Sunflower cake in diets for beef cattle: digestibility, kinetics and *in vitro* ruminal fermentation parameters

Rafael Henrique de Tonissi e Buschinelli de Goes[1]*, **Luiz Henrique Xavier da Silva[2]**, **Tatiana Garcia Diaz[3]**, **Antonio Ferriani Branco[3]**, **Ana Lúcia Teodoro[4]** and **Gislaine Ribeiro Ferreira[1]**

[1]Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Rodovia Dourados – Itahum, Km 12, 79804-970, Dourados, Mato Grosso do Sul, Brasil. [2]Departamento de Zootecnia, Universidade Federal de Goiás, Goiânia, Goiás, Brasil. [3]Departamento de Zootecnia, Universidade Estadual de Maringá, Maringá, Paraná, Brasil. [4]Instituto Federal de Educação, Ciência e Tecnologia do Piauí, Corrente, Piauí, Brasil. *Author for correspondence. E-mail: rafaelgoes@ufgd.edu.br.

ABSTRACT. The objective of this study was to evaluate the effect of the inclusion of sunflower cake replacing soybean meal in beef cattle diets on the *in vitro* digestibility of dry matter (IVDDM), organic matter (IVDOM), crude protein (IVDCP) and the ruminal fermentation kinetics and parameters. The experiment was analyzed according to a completely randomized design. The treatments consisted of four levels of sunflower cake, 0, 200, 400, 600 g kg^{-1}, replacing soybean meal in the concentrate of beef cattle diets. The coefficients of IVDDM, IVDOM and IVDCP presented a quadratic effect with the addition of sunflower cake. The soluble fraction (fraction B) degradation rate and total gas production decreased linearly with the inclusion of sunflower cake. Values of pH in ruminal fluid were higher for levels 0, 200 and 600 g kg^{-1} sunflower cake. Sunflower cake can replace soybean meal by up to 280 g kg^{-1} in the concentrate of beef cattle diets, improving the *in vitro* digestibility of dry matter organic, matter and crude protein. Levels above 400 g kg^{-1} reduce ruminal digestion rate, digestibility and release of final fermentation products.

Keywords: ammonia; co-products; ruminal fermentation; oilseed; pH.

Introduction

New trends in ruminant production are aimed at increasingly competitive, sustainable and economically viable production systems (Mesacasa et al., 2012). However, high costs of ingredients such as corn and soybean meal, widely used in ruminant nutrition, lead to increased production costs. Because of this, it is necessary to use alternative, good quality foodstuffs that allow the reduction of the costs associated with food, and at the same time, to improve the efficiency and competitiveness of the production systems.

Alternative foods include the co-products originated from biodiesel production, mainly from oilseed species, such as sunflower. The main products resulting from the sunflower oil extraction are the meal and the cake, which present great potential for use in the feeding of ruminant animals (Goes et al., 2010; Oliveira, Mota, Barbosa, Stein, & Borgonovi, 2007).

Sunflower cake is obtained by extracting the oil by mechanical process (cold pressing), without cooking or using solvent, generating a product with high crude protein and ether extract contents. In general, sunflower cake contains 22.0% protein content and 22.5% ether extract (Oliveira & Vieira, 2004), and can be considered as protein and energy food. However, it requires special care with ether extract content, since diets rich in fatty acids can cause digestive disturbances and reduced intake (Palmquist & Jenkins, 1980).

The use of sunflower cake in ruminant feed, replacing conventional foods (corn and/or soybean), is still poorly explored, possibly due to the lack of nutritional information of this food, due to very variable characteristics, mainly in the chemical composition (Oliveira & Cáceres, 2005). These changes depend mainly on the amount of shell hat is removed during the oil extraction process, the genetic variety of grain used, the type of soil and climate (Goes et al., 2008).

The information found in the literature on the use of sunflower cake in ruminant diets is still scarce, making it necessary to conduct research evaluating the effects on the fermentation process and the best

level of inclusion in the diet without causing deleterious health effects animal and consequent economic loss.

Considering the above, the objective of this study was to determine the effect of inclusion of sunflower cake replacing soybean meal in diets for ruminants on the in vitro digestibility of dry matter (IVDDM), organic matter (IVDOM), crude protein (IVDCP), as well as the ruminal fermentation kinetics and parameters (ammonia and pH).

Material and methods

Experimental design and treatments

The experiment was conducted at the Laboratory of Food Analysis (LANA), Animal Science Department, Federal University of Grande Dourados (UFGD) and State University of Maringá (UEM), located in the city of Dourados, State of Mato Grosso do Sul and Maringá, State of Paraná, between September and December 2012 and from March to May 2013.

The experiment was a completely randomized design, with four diets, in a forage: concentrate of 60:40. The treatments consisted of four levels of replacement of soybean meal with sunflower cake: 0, 200, 400 and 600 g kg^{-1}. *Brachiaria brizanth*a cv Marandu was used as forage (Table 1).

Preparation of rumen inoculum and the buffer solution

The ruminal inoculum used in the *in vitro* procedures was obtained from two castrated Holstein cattle, with an average body weight of 380 kg, rumen fistulated. Animals were fed a diet composed of 80% forage (corn silage) and 20% concentrate (corn, soybean meal and mineral supplement), twice a day at 08:00 and at 16:00 h.

Rumen fluid was collected before the first meal through the ruminal fistula. Four liters of rumen fluid (two liters per animal) were taken, including a solid fraction of the rumen content. The collected material was transferred to a preheated thermos flask, previously purged with CO_2 to facilitate transport to the laboratory. The material was homogenized using a blender for 10 seconds and then filtered through four layers of cotton cloth (cheesecloth).

The buffer solution, consisting of solution A and B, was prepared with the following reagents: Solution A (g L^{-1}) composed of: 10.0 g potassium dihydrogen phosphate (KH_2P0_4); 0.5 g magnesium sulfate ($MgSO_47H_2O$); 0.5 g Sodium chloride (NaCl); 0.1 g calcium chloride dehydrate ($CaCl_2.2H_2O$); 0.5 g urea. Solution B (g/100 mL) was composed of: 15.0 g sodium carbonate (Na_2CO_3) and 1.0 g sodium sulfide ($Na_2S.9H_2O$). The solutions were mixed in the ratio 1: 5 reaching pH 6.8 at the constant temperature of 39°C.

In vitro digestibility

True *in vitro* dry matter digestibility (IVDDM) was determined according to the methodology described by Tilley and Terry (1963) modified by Holden (1999), using the artificial rumen (DaisyII Fermenter®, Ankom).

Table 1. Inclusion levels of sunflower cake and chemical composition of the ingredients and the diets used in the experiment, g kg^{-1}.

Item	Inclusion of sunflower cake, g kg^{-1}			
	0	200	400	600
Ingredients				
Brachiaria brizantha cv Marandu	600.00	600.00	600.0	600.0
Soybean meal	209.60	167.6	126.0	84.0
Sunflower cake	-	69.6	139.0	208.8
Corn	170.4	142.8	115.0	87.2
Mineral supplement	20.0	20.0	20.0	20.0
Chemical composition				
Dry matter	448.2	454.2	460.5	466.1
Mineral matter	47.9	46.6	45.4	44.1
Crude protein	150.2	157.1	154.2	151.0
Ether extract	11.2	20.8	30.4	40.1
Neutral detergent fiber	544.5	556.0	567.7	579.2
Acid detergent fiber	277.3	289.1	301.0	312.8
Lignin	51.8	92.5	137.5	195.5
Total carbohydrates	780.7	775.4	769.9	764.7
Non-fiber carbohydrates	236.2	219.4	202.2	185.5

Weighed inside 0.5 g sample of each diet were TNT-100 g m $^{-1}$ bags, 5.0 x 5.0 cm size, according to Casali et al. (2009). Bags with samples were uniformly distributed from the jars of the artificial rumen, with 10 bags/jar (8 bags with samples 2 blank bags), totaling 80 bags. The blank bags (without sample) were used to correct the data. 615.4 mL buffer solution, 154 mL rumen inoculum and CO_2 were added to each jar to maintain the anaerobic environment. The jars remained in the artificial rumen at 39°C for 48h under continuous stirring. After 48h, incubation was stopped, and the bags were washed and treated with neutral detergent solution, resulting in a residue consisting of only indigestible cell wall, as described by Goering and Van Soest (1970), allowing to determine the true *in vitro* dry matter digestibility. The bags obtained at the end of the incubation were used to determine the *in vitro* digestibility of crude protein (IVDCP) and organic matter (IVDOM). The CP and ash in the residues were determined as described by Association Official Analytical Chemist (AOAC, 2006). The IVDDM, IVDOM and IVDCP were calculated by the difference between the concentration of the nutrient in the sample before and after incubation.

Rumen fermentation kinetics

The automated *in vitro* gas production technique was used to determine the rumen fermentation kinetics parameters. 0.5 g sample of each diet were weighed in duplicate in glass vials, with a capacity of 250 mL. In each flask, 100 mL buffer solution, 25 mL rumen inoculum and CO_2 were added. For each incubation, two flasks were used as blank, containing only rumen inoculum and buffer solution, in order to adjust the pressure values. Pressure values were measured using the automated system RF: Gas Production System® (ANKOM). Gas pressure values were recorded in pounds per square inch (psi), through pressure sensors on the bottle caps (modules), which sent the information from each vial to the coordinating base connected to a computer. The readings were recorded at 5-minute intervals for 24h incubation.

In vitro ruminal parameters (ammonia and pH)

In order to determine the concentration of ammonia (NH_3) and pH *in vitro*, caps were fitted with a three-way system to allow the collection of buffered rumen fluid and a Büssen valve to release gases produced during fermentation. In each vial was weighed 10 g sample from each diet in duplicate, together with 1600 mL buffer solution and 400 mL rumen inoculum. Jars were kept in an environment at 39°C under continuous stirring for 8 h incubation. 20 mL samples of the buffered rumen fluid were collected hourly until an 8h measurement was completed using a syringe and the three-way tap installed in the cap of each jar. The pH was measured immediately after each collection in 10 mL buffered rumen fluid, using a digital potentiometer. For determination of NH_3, a 10-mL aliquot of rumen fluid was acidified with 1 mL sulfuric acid (1: 1) and stored at -20° C for further analysis.

Chemical analysis

Samples of forage and foods of experimental diets were analyzed for dry matter (DM, 934.01), ash (942.05), organic matter (100-ash), crude protein (CP, Nx6.25; 984.13), ether extract (EE; 920.39) according to the techniques described by AOAC (2005). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG) were determined according to Van Soest, Robertson, and Lewis (1991). In the determination of NDF, heat-stable α-amylase and no sodium sulfite addition were used. The determination of the ammonia content in the rumen liquid (NH_3) were performed according to the INCT-Animal Science method and described by Detmann et al. (2012).

Calculations and statistical analysis

Non-fiber carbohydrates were calculated by the following equation (Sniffen, O'Connor, Van Soest, Fox, & Russell, 1992): NFC = 100 − (% NDF + % CP + % EE + % ash). Total carbohydrates (CHO) were obtained by the equation proposed by Sniffen et al. (1992): CHO = 100 − (%CP + % EE + % ash).Data of gas pressure were measured in psi, and were transformed to moles of gas by means of the ideal gas equation. Subsequently, data in moles were converted into mL of gas produced under standard conditions of temperature and pressure (STP) using the corrected pressure of the bottles, the atmospheric pressure of the region (96.538 kPa) and the atmospheric pressure under normal conditions (101.325 kPa). The logistic bicompartmental model proposed by Schofield, Pitt, and Pell (1994), was used to determine the kinetic parameters of rumen fermentation. The fit of the curves and the estimation of the parameters of biological

interest were performed using the Gauss-Newton iterative process by means of the non-linear model of the software SAEG (2007).

Data obtained from each parameter were broken down into orthogonal polynomials in order to allow the analysis of variance and regression, according to their distributions, using the software SAEG (2007). We adopted $\alpha = 0.05$ probability.

The mathematical model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where: Yi = observed variable i; μ = overall mean; Ti = effect of levels of sunflower cake, ranging from 1 to 4 (0, 200, 400 and 600 g kg^{-1}); eij = experimental error.

The pH and ammonia-N variables were distributed in a split-plot and the analysis of variance and regression at $\alpha = 0.05$. The statistical procedures were run using SAEG (2007).

Results and discussion

The inclusion levels of sunflower cake in beef cattle diets as a substitute for soybean meal showed a quadratic effect ($p < 0.001$) on *in vitro* digestibility of dry matter (IVDDM) and organic matter (IVDOM), with the point of maximum digestibility reached with the inclusion of 287.5 and 266.6 g kg^{-1} of sunflower cake in the diet, respectively. However, increasing sunflower cake levels in the diet up to 600 g kg^{-1} resulted in lower IVDDM and IVDOM coefficients.

The reduction in IVDDM observed can be attributed to dietary characteristics, which presented higher levels of acid detergent fiber (ADF, cellulose and lignin) with increasing levels of sunflower cake in the diet (Table 1). This directly influences digestibility, since it increases the indigestible fraction, thus reducing the potentially digestible fraction of the diet (Mizubuti et al., 2011; Nussio, Campos, & Lima, 2011; Oliveira et al., 2007).

Silva et al. (2015) observed reduced IVDDM and IVDOM with the inclusion of levels (0, 10, 20 and 30%) of sunflower cake in the concentrate for beef cattle. The authors attributed the reduced digestibility to high ether extract (EE) in the diets evaluated (ranging from 12.7 to 71.8 g kg^{-1} DM), since the high concentration of oil in the ruminant diet can form a hydrophobic coating that hinders the adhesion of microorganisms to food particles, reducing their degradation. In addition, oil levels in ruminant diets above 7% may produce a toxic effect, causing death of ruminal microorganisms, such as fibrolytic bacteria, which may cause a decrease in fiber degradation and interfere with fiber intake by the animal (Kozloski, 2011; Van Soest, 1994). The EE levels observed in the diets evaluated in this experiment were 11.2 to 40.1 g kg^{-1} DM, within the recommended mean levels for ruminant diets.

In contrast to the results observed in this experiment, Garcia et al. (2004) did not observe effect on IVDDM with the inclusion of sunflower cake levels (0, 15, 30 and 45%) in the diet for cattle with 45% concentrate. The divergences in the literature regarding the digestibility of diets for ruminants with the addition of sunflower cake are due to the process of obtaining the cake (press regulation) and to the variation in the chemical composition of the cake, according to the genetic variety of grain, type of soil and climate (Beran et al., 2007; Goes et al., 2008), factors that directly affect the nutritional quality of this food.

The *in vitro* digestibility coefficients of crude protein (IVDCP) presented a linear ($p < 0.001$) and quadratic ($p < 0.001$) effects with the inclusion of sunflower cake. The highest value of IVDCP was estimated for the inclusion level of 467.5 g kg^{-1} sunflower cake in the diet. It was observed that the inclusion of 200, 400 and 600 g kg^{-1} levels of sunflower cake in the diet increased by, on average, 40% the IVDCP, relative to the control diet (Table 2).

The increase in the IVDCP coefficient observed herein may be due to the high total digestibility (95.6%) of the sunflower cake protein (Mupeta, Weisbjerg, Hvelplund, & Madsen, 1997). Similarly, Beran et al. (2007) indicated that the sunflower cake protein is characterized by being extensively degradable in the rumen, and the rumen undegradable protein content is less than 10%. On the other hand, Goes et al. (2010) reported values of protein degradation at 50%. Oliveira et al. (2007) observed IVDCP of 63.16 and 71.62% with addition of 25 and 50% sunflower cake in cattle diet evaluated in vitro. These values were higher than those found in this work (Table 2), which may indicate variation in the characteristics of the cake used in the diets.

The coefficients of determination (r^2) obtained in the gas production analyses, higher than 0.98, indicate an adequate fit to the estimates of gas production parameters obtained with the bicompartmental logistic model (Table 3). Fractions A and D (rapid and slow digestion fractions, mL g^{-1}) had no effect ($p > 0.05$) with the inclusion of sunflower cake in the diet replacing soybean meal. However, there was a decreasing linear ($p < 0.05$) on total gas production (fraction A + D, mL gas^{-1}; Table 3). This effect can be attributed to the fiber carbohydrate content, which comprise available and unavailable fibers, represented by cellulose, hemicellulose and lignin, being partially available for rumen microorganisms, reducing the degradation rate, as well as gas production (Mertens, 1997).

The increase in the ether extract (EE) content in the diets evaluated (Table 1) could also explain the lower gas production verified, since the contribution of fat to gas production is insignificant (Wascheck et al., 2010), in addition, it may hinder the process of adhesion of microorganisms to dietary fibers and, the digestion of food, as discussed above.

The highest gas production was found for the control diet (10.26 mL g^{-1}), without addition of sunflower cake, possibly due to the higher concentration of non-fiber carbohydrates (NFC, pectin, β-glucans, starch) present in this diet (236.2 g kg^{-1}), compared to diets that included sunflower cake (219.4, 202.2 and 185.5 g kg^{-1}, respectively). The higher content of NFC in the diet increases the amount of digestible nutrients, which are easily fermented by rumen microorganisms, releasing more short chain fatty acids (Nussio et al., 2011).

The fraction B (degradation rate of fraction A, h^{-1}) presented a linear decreasing effect ($p < 0.05$) with the increase of the levels of sunflower cake (Table 3). The reduction in digestion rate may be due to the increase of fiber carbohydrates in the diets with the inclusion of the sunflower cake (Table 1), since the degradation rate is directly related to the solubility of substrates (Kozloski, 2011). Thus, the higher concentration of soluble carbohydrates is degraded faster by bacterial enzymes than fiber carbohydrates (Azevêdo et al., 2003).

Mizubuti et al. (2011) evaluated the rumen fermentation kinetics of different biodiesel co-products and observed that the sunflower cake had the lowest degradation rate (0.057 h^{-1}), compared to the crambe meal, cottonseed meal, crambe cake and soybean cake (0.127, 0.288, 0.135, 0.0935, respectively). The authors state that this characteristic may have an effect on ruminal repletion or time that the food remains in the rumen, which may have a direct influence on dry matter intake and animal production.

Although the fraction E (slow degradation fraction, h^{-1}) was lower in the diets as the inclusion levels of sunflower cake in the diets increased, no effect ($p > 0.05$) was detected in this parameter.

The time of colonization or lag time (fraction C) was the same for all diets evaluated (0.99), except for the diet with 40% sunflower cake, which presented a shorter colonization time (0.51) compared to the other treatments (Table 3). The shorter colonization time indicates a higher adherence of the ruminal microorganisms to the fiber particles, allowing a faster onset of food degradation and release of the final products of the ruminal fermentation (AGCC, CO_2, methane) (Mertens, 1997). On the contrary, the value of lag time tends to be higher, the lower the nutritional value of the food, due to the thickening of the cell wall and the difficulty of microbial adherence (Azevêdo et al., 2003; Kozloski, 2011; Mizubuti et al., 2011). However, there was no effect ($p > 0.05$) on the time of colonization (fraction C) with the inclusion of sunflower cake replacing soybean meal in diets.

Minimum values of *in vitro* ruminal pH for all treatments were observed within 1h after the onset of incubation (Figure 1). This response is due to the higher accumulation of organic acids in the rumen, such as AGCC, CO_2 and lactate, mainly produced during the rumen fermentation process, causing ruminal pH to drop (Nussio et al., 2011; Owens & Goetsch, 1993). The lowest ruminal pH value was 6.62, indicating that the pH drop was not very marked, possibly due to the characteristics of the diet, which contained 60% forage volume, *Brachiaria brizantha* cv Marandu.

Table 2. *In vitro* digestibility coefficients of dry matter (IVDDM), organic matter (IVDOM) and crude protein (IVDCP) in g g^{-1} of diets with levels of sunflower cake replacing soybean meal.

Variables, g g^{-1}	Inclusion of sunflower cake, g kg^{-1}				CV%	P <	
	0	200	400	600		L	Q
IVDMD	0.7604	0.7902	0.7823	0.7447	1.8	ns	0.001[1]
IVDOM	0.8023	0.8283	0.8086	0.7828	1.6	ns	0.001[2]
IVDCP	0.1956	0.5613	0.5842	0.6260	10.04	0.001[3]	0.001[4]

L – linear defect.; Q – quadratic effect, NS – non-significant, CV – Coefficient of Variation, [1]$Y = 0.7608 + 0.00023 x - 0.0000004x^2, r^2 = 0.99$; [2]$Y = 0.8043 + 0.00016x - 0.0000003x^2, r^2 = 0.92$; [3]$Y = 0.2947 + 0.0007x; r^2 = 0.72$; [4]$Y = 0.2137 + 0.00187x - 0.000002x^2, r^2 = 0.94$

The increase in the levels of sunflower cake replacing soybean meal in the diet showed a quadratic effect (p > 0.001) for mean *in vitro* ruminal pH values. The minimum ruminal pH value was estimated for 518.5 g kg^{-1} inclusion level of sunflower cake (Table 4). It should be noted that although there was a drop in pH values, in relation to the control diet, the mean ruminal pH value for all treatments was above 6.20. This value can be considered as excellent for the development of ruminal bacteria, mainly cellulolytic bacteria (*Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*), which favor the digestion of fiber component of the diet (Van Soest, 1994). The minimum supply of fiber carbohydrates is important in the ruminant diet, since they stimulate rumination and increased salivation of the animal, and helps in pH buffering in the rumen (Nussio et al., 2011).

The ruminal ammonia (NH_3) concentration did not show effect (p > 0.05) with the inclusion of sunflower cake in the diet replacing soybean meal (Table 4). Similarly, Domingues et al. (2010) reported no effect on rumen NH_3 concentrations in cattle as a function of the levels of substitution (0, 25, 50, 75 and 100%) of the cottonseed meal for the sunflower cake. On the other hand, Silva et al. (2015) found a linear effect with the inclusion of 0, 100, 200 and 300 sunflower cake in the concentrate.

The mean values of ammonia in all treatments were around 25.01 mg NH_3 dL^{-1}. According to Owens and Bergen (1983), ruminal NH_3 concentrations to maximize microbial synthesis range from 0.35 to 29 mg NH_3 dL^{-1}, depending on the protein and fermentable carbohydrate contents in the diet, indicating that the values of ruminal NH_3 verified in this study were suitable for microbial growth.

Table 3. Ruminal fermentation kinetic parameters of diets containing different levels of sunflower cake replacing soybean meal.

Parameters	Inclusion of sunflower cake, g kg^{-1}				CV (%)	P <	
	0	200	400	600		L	Q
A (mL gas^{-1})	4.32	6.15	3.58	4.84	50.29	ns	ns
B (h^{-1})	0.56	0.53	0.43	0.43	15.34	0.05[1]	ns
C (hours)	0.99	0.98	0.51	0.99	39.48	ns	ns
D (mL gas^{-1})	5.94	3.77	2.95	2.43	51.22	ns	ns
E (h^{-1})	0.46	0.45	0.41	0.42	8.94	ns	ns
A+D (mL gas^{-1})	10.26	9.92	6.53	7.27	16.8	0.04[2]	ns

L – linear effect; Q – quadratic effect; NS – non-significant; CV – Coefficient of Variation; [1]$Y = 0.561 − 0.0002x$; $r^2 = 0.87$; [2]$Y = 10.349 − 0.0062x$; $r^2 = 0.72$.. The fermentation kinetic parameters obtained from *in vitro* gas production were analyzed on 100 mg substrate according to the model $Y = A/(1 + exp\ [2 + 4B(C − t)]) +\ D/(1 + exp[2 + 4E(C − t)])$, where y is the total volume of gas (mL); A and Dare the volume of gas (mL) from rapid digestion (soluble carbohydrates and starch) and slow digestion (cellulose and hemicellulose), respectively; B and E correspond to the degradation rates of the fractions of rapid and slow degradation (h^{-1}), respectively; C is lag time (h), time of bacterial colonization. $r^2 = 0.99$ – fit of the bicompartmental logistic model to describe the fermentation for all experimental diets.

Table 4. Mean values of pH and ammonia concentration in rumen fluid *in vitro* of diets with sunflower cake replacing soybean meal.

Variables	Inclusion of sunflower cake, g kg^{-1}				CV, %	P<		
	0	200	400	600		T	H	TxH
pH	6.81	6.78	6.74	6.75	0.94	Q[1]	L	ns
NH_3, mg dL^{-1}	24.50	25.92	23.90	25.72	14.96	ns	Q	ns

Q: quadratic regression (p < 0.05); ns: non-significant; CV (%): Coefficient of variation; T: treatment; H: collection times; TxH: Interaction treatment x collection times; [1]$Y = 6.8092 − 0.00028x − 0.00000027x^2, r^2 = 0.87$.
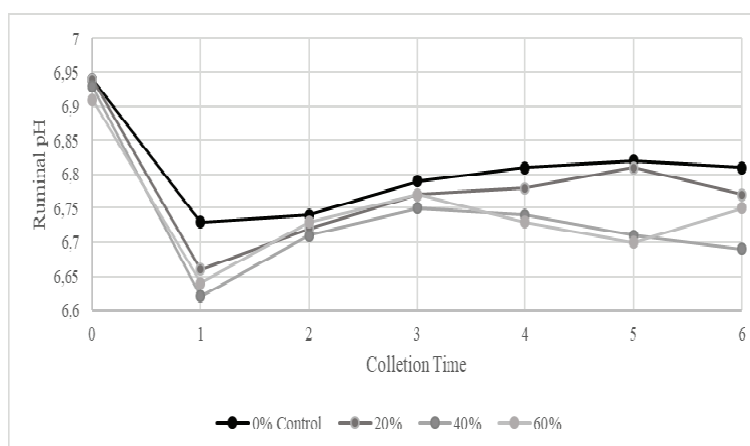

Figure 1. *In vitro* pH values of diets with sunflower cake replacing soybean meal.

There was a quadratic effect ($p < 0.05$) of ruminal NH_3 concentrations in relation to collection times, with an increase in concentrations around 3 to 4 hours after incubation. This behavior is considered normal, since the ruminal NH_3 concentration varies over time, due to the fermentation of the food by the action of ruminal microorganisms. The increase in NH_3 concentrations indicates the maximum fermentation activity, which can occur 3 to 5 hours after feeding when true protein sources are provided, varying with ruminal degradability and passage rate (Santos & Pedroso, 2011).

Conclusion

Sunflower cake can replace soybean meal up to 280 g kg^{-1} in the concentrate of beef cattle diets, improving the *in vitro* digestibility of dry matter, organic matter and protein. In this way, it can be considered an alternative to replace soybean and reduce production costs. Levels above 400 g kg^{-1} reduce ruminal digestion rate, digestibility and release of final fermentation products, such as short chain fatty acids, the main energy source for ruminants.

References

Association Official Analytical Chemist [AOAC]. (2006). *Official Methods of Analysis* (15th ed.). Arlington, VA: AOAC International.

Azevêdo, J. A. G., Pereira, J. C., Queiroz, A. C., Carneiro, P. C. S., Lana, R. P., Barbosa, M. H. P., ... Rennó, F. P. (2003). Composição químico-bromatológica, fracionamento de carboidratos e cinética da degradação *in vitro* da fibra de três variedades de cana-de-açúcar (*Saccharum* spp.). *Revista Brasileira de Zootecnia, 32*(6), 1443-1453. doi: 10.1590/S1516-35982003000600019.

Beran, F. H. B., Silva, L. D. F., Ribeiro, E. L. d. A., Rocha, M. A., Ezequiel, J. M. B., Correa, R. A., ... Silva, K. C. F. (2007). Avaliação da digestibilidade de nutrientes, em bovinos, de alguns alimentos concentrados pela técnica de três estádios. *Revista Brasileira de Zootecnia, 36*(1), 130-137. doi: 10.1590/S1516-35982007000100016.

Casali, A. O., Detmann, E., Valadares Filho, S. C. Pereira, J. C., Cunha, M., Detmann, K. S. C,. & Paulino, M. F. (2009). Estimação de teores de componentes fibrosos em alimentos para ruminantes em sacos de diferentes tecidos. *Revista Brasileira de Zootecnia, 38*, 130-138. doi: 10.1590/S1516-35982009000100017.

Detmann, E., Souza, M., Valadares Filho, S., Queiroz, A., Berchielli, T., Saliba, E., ... Azevedo, J. (2012). *Métodos para análise de alimentos*. Visconde do Rio Branco, MG: Suprema.

Domingues, A. R., Silva, L. D. F., Azambuja, R., Edson Luis, Castro, S. V. R., Barbosa, M. A. A. F., Mori, R. M., ... Silva, J. A. O. (2010). Consumo, parâmetros ruminais e concentração de uréia plasmática em novilhos alimentados com diferentes níveis de torta de girassol em substituição ao farelo de algodão. *Semina: Ciências Agrárias, 31*(4), 1059-1070.

Garcia, J. A. S., Figueiredo, P. V., Cecon, P. R., Melo, G. M. P., Martins, A. S., & Setti, M. C. (2004). Digestibilidade aparente do farelo de girassol na alimentação de bovinos leiteiros em fase de crescimento. *Ciência Animal Brasileira, 5*(3), 123-129.

Goering, H. K., & Van Soest, P. J. (1970). *Forage Fiber Analysis.* Washington, DC: USDA.

Goes, R. H. B. T., Souza, K. A., Patussi, R. A., Cornelio, T. C., Oliveira, E. R., & Brabes, S. K. C. (2010). Degradabilidade in situ dos grãos de crambe, girassol e soja, e de seus coprodutos em ovinos. *Acta Scientiarum. Animal Sciences, 32*(3), 271-277. doi: 10.4025/actascianimsci.v32i3.7913.

Goes, R. H. T., Tramontini, R. d. C. M., Almeida, G. D., Cardim, S. T., Ribeiro, J., Oliveira, L. A., ... Oliveira, E. R. (2008). Degradabilidade ruminal da matéria seca e proteína bruta de diferentes subprodutos agroindustriais utilizados na alimentação de bovinos. *Revista Brasileira de Saúde e Produção Animal, 9*(4), 715-725.

Holden, L. A. (1999). Comparison of methods of *in vitro* dry matter digestibility for ten feeds. *Journal of Dairy Science, 82*(8), 1791-1794. doi: 10.3168/jds.S0022-0302(99)75409-3.

Kozloski, G. V. (2011). *Bioquímica dos ruminantes* (3a ed.). Santa Maria, RS: Universidade Federal de Santa Maria.

Mertens, D. R. (1997). Creating a system for meeting the fiber requirements of dairy cows. *Journal of Dairy Science, 80*(7), 1463-1481. doi: 10.3168/jds.S0022-0302(97)76075-2.

Mesacasa, A. C., Zervoudakis, J. T., Zervoudakis, L. K. H., Cabral, L. S., Abreu, J. G., Leonel, F. P., ... Silva, R. F. G. (2012). Torta de girassol em suplementos múltiplos para bovinos em pastejo no período seco do ano: desempenho produtivo e viabilidade econômica. *Revista Brasileira de Saúde e Produção Animal, 13*(4), 1116-1179. doi: 10.1590/S1519-99402012000400022.

Mizubuti, I. Y., Ribeiro, E. L. A., Pereira, E. S., Pinto, A. P., Franco, A. L. C., Syperreck, M. A., ... Muniz, E. B. (2011). *In vitro* rumen fermentation kinetics of some co-products generated in the biodiesel production chain by gas production technique. *Semina: Ciências Agrárias, 32*(Suplemento), 2021-2028. doi: 10.5433/1679-0359.2011v32n4Sup1p2021.

Mupeta, B., Weisbjerg, M. R., Hvelplund, T., & Madsen, J. (1997). Digestibility of amino acids in protein rich tropical feeds for ruminants estimated with the mobile bag technique. *Animal Feed Science and Technology, 69*(1-3), 271-280. doi: 10.1016/S0377-8401(97)81641-0.

Nussio, L. G., Campos, F. P., & Lima, M. L. M. (2011). Metabolismo de carboidratos estruturais. In T. T. Berchielli, A. V. Pires, & S. G. Oliveira (Eds.), *Nutrição de Ruminantes* (p. 183-228). Jaboticabal, SP: FUNEP.

Oliveira, M. D. S., & Cáceres, D. R. (2005). *Girassol na alimentação de bovinos*. Jaboticabal, SP: Funep.

Oliveira, M. D. S., Mota, D. A., Barbosa, J. C., Stein, M., & Borgonovi, F. (2007). Composição bromatológica e digestibilidade ruminal *in vitro* de concentrados contendo diferentes níveis de torta de girassol. *Ciência Animal Brasileira, 8*(4), 629-638.

Oliveira, M. F., & Vieira, O. V. (2004). *Extração do óleo de girassol utilizando miniprensa*. Londrina, PR: EMBRAPA-CNPSO.

Owens, F. N., & Bergen, W. G. (1983). Nitrogen metabolism of ruminant animals: historical perspective, current understanding and future implications. *Journal of Animal Science, 57*(Suppl2), 498-518.

Owens, F. N., & Goetsch, A. L. (1993). Fermentación ruminal. In D. C. Church, *El rumiante fisiología digestiva y nutrición* (p. 159-190). Zaragoza, ES: Acribia.

Palmquist, D. L., & Jenkins, T. C. (1980). Fat in lactation rations: Review. *Journal of Dairy Science, 63*(1), 1-14.

Santos, F. A. P., & Pedroso, A. M. (2011). Metabolismo de carboidratos não estruturais. In T. T. Berchielli, A. V. Pires & S. G. Oliveira (Eds.), *Nutrição de Ruminantes* (p. 265-298). Jaboticabal, SP: Funep.

SAEG. (2007). *Sistema para análises estatísticas*, *versão 9.1*. Viçosa, MG: UFV.

Schofield, P., Pitt, R. E., & Pell, A. N. (1994). Kinetics of fiber digestion from *in vitro* gas production. *Journal of Animal Science, 72*(11), 2980-2991.

Silva, L. H. X., Goes, R. H. T. B., Carneiro, M. M. Y., Burin, P. C., Oliveira, E. R., Souza, K. A., ... Oliveira, R. T. (2015). Produção total de gases e degradabilidade *in vitro* de dietas com torta de girassol. *Archivos de Zootecnia, 64*(248), 365-371. doi: 10.21071/az.v64i248.421.

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science, 70*(11), 3562-3577.

Tilley, J. M. A., & Terry, R. A. (1963). A two stage technique for the in vitro digestion of forage crops. *Grass and Forage Science, 18*(2), 104-111.

Van Soest, P. J. (1994). *Nutritional ecology of the ruminant* (2nd ed.). Ithaca, NY: Cornell University Press.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*(10), 3583-3597.

Wascheck, R. C., Rezende, P. L. P., Moreira, P. C., Reis, R. B., Rosa, S. R. A., & Mendonça, A. C. (2010). Degradabilidade e produção de gases in vitro de fontes energéticas alternativas na alimentação de ruminantes. *Acta Scientiarum. Animal Sciences, 32*(4), 425-430. doi: 10.4025/actascianimsci.v32i4.8835.