	0.182	0.191	0.005	0.156	0.201
NDFI	0.239	0.230	0.243	0.250	0.011	0.374	0.447
Digestibility (g.kg^{-1} of DM)							
DMD_a	734.2	753.3	744.1	760.8	9.68	0.144	0.706
OMD_a	768.4	783.4	771.0	784.0	8.00	0.334	0.862
CPD_a	649.8	705.1	679.1	704.2	1.786	0.087	0.378
NDFD	339.7	390.1	328.9	390.9	2.299	0.379	0.802

DMI - dry matter intake; DMI_{BW} - DMI expressed as % of body weight; OMI - organic matter intake; CPI - crude protein intake; NDFI - neutral detergent fiber intake; DMD_a - apparent digestibility of dry matter; OMD_a - apparent digestibility of organic matter; CPD_a - apparent digestibility of crude protein; NDFD - neutral detergent fiber digestibility; SE - standard error; L - linear effect; Q - quadratic effect.

Regarding the *in vitro* digestibility, the DSBM levels did not affect IVDMD or IVCPD at 48 h of incubation, which showed mean values of, respectively, 826.8 g.kg^{-1} (P = 0.471) and 935.7 g.kg^{-1} (P = 0.523). The increase of DSBM levels in diets caused a linear decrease (P<0.05) on ammonia concentration in fluid from the flasks at 48 h of *in vitro* incubation (Table 6).

In the *in vivo* trial, there was no effect (P>0.05) of DSBM levels in the diets on DMI and on apparent digestibility of DM, OM, CP, and NDF digestibility (Table 7). Consequently, the inclusion of the DSBM levels replacing NSBM in the diets did not cause effect (P>0.05) on TBWG, ADG, CG, DMI, FE, or CY (Table 8).

Table 8 - Animal performance estimates of growing-finishing lambs fed diets containing damaged soybean meal (DSBM) replacing normal (NSBM) soybean meal

Item	DSBM level replacing NSBM (g.kg^{-1} of NSBM)				SE	P-value	
	0	333	667	1,000		L	Q
IBW (kg)	25.21	24.83	25.60	25.32	0.376	0.516	0.894
FBW (kg)	42.56	42.54	41.77	44.23	1.096	0.392	0.263
TBWG (kg)	17.35	17.70	16.16	18.90	1.026	0.498	0.251
ADG (g.d^{-1})	284.6	290.3	264.9	310.0	0.016	0.502	0.248
WCW (kg)	21.64	21.08	21.04	21.60	0.574	0.952	0.341
CY (%)	50.83	50.00	50.49	48.82	0.702	0.085	0.557
CG (kg)	8.48	7.92	7.88	8.44	0.574	0.952	0.341
FE (dmls)	0.219	0.236	0.214	0.237	0.009	0.429	0.716

IBW - initial weight; FBW - final weight; TBWG - total weight gain; ADG - average daily gain; WCW - warm carcass weight; CY - carcass yield; CG - carcass gain; FE - feed efficiency (dmls - dimensionless); SE - standard error; L - linear effect; Q - quadratic effect.

4. Discussion

Soybean and SBM are important sources of protein for human and animal nutrition worldwide, and thus there is a great interest in producing high quality grains to meet this demand and to preserve the grains and their byproducts to meet nutritional and food safety characteristics.

The occurrence of damages in soybean grains could cause many issues associated to chemical changes and toxins accumulation; however, some researchers (Lehmkuhl, 2011; Andrade, 2016) did not find any important changes in chemical composition or the presence of toxins in damaged soybean grains produced in Mato Grosso State. According to Lehmkuhl (2011), the presence of damage and the incidence of fungi do not have any influence on the nutritional composition of soybean grains in terms of oil and CP content.

In this study, the chemical composition between NSBM and DSBM (79.7% of damaged soybeans) was numerically similar (Table 3), an observation also verified by Lehmkuhl (2011). Additionally, the average values found for both types of SBM are close to those presented on CQBAL 3.0 (Valadares Filho et al., 2016) for DM, OM, ash, and CP of 886.3, 933.9, 64.7, and 489.0 g.kg^{-1}, respectively. These results indicate that even when presenting a high percentage of damage (79.7%), soybean grains used to produce soybean meal did not change the major chemical compounds such as OM, CP, and NDF.

Because SBM presents a high CP content, even when included in a small percentage of the diet as in this study (14% DM basis), its CP represents at least 50% of total CP in the diet. In this way, for ruminants, every CP source needs to be evaluated considering three different aspects: its proportion of RDP and, consequently, of RUP, the intestinal digestibility of RUP, and its AA profile (Santos et al., 1998). Thus, any change in composition of its CP can affect the N availability for the rumen microbial population (RDP) or AA absorption in the small intestine (RUP) (Van Soest, 1994; Broderick, 2018).

Thus, to evaluate the effects of damage occurring in soybean grains used for producing SBM, the objective of this study was to evaluate DSBM to try to find any change in its chemical composition, but especially some alteration related to its CP, by determining the *in vitro* and *in situ* rumen degradability and estimating its RUP and intestinal digestibility, the AA profile of each SBM, as well as its effect on animal performance.

When NSBM and DSBM were evaluated by *in vitro* incubation with rumen inoculum, we observed that DSBM was digested in rumen to a lesser extent compared with NSBM, which is confirmed by lower total gas production and IVDMD (Table 4 and Figure 1). However, when HCl-pepsin solution was added into the incubation flasks to simulate the effects of abomasum secretion, the differences ($P>0.05$) found using only rumen inoculum disappeared. This indicates that DSBM presents some compound that is difficult to digest or some negative effect on rumen microbial population compared with NSBM, which does not happen when DSBM was subjected to acid and enzymatic secretion from an animal.

The same behavior observed *in vitro* was also found *in situ* when both types of SBM were incubated for 27 h in the rumen of two beef cattle in grazing to estimate RDP and RUP. The DSBM presented a lower fraction of RDP (about 7%) and, consequently, a higher fraction RUP and digestible RUP (about 8%) when compared with the NSBM (Table 5). The RDP and RUP values estimated for NSBM are similar to those observed by Cabral et al. (2001) of 508.6 and 491.4 $g.kg^{-1}$ of CP, but higher than the values estimated by Erasmus et al. (1994) of 462.0 and 532.0 $g.kg^{-1}$ of CP, respectively. The similarity of values for RDP and RUP obtained in this study to values estimated by another author in Brazil (Cabral et al., 2001) and the difference from values observed by authors (Erasmus et al., 1994) from other countries reflects some peculiarities associated to the type of soybean planted as well as to the processing methods used for producing SBM in each country.

Although the major type of damage in the soybean used for producing the DSBM in this study was fermented grains, the percentage of heat-damaged grains was 4.1%, which partially helps to explain the lower RDP observed for DSBM compared with NSBM. In the literature, the only type of damage that has been evaluated is the one caused by heating soybean or SBM to decrease RDP and to increase RUP, aiming to improve N efficiency use by the animal (Erasmus et al., 1994; Broderick, 2018).

Mjoun et al. (2010) estimated lower RDP for expeller SBM (463.0 $g.kg^{-1}$ of CP) compared with solvent SBM (677.0 $g.kg^{-1}$ of CP), while Demjanec et al. (1995) estimated RUP ranging from 349.0 to 929.0 $g.kg^{-1}$ of CP of SBM untreated or roasted at increased temperatures. The last authors suggested that RUP cannot be assumed as a fixed value, since it can be affected by the quality of the grain, processing method, method used for obtaining the estimate, and effect of incubation time and particle size. Bach et al. (2005) highlighted that many factors can affect the proportion of RDP and RUP of feeds used for ruminants such as protein solubility, AA profile, presence of sulfur bonds, previous treatment of feed with heating or formaldehyde, rumen pH, and digestion passage rate of the particles from the rumen.

These results regarding the *in situ* and *in vitro* digestibility of DM and CP of DSBM are interesting because they can be interpreted from two different perspectives. The first one would be to consider the decrease in RDP, which can affect N (AA and NH_3-N) availability for the rumen microbial population. However, considering that SBM frequently presents a CP that is quickly degraded in the rumen and, thus, can allow N losses by urinary excretion, the decrease in RDP can help improve N use efficiency by the animal (Van Soest, 1994).

To verify if the DSBM would cause a shortage of N availability in the rumen when incubated alone or as a part of the diet, the NH_3-N concentration in the fluid from the *in vitro* incubation flasks was measured after 48 h of incubation with rumen inoculum. The linear drop in NH_3-N content in 48 h of *in vitro* incubation (Table 6), as the level of DSBM was increased in the diets, is justified by the lower PDR content of DSBM in relation to NSBM that we found (Table 5), since the two variables in question have a direct relationship. It is also important to emphasize that the absence of effect of the NH_3-N content

in SBM after 48 h of incubation (Table 4) could be because the method is highly variable, leading to large standard error of the values observed for NH_3-N.

The *in vitro* NH_3-N concentrations observed for experimental diets that had higher DSBM levels, 4.73 mg.dL^{-1}, seems to be close to the minimum concentration suggested by Satter and Slyter (1974) of 5 mg.dL^{-1} for maximizing microbial growth *in vitro* and, thus, it could be said that even presenting a lower RDP than NSBM, DSBM was able to maintain adequate ammonium concentration for rumen microbial growth.

The amount of NH_3-N in the rumen is a result of AA fermentation as well as urea hydrolysis (from the diet or endogenous origin) by rumen microorganisms and represents an important source of N for many organisms in the rumen, especially for fibrolytic ones that seem to use only NH_3 as N source for growth (Russell et al., 1992). Thus, NH_3-N concentrations are used as an indicator of N availability in the rumen, for which we intend to prevent too low (< 5 mg.dL^{-1}) or too high (> 20 mg.dL^{1}) concentrations, which are associated to shortage or excess of N in the rumen that can limit the microbial protein synthesis or increase the N losses from the diet by urine, respectively (Broderick, 2018).

Bach et al. (2005), by mixed model regression analysis using data from the literature (n = 285), pointed out that the efficiency of microbial protein synthesis (EMPS) was not affected by NH_3-N concentration in the rumen. The same authors also highlighted this in a report of the NRC (2001), which suggested that with an abundance of N in the rumen, the EMPS tends to be lower than when N availability is limiting for bacterial growth. Taking all this information together, it would be good to remember that EPMS should not be confused with the flow of microbial protein synthesis, which represents the actual amount of microbial protein that the small intestine is able to digest by enzymatic secretion by the animal and that contributes to meeting the animal's requirements of metabolizable protein (NRC, 2001). Although the terms apparently mean the same thing, it is not true, as often a higher microbial protein synthesis is not necessarily observed in higher EPMS.

The *in vitro* NH_3-N concentrations are just an indicator of protein degradation and its use by rumen microorganisms, which needs to be interpreted carefully, because the N recycling from the liver, which happens in the animal, does not occur in the flasks, and can contribute a significant amount of N for microbial growth, which depends on N intake, assuming a higher percentage of N intake in low-CP diets and a lower percentage in high-CP diets (NRC, 1985).

The lower digestibility of DSBM by rumen microorganisms can also be related to lower AA content in DSBM compared with NSBM, especially for branched AA that, when fermented, produce branched-chain fatty acids, which are known to play an important role in the ruminal environment (Tedeschi et al., 2000), being considered essential for many rumen organisms, including most fiber-degrading microorganisms (Yang, 2002).

When we evaluated the DSBM included in the diets and its effects on *in vitro* incubation (Table 6), we observed that the inclusion of DSBM levels up to 100% replacing NSBM had no effect on total *in vitro* gas production, but there was a quadratic effect on digestion rates and on gas production at specific reading times (from 6 until 36 h), but there was no effect of DSBM levels at 48 h (Table 6). It could be that negative effects of DSBM on gas production were observed only at initial digestion events, which disappeared at 48 h of incubation. In addition, IVDMD and IVCPD were not affected by DSBM levels in the diet, but were measured only at 48 h of incubation, coinciding with the same incubation times that DSBM levels did not affect gas production, suggesting that these events should be monitored at early incubation times as well.

To measure the real effects of DSBM levels in the feedlot lamb diets, we fed 48 growing/finishing lambs over 60 days and measured DMI, nutrient digestibility, and animal performance. The DMI, expressed in both as kg.day^{-1} and percentage of BW, and apparent digestibility of DM were not affected by the inclusion of DSBM (Table 7), with the DMI values being similar to those predicted by Cabral et al. (2008a). Following the results of DM, we also did not find deleterious effect of DSBM levels on the intake and digestibility of other nutritional fractions analyzed (Table 7). Therefore, the nutritional value

of DSBM is no different than NSBM. It is important to note that the intake and digestibility of nutrients are elementary variables to estimate the nutritional value of a given feedstuff (Van Soest, 1994).

The DMI is the most important variable affecting animal productivity, since 60 to 90% of the variation observed in digestible energy intake was explained by DMI variation, while the digestibility only explains from 10 to 40% of this variation (Crampton et al., 1960; Reid, 1961; Mertens, 1987). Thus, the absence of a negative effect of DSBM on DMI is a good indicator related to its potential to be used in diets for ruminants.

It would be expected that replacement of NSBM by DSBM could cause negative effects on DMI, nutrient digestibility, or animal performance, considering its lower DM and CP digestibility in the presence of rumen microbial population *in vitro* or *in situ*. Considering that the rumen constitutes a major part of the gastrointestinal tract in ruminant animals, in which around 60 to 70% of all dietary compounds are digested by the rumen microbial population (Van Soest, 1994), any negative effect on rumen microbial population could affect total digestion of nutrients as well as animal performance.

Additionally, the lower RDP estimated for DSBM could cause a shortage in N for microbial growth and, consequently, limit the flow of microbial protein to the duodenum. Considering that microbial growth is the major source of AA to ruminant animals, representing around 50 to 85% of metabolizable protein in the duodenum (Storm and Ørskov, 1983), a limitation of N availability in the rumen could present negative effects on animal performance. However, even presenting lower RDP, if the diets containing DSBM do not cause a shortage in N available in the rumen, the higher RUP of DSBM could improve the efficiency of N use by the animal assuming that more of its CP would be digested in the intestine than in the rumen, where significant losses of N associated with rumen fermentation of AA could occur (Broderick, 2018). In this way, Demjanec et al. (1995) highlighted that even using high temperatures for processing SBM that increase the RUP, if rumen NH_3-N availability is not limiting for microbial growth, probably it will increase the N flow to the duodenum and, consequently, will increase animal performance, especially for animals presenting a high demand of metabolizable protein such as high-producing dairy cows and fast-growth animals.

Borucki Castro et al. (2007) evaluated four different methods (solvent-extracted SBM, expeller SBM, lignosulfonate SBM, and heat and soyhulls SBM) for treating SBM on rumen degradability and intestinal digestibility of AA by a combination of *in situ* and *in vitro* techniques and by mobile nylon bag technique using rumen- and duodenal-fitted dairy cows. Soybean meal subjected to expeller, lignosulfonate, and heat + soybean hulls treatment methods presented more CP and AA protected from ruminal degradation than solvent-extracted SBM, in which the RDP increased from 420.0 to 680.0 $g.kg^{-1}$ of CP. The authors concluded that based on *in situ* (rumen and small intestine) procedures, heat and chemical treatment of SBM increased AA availability compared with solvent-extracted SBM, and thus these methods present a higher potential to enhance the AA supply to the small intestine of high-producing dairy cows.

It is also important to highlight that this is the first study that aimed to evaluate the effects of naturally caused damage of soybean grain and its effects on the nutritive value of SBM, considering that in the literature, there is some information related to damage caused by heating soybean or SBM to evaluate it to control protein degradation in the rumen (Van Soest, 1994; Broderick, 2018).

In this study, we evaluated SBM produced from damaged grains by *in vitro* and *in situ* and *in vivo* studies and, even distinguishing between two types of SBM (DSBM and NSBM) related to *in vitro* or *in situ* degradability, in which the DSBM presented lower digestibility than NSBM, we did not find any effect when we evaluated the DSBM in lamb diets. Although it is known that SBM has been included in a small percent of the diet (14%), typical for CP sources, the CP from SBM contributes 51% of total CP in the diet. Thus, when we replaced CP from NSBM by CP from DSBM, its CP contributed 0, 17, 34, and 51% of total CP in the diet, respectively, for diets containing 0, 333, 667, and 1,000 $g.kg^{-1}$ of NSBM replaced by DSBM. However, even replacing around 51% of dietary CP from NSBM with CP from DSBM, which presents lower RDP, we did not observe any negative effect on intake, digestibility, and animal performance.

5. Conclusions

Damaged SBM presents similar chemical composition, but lower rumen degradable protein, total gas production, and *in vitro* dry matter digestibility than normal SBM. However, considering the absence of negative effects of inclusion of levels 0, 333, 667, and 1,000 $g.kg^{-1}$ of damaged SBM in feedlot diets on nutritional and animal performance variables, the same may replace normal soybean meal in feedlot diets for ruminants.

Conflict of Interest

The authors declare no conflict of interest.

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