



Acta Scientiarum. Animal Sciences

ISSN: 1806-2636

ISSN: 1807-8672

Editora da Universidade Estadual de Maringá - EDUEM

Tres, Tamara Tais; Jobim, Clóves Cabreira; Diaz, Tatiana
Garcia; Daniel, João Luiz Pratti; Jacovaci, Fernando Alberto
Okara or soybean grain added to the rehydrated corn grain silage
for cattle: digestibility, degradability and ruminal parameters
Acta Scientiarum. Animal Sciences, vol. 42, e48586, 2020
Editora da Universidade Estadual de Maringá - EDUEM

DOI: <https://doi.org/10.4025/actascianimsci.v42i1.48586>

Available in: <https://www.redalyc.org/articulo.oa?id=303162575051>

- How to cite
- Complete issue
- More information about this article
- Journal's webpage in redalyc.org

EDUEM
redalyc.org

Scientific Information System Redalyc

Network of Scientific Journals from Latin America and the Caribbean, Spain and
Portugal

Project academic non-profit, developed under the open access initiative

Okara or soybean grain added to the rehydrated corn grain silage for cattle: digestibility, degradability and ruminal parameters

Tamara Tais Tres^{*ID}, Clóves Cabreira Jobim, Tatiana Garcia Diaz, João Luiz Pratti Daniel and Fernando Alberto Jacovaci

Universidade Estadual de Maringá, Avenida Colombo, 5790, Jardim Universitário, 87020-900, Maringá, Paraná, Brasil. *Author for correspondence. E-mail: tamarattres@gmail.com

ABSTRACT. The experiment was carried out to evaluate total and partial digestibility of nutrients, the efficiency of microbial protein synthesis, *in situ* degradability and ruminal parameters in cattle fed diets with rehydrated corn grain silages, okara or soybean grain. Three Holstein steers were distributed in a 3 x 3 Latin square design. The treatments evaluated were: SO (corn grain silage + 30% okara), SSG (corn grain silage + 20% soybean grain) and CG (dry corn grains). The ruminal digestibility of non-fiber carbohydrates (NFC) increased in SO (88.34%) and SSG treatments (87.87%), compared to the CG treatment (63.48%). The minimum ruminal pH value was 6.01, observed 4.13 hours after feeding a diet with SO. The highest ammonia-N contents were 15.25 and 15.07 mg dL⁻¹ observed in SSG and SO, respectively, 2.45 and 2.61 hours after feeding. Treatments SO and SSG showed higher fraction A content (readily degradable fraction) and C (constant rate of degradability of fraction B). The effective degradability (ED) of dry matter (DM) was higher for the diets SO and DE of CP was higher for treatments SO and SSG. SSG and SO result in better utilization of nutrients by animals.

Keywords: byproduct; fermentation; microbial synthesis; pH; soybean.

Received on July 3, 2019.
 Accepted on February 5, 2020.

Introduction

The use of dry corn can lead to serious grain storage problems in properties, usually with high qualitative and quantitative losses, significantly increasing food costs (Jobim, Branco, Gai, Calixto Junior, & Santos, 2010). Thus, rehydrated corn silage has become an interesting alternative to replace ground corn, reduce production costs and improve efficiency in the use of starch. Rehydration of corn grains consists of returning the dry grain (87 - 90% DM) to adequate moisture (30 - 35%) for the fermentation process in the silo (Silva et al., 2018). This technique allows flexibilization of the ensiling process, and benefits producers who do not have corn harvesting equipments, area for cultivation or adequate structure for storing dry grain. In addition, it can increase digestibility compared to dry milled grain and minimizes the market effects on price fluctuations (Arcari, Martins, & Santos 2016).

This technology also allows the addition of other grains to silage besides corn, such as soybean, which improve the chemical composition of silage, mainly in relation to protein and energy contents (Calixto Junior et al. 2017). Another ingredient that can be added in corn grain silage to increase its nutritional value is okara, a byproduct of soybean processing to obtain the aqueous extract and tofu (Bowles & Demiate 2006).

The major interest in the inclusion of agroindustrial byproducts in animal nutrition is related to the low price in relation to the ingredients commonly used in animal diets, such as corn and soybean meal, for example (Harthan & Cherney, 2017). However, the disadvantage of using such byproducts refers to the seasonality of supply and possible variations in nutritional composition (Mirzaei-Aghsaghli & Maheri-Sis, 2008). Okara extraction process involves a wet milling of soybean, where the residue consists of a slurry with about 72% moisture content. Thus, large volumes of solid byproduct are produced. However, the high moisture content and the high cost of drying make its use unfeasible (Redondo-Cuenca et al., 2008). In this way, the use of wet okara added to dry corn grains to be stored as silage may be an alternative to the use of this byproduct.

Given the above, this study aimed to evaluate the partial and total digestibility of nutrients, the microbial synthesis efficiency, in situ degradability and ruminal parameters of cattle fed diets containing rehydrated corn grain silages with the inclusion of soybean grain or okara.

Material and methods

The experiment was conducted at the Iguatemi Experimental Research Farm, State University of Maringá (UEM), Maringá, PR, Brazil. The Research Farm is located at the latitude 23° 25' South and longitude 51° 57' West of Greenwich, with an average altitude of 540 m. All procedures were performed in accordance with the National Council for the Control of Animal Experimentation (CONCEA) guidelines and were authorized by the Ethics Committee for Animal Use of the State University of Maringá, State of Paraná, Brazil (Protocol 6372301115).

Three Holstein steers weighing on average 477 ± 76 kg, fitted with a rumen and duodenal cannula, were distributed in a 3 x 3 Latin square design. Animals were housed in a roofed masonry facility with 8.8 m² floor space, equipped with a feeder and automatic drinking fountain. The treatments consisted of three concentrate formulations: SO: rehydrated corn grain silage added with 30% okara, SSG: concentrate with rehydrated corn grain silage added with 20% soybean grain and GS: dry corn grain concentrate (control treatment).

For the SO production, dry corn grains were milled (10 mm) and then the okara was added at the level of 30% DM. The byproduct (okara) was provided by Cocamar Cooperativa Agroindustrial, a unit in Maringá, State of Paraná. The chemical composition of okara before ensiling was 19% dry matter, 28.6% crude protein, 18.1% ether extract, 4.16% ash, and 27.66% neutral detergent fiber. Upon ensiling, the DM contents of okara were determined in a microwave oven (Silva & Queiroz, 2002), in order to adjust the inclusion of the byproduct at the established level. For the SSG production, corn and soybean grains were milled through a 10mm sieve. Subsequently, 20% soybean grain (on a natural matter basis) were added to corn and homogenized. Thereafter, water was added to the mixture to rehydrate the grains to a moisture content of 35% to allow adequate fermentation.

For production of silages (SO and SSG), the microbial inoculant based on *Lactobacillus plantarum* MA 18/5U and *Propionibacterium acidipropionici* MA 26/4U (Lallemand Animal Nutrition) was applied with a backpack sprayer, aiming at a uniform distribution. Silos were plastic drums with capacity for 200 L (5 drums for each silage). Silos were stored in roofed area and remained sealed for 217 days. Part of the dry corn grain used for silage production was stored for formulation of the CG concentrate and was also processed through a 10 mm sieve. Forage was 40% corn whole plant silage. Diets were formulated to be isoproteic and isoenergetic, as presented in Table 1. The animals were fed twice a day at 08h00min and 16h00min. Diets were offered in a sufficient amount, allowing for 10% leftovers, which were collected daily from the feeders and weighed for individual consumption adjustment. The experimental periods lasted 14 days, consisting of 11 days for adaptation and 3 days for sampling.

Table 1. Chemical composition of the experimental diets.

Item	SO	SSG	CG
Ingredient (% DM)			
Corn grain silage with 20% soybean	0.00	33.00	0.00
Corn grain silage with 30% okara	32.00	0.00	0.00
Corn grain (ground)	0.00	0.00	27.09
Soybean meal	7.22	5.00	11.00
Limestone	0.70	0.80	0.80
Kaolin	0.00	1.12	1.00
Common salt	0.08	0.08	0.11
Corn silage	60.00	60.00	60.00
Nutrients (% DM)			
CP	12.20	12.30	12.20
EE	3.65	4.19	2.46
NDF	39.32	38.89	39.33
ADF	23.28	23.06	22.68
TDF	71.70	71.10	71.60
Phosphorus	0.28	0.27	0.26
Sodium	0.08	0.08	0.08

SO= diet with the inclusion of corn grain silage with 30% okara; SSG= diet with the inclusion of corn grain silage with 20% raw soybeans; CG= diet with dry corn grain.

Samples of duodenal digesta and feces were collected from the 10th days, for 3 days for the evaluation of the apparent digestibility coefficients of nutrients at different times 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 hours after the first meal, totaling 12 samples of duodenal digesta and 12 samples of feces per treatment/period. Additionally, samples of feeds and leftovers were collected and frozen (-20°C). Afterwards, the samples were thawed, homogenized, and dried in a forced-air oven at 55°C for 72 hours and ground in a Wiley mill with a 1 mm sieve. Then, the samples were mixed in the same ratio (10% of the sample based on the dry weight), and was made a composite sample of feces, duodenum, feeds and leftovers per animal/period.

The internal indicator was used for evaluation of duodenal flow and fecal excretion was the indigestible neutral detergent fiber (iNDF), as described by Casali et al. (2008) and samples of feeds, leftovers, duodenal digesta and feces were analyzed by means of an *in situ* incubation for 288 hours; the samples were processed through a 2 mm sieve and incubated in the rumen in F57 bags (Ankom®).

Samples of the feeds, leftovers, feces and duodenal digesta were analyzed for DM (method 934,13), ash (method 942,05), crude protein (method 984,13), ether extract (method 920,39 Soxhlet extraction with petroleum ether) according to techniques described by Association of Official Analytical Chemists (AOAC, 2006). Neutral detergent fiber (NDF) with addition of sulphite (Na₂SO₃) and heat stable α -amylase according to Van Soest, Robertson, and Lewis (1991) and acid detergent fiber (ADF), according to Mertens (2002). Total digestible nutrients (TDN) concentrations was calculated according to Sniffen, O'Connor, Van Soest, Fox, and Russel (1992).

Rumen fluid was collected through the ruminal cannula to determine pH and ammonia-N concentration at 0, 2, 4 and 6 hours after feeding. Ruminal pH was measured immediately using a portable pH meter (MA522 model, Marconi Laboratory Equipment, Piracicaba, São Paulo, Brazil). For ammonia-N analysis, 50 mL ruminal liquid was preserved by adding 1 mL sulfuric acid (H₂SO₄, 1:1) and stored at -20°C. Rumen fluid samples was thawed at room temperature and centrifuged at 3,000 x g for 15 min and the supernatants were used to determine the ammonia-N concentrations according to Chaney and Marbarch (1962).

On the 14th days of each experimental period, 1.5 kg ruminal contents were collected manually from the cranial, ventral, and caudal areas of the rumen from each animal 4 hours after each feeding for analysis of microbial synthesis efficiency. Samples collected were mixed with 500 mL saline (9 g NaCl L⁻¹) homogenized using a blender for 1 min, then filtered through four layers of cotton cheesecloth and stored at -20°C. Samples were analyzed according to Cecava et al. (1990). The bacterial pellets resulting from the centrifugations were dried at 55°C for 48 hours and macerated for determining the total DM, total N, total purines, and microbial synthesis efficiency according to Ushida, Lassalas, and Jouany (1985).

In situ ruminal degradability of DM and CP was determined using the nylon bag technique. Three Holstein steers, previously adapted to the diets described in Table 1, were used for a period of 10 days before the start of the incubation. The ground samples (6 g) were heat-sealed in nylon bags (Forrage Bag Ankom®) with 50µm porosity and incubated. Each feed sample was incubated in three replicates. The incubation times were 0, 2, 6, 12, 24 and 64 hours. The bags were removed at the determined time and washed in a washing machine in 5 cycles of 1 min and dried at 55°C for 48 hours in a drying oven. The DM content of the residue in each bag was determined at 105°C in a forced-air oven for 2 hours and CP content was determined according to Association of Official Analytical Chemists (AOAC, 1990).

Blood samples were collected from the 11th days by puncturing the jugular vein in vacutainer tubes containing EDTA. The blood was centrifuged at 3000 x g at 4°C for 15 min to separate the plasma, which was stored in Eppendorf tubes at -20°C for further analysis. Determination of BUN concentrations were made with commercial kits (Analisa®, Belo Horizonte, MG), and spectrophotometer readings (Bentley Instrument Inc., Chaska, MN) at 600 nm. The BUN was obtained by means of the product of the concentration of urea by the value 0.4667; corresponding to the N content in urea.

All data analyses were performed according to Rossi (2011) by means of Bayesian Inference. Total and partial nutrient digestibility data was considered that the response (Y_{ij}) followed normal distribution, that is, $Y_{ij} \sim N(\mu_j, \sigma_j^2)$. For each μ_j and σ_j^2 , non-informative distributions, respectively, $\mu_j \sim N(0, 10^{-6})$ and $\sigma_j^2 \sim \text{Gamma}(10^{-3}, 10^{-3})$ were considered a priori, according to the OpenBUGS parameterization. For pH and NH₃, we considered that the univariate response (y) of pH and NH₃ (mg dL⁻¹) ($i = 1, 2, \dots, n$) in a 3 x 3 Latin square experiment follows a normal distribution, i.e., $y_i \sim N(f(\beta, X); \sigma_e^2)$, assuming $\varepsilon_i \sim N(0; \sigma_e^2)$, for the multiple linear model: $y_i = X\beta + \varepsilon_i = \mu + p + a + H + T + T^2 + \varepsilon_i$, where: X: incidence matrix for, p: period, a:

animal, H: effect of treatments (Diets: SO, SSG and CG), T: cubic effect of time (0, 2, 4 and 6 h) corrected by the mean. For simplicity, operability and with the objective of comparisons between levels of treatments, as: $y_{ijk} = X\beta + \varepsilon_i = \mu + p + a + (T + T^2)_k + \varepsilon_{ik}$, k: effect within the k-th treatment (k=1,2,3).

Data for DM and CP degradability were adjusted by non-linear regression, which predicts the potential degradability ($y = PD$) of the food by means of the model proposed by Mehrez and Orskov (1977), as follows: $y_{ijk} = a_{ik} + b_{ik}(1 - e^{-c_{ik}t_j})$, i-animal; j-time, J; k-treatment, y is the fraction (%) of the nutrient degraded after time t (in hours); a is the soluble fraction (%) of the material contained in the nylon bag (the curve intercept); b is the potentially degradable fraction (%) of the material contained in the nylon bag after time zero; c is the constant fractional rate (%) of degradation of the potentially degradable fraction; t is the incubation time in the rumen, in hours. To estimate the effective degradability (ED), we used the Orskov and McDonald model (1979): $DE = a + \frac{bc}{c + tax_a}$, where the passage rate of solids in the rumen has values fixed at 2, 5 and 8% per hours. In the Bayesian procedure described by Rossi (2011), it was considered in the modeling that the regression parameter vector β_p is uncorrelated. Non-informative distributions were assumed a priori for all parameters of the model, i.e., for $c = 1,000$, according to the OpenBugs parameterization: $\beta_p | \sigma_e^2 \text{ iid} \sim N\left(0, \frac{1}{c^2}\right)$. As $\sigma_e^2 = \tau_e^{-1}$, it was assumed a non-informative Gamma distribution for τ_e , that is, $\tau_e \sim \text{Gama}\left(\frac{1}{c}, \frac{1}{c}\right)$.

The initial values for each μ_j , respectively, were the sample mean of the j-th treatment. The *a posteriori* marginal distributions for all parameters involved in the model were obtained through the *BRugs* package of R (R Development Core Team, 2015). 11,000 values were generated in an MCMC (Monte Carlo Markov Chain) process, and, considering a sample discard period of 1,000 initial values, the final sample taken at intervals of size equal to 1 (no autocorrelation) contained 10,000 values generated. Chain convergence was checked by using Heidelberger and Welch (1983) and Geweke (1992) criteria in the coda package of R (R Development Core Team, 2015).

Results and discussion

Dry matter intake (DMI), ruminal digestibility and total digestibility of DM, OM, NDF, CP, and TDN were not influenced ($p > 0.05$) by the intake of SO, GP and CG in the diet. There was no effect ($p > 0.05$) of treatments on total-tract digestibility. However, the ruminal digestibility of NFC was higher ($p < 0.05$) with the use of SO (88.34%) and SSG (87.87%) in the diet, compared to the inclusion of GC (63.48%; Table 2). These effects may be related to the fermentation process that takes place during ensiling, since the prolamin protein content is reduced. These proteins, which form the hydrophobic protein matrix around the starch granules, are responsible for limiting the starch degradation by the action of ruminal microorganisms (Hoffman et al., 2011). The decrease in the content of these proteins may occur during the silage fermentation, through solubilization of organic acids such as lactic and acetic acids, as well as by the proteolytic activity of bacterial enzymes or proteases present in the ensiled mass (Kung, Shaver, Grantt, & Schmidt, 2018). Thus, the reduction of these proteins increases starch availability for rumen degradation, which may increase NFC digestibility and DM degradation. Additionally, wet corn grains presented better response in relation to dry grain, which can be attributed to, among other factors, the composition of starch in corn grains (Reis et al., 2001).

The effect of ensiling on degradation of the protein matrix surrounding starch granules is also evident in the analysis of *in situ* degradability of CP, since it was observed a similar behavior to the degradation of DM (Table 3). In this way, it was possible to observe an increase ($p < 0.05$) in degradation of fraction A for the animals that received concentrate with SSG inclusion, followed by the animals that received the concentrate with inclusion of SO and consequent lower degradation of fraction B for these concentrates, when compared to the treatment CG. The potential degradability of CP was also higher ($p < 0.05$) for SO and SSG. The results for silage degradability were also reported in other studies with grain silages due to the enzymatic hydrolysis of proteins, starch and other carbohydrates that occur due to the fermentation in silos (Silva et al., 2014; Reis et al., 2001).

The supply of the concentrate with addition of CG resulted in lower ruminal digestibility of NFC and lower ($p < 0.05$) degradation of fraction A (soluble fraction; Table 3), possibly because it was composed of dry and ground corn grains (27.09% concentrate DM) and soybean meal (11% concentrate DM). This form of

grain processing influences the density of the particles increasing the ruminal passage rate. Thus, ground foods remain less time in the rumen to undergo degradation by bacterial enzymes (Bürger et al., 2000). Thus, higher amounts of these foods rapidly reach the posterior digestive tract and may undergo the action of intestinal enzymes (Passini et al., 2002). This fact indicates a possible compensatory effect of starch utilization, which was corroborated in this study, since no treatment effect was detected on total tract digestibility. The total digestibility of EE showed a diet effect ($p < 0.05$), with higher values for the SSG treatment, which can be attributed to the higher EE of the okara byproduct that resulted in a increased EE of this diet (4.19%), which may provide greater energy availability for the animals.

Table 2. Bayesian estimates for dry matter intake (DMI), total and ruminal apparent digestibility of nutrients, total digestible nutrients (TDN), microbial synthesis efficiency (Mic. Syn.) and blood urea nitrogen (BUN) of Holstein steers fed diets based on corn grain silage added with okara (SO), corn grain silage added with soybean grain (SSG) or diets based on dry corn grain (CG).

Variable	Diet		
	SO	SSG	CG
Intake			
DMI (kg)	9.66 ± 3.84	9.11 ± 2.87	9.95 ± 4.7
DMI (g kg ⁻¹ BW ^{0.75})	93.95 ± 23.60	89.97 ± 11.33	96.26 ± 14.15
Apparent ruminal digestibility (%)			
DM	44.49 ± 2.25	46.79 ± 6.25	38.64 ± 6.37
OM	49.79 ± 2.03	51.86 ± 5.62	44.51 ± 5.85
NFC*	88.34 ± 6.70a	87.87 ± 5.29a	63.48 ± 9.42b
NDF	25.96 ± 7.82	32.35 ± 12.23	33.19 ± 4.38
Total apparent digestibility (%)			
DM	65.83 ± 5.97	66.79 ± 2.65	61.44 ± 8.05
OM	67.93 ± 5.61	68.82 ± 2.51	63.81 ± 7.64
NFC	94.16 ± 0.68	97.32 ± 3.04	87.36 ± 12.13
NDF	37.44 ± 10.25	37.38 ± 2.33	35.89 ± 11.20
EE*	89.91 ± 8.23ab	91.02 ± 3.54a	73.34 ± 12.59b
CP	63.48 ± 8.25	62.60 ± 7.80	58.30 ± 12.30
TDN (%)	72.52 ± 5.82	74.57 ± 1.74	63.74 ± 8.70
Mic. Syn. (g Nmic kg ⁻¹ DOMR)	30.93 ± 13.60	30.59 ± 9.24	32.46 ± 21.01
BUN (mg dL ⁻¹)	10.31 ± 2.31	8.67 ± 1.78	8.55 ± 2.27

*Different letters, in the same row, indicate significantly different mean values by Bayesian comparisons, * $p < 0.05$; of a *posteriori* and populational mean estimated. Data are expressed in mean ± SD (standard deviation). DOMR = degraded organic matter in rumen.

Table 3. Mean Bayesian estimates (standard deviation) for the parameters of models (1) and for data of in situ degradability of dry matter and crude protein and effective degradability (ED) with passage rates of, respectively, 2, 5 and 8% of diets based on corn grain silage added with 30% okara (SO), corn grain silage added with 20% soybean grain (SSG) or diets based on dry corn grain (CG).

Parameters	Diet		
	SO	SSG	CG
Degradability of dry matter			
a ^{1*}	29.51 ± 4.54a	28.18 ± 3.45a	15.91 ± 1.34b
b ^{2*}	63.19 ± 4.69c	66.31 ± 3.83b	82.32 ± 1.81a
c ^{3*}	0.27 ± 0.07a	0.14 ± 0.03b	0.05 ± 0.01c
ED k=2%*	88.16 ± 2.16a	86.05 ± 1.85b	74.14 ± 0.81c
ED k=5%*	82.49 ± 1.76a	76.84 ± 1.51b	56.40 ± 0.81c
ED k=8%*	77.86 ± 1.78a	70.19 ± 1.57b	46.94 ± 0.81c
σ ² _e	55.51	40.56	11.19
Degradability of crude protein			
a*	45.88 ± 2.16b	46.79 ± 2.38a	14.41 ± 3.53c
b*	52.31 ± 2.54b	51.32 ± 2.81c	82.98 ± 5.04a
c*	0.076 ± 0.01a	0.070 ± 0.01b	0.073 ± 0.01ab
ED k=2%*	86.77 ± 1.07a	86.62 ± 1.18a	79.13 ± 2.30b
ED k=5%*	76.72 ± 1.15a	76.65 ± 1.28a	63.18 ± 1.89b
ED k=8%*	70.64 ± 1.21a	70.68 ± 1.34a	53.58 ± 1.93b
σ ² _e	25.75	31.71	63.76

*Different letters, in the same row, indicate significantly different mean values by Bayesian comparisons comparisons * $p < 0.05$; Data are expressed in mean ± SD (standard deviation); ¹a is the soluble fraction (%) of the material contained in the nylon bag (the curve intercept); ²b is the potentially degradable fraction (%) of the material contained in the nylon bag after time zero; ³c is the constant fractional rate (%) of degradation of the potentially degradable fraction.

The microbial protein synthesis had a mean of 31.32 g Nmic kg⁻¹ OMDR, however, it did not show effect ($p > 0.05$) from the use of SO, SSG and CG added in the diet (Table 2). Microbial protein synthesis depends

on several factors, one of the most important is the adequate availability of carbohydrates and N in the rumen, so that microbial growth can be maximized by the synchronization between the availability of fermentable energy and degradable nitrogen in the rumen (National Research Council [NRC], 2000). This parameter is of utmost importance for the protein metabolism in ruminants, since most of the amino acids absorbed in the small intestine comes from the microbial protein (NRC, 2000). In this study, the microbial protein synthesis no effect of the treatments was found, indicating that the diets evaluated had an adequate protein and TDN balance.

The BUN concentration is a parameter that allows evaluating the protein nutrition of ruminants, through the metabolic response to a diet. Swenson and Reece (1996) consider adequate BUN concentrations those between 10 and 30 mg dL⁻¹. Thus, higher concentrations may characterize inefficiency in protein utilization and higher energy losses (Rodríguez, Sosa, & Rodríguez, 2007). In this study, the mean value was 9.17 mg dL⁻¹, and no effect ($p > 0.05$) was found for the treatments (Table 2). The low BUN concentrations observed indicate adequate synchronization between dietary protein and energy availability, indicating a higher efficiency in the use of these nutrients by ruminants (Powell, Wattiaux, & Broderick, 2011).

Another parameter directly related to the digestible protein fraction and the protein digestion rate is the ammonia-N in the rumen. Maximum ammonia-N concentrations were similar ($p > 0.05$; Table 4) in all treatments since the formulated diets were isoproteic and isoenergetic (Table 4). At different evaluation times, ammonia-N concentrations were within the minimum recommended range (5 mg dL⁻¹) by Satter and Slyter (1974) to provide adequate ruminal fermentation and microbial protein synthesis.

Table 4. Estimates of the critical points of the quadratic regressions fit for ruminal NH₃ and pH as a function of time (h) after feeding Holstein steers with diets based on corn grain silage added with 30% okara (SO), corn grain silage added with 20% soybean grain (SSG) or diets based on dry corn grain (CG), obtained by the Bayesian method for the diets evaluated.

Parameter	Diet		
	SO	SSG	CG
Ruminal ammonia (NH ₃)			
NH ₃ max (mg dL ⁻¹) *	15.07 ± 3.00	15.25 ± 5.45	15.03 ± 2.17
T _{max} NH ₃ max (h) *	2.61 ± 3.61ab	2.45 ± 8.37b	2.66 ± 1.39a
Ruminal pH			
pH _{min} *	6.01 ± 0.04c	6.02 ± 0.04b	6.03 ± 0.03a
T _{min} pH _{min} (h) *	4.13 ± 0.66a	3.88 ± 0.43b	3.54 ± 0.24c

*Different letters, in the same row, indicate significantly different mean values by Bayesian comparisons * $p < 0.05$. Data are expressed in mean ± SD (standard deviation).

The minimum ruminal pH value had an effect ($p < 0.05$) of the treatments used (Table 4). The ruminal pH values are associated with the concentrations of short chain fatty acids (acetic, propionic and butyric acids) released into the rumen as the main ruminal fermentation products (Vargas Junior et al., 2011). The greater amount of soluble carbohydrates available and the higher digestibility observed in the treatments with inclusion of SO and SSG are associated with higher concentrations of these acids, which when produced at a higher fermentation rate leads to a drop in ruminal pH. According to Hoover and Stockes (1991), moderate reductions in ruminal pH to values up to 6.2 do not cause negative impacts on the ruminal fermentation process or the digestion of fiber carbohydrates.

Conclusion

The ruminal digestibility of NFC, total digestibility of EE, as well as the degradation of the soluble fraction and the effective degradability of DM and CP of the food can be improved by the process of ensiling corn grains, compared to the use of dry grains in the diet for cattle. The inclusion of okara in corn grain ensiling can be considered a viable alternative to the use of this soybean processing byproduct, since it results in benefits in the nutrient digestibility and ruminal fermentation process, similar to those obtained with soybean grains.

References

- Association of Official Analytical Chemists [AOAC]. (1990). *Official Methods of Analysis* (15th ed.). Washington, DC: AOAC International.
- Association of Official Analytical Chemists [AOAC]. (2006). *Official Methods of Analysis* (18th ed.). Gaithersburg, MD: AOAC International.

- Arcari, M. A., Martins, C. M. M. R. T. T., & Santos, M. V. (2016). Effect of the ensiling time of hydrated ground corn on silage composition and in situ starch degradability. *Brazilian Journal of Veterinary Research and Animal Science*, 53(1), 60-71. doi:10.11606/issn.1678-4456.v53i1p60-71
- Bürger, P. J., Pereira, J. C., Coelho da Silva, J. F. C., Valadares Filho, S. C., Cecon, P. R., Jordão, C. P., & Braz, S. P. (2000). Taxas de Passagem e Cinética da Degradação Ruminal em Bezerros Holandeses Alimentados com Dietas Contendo Diferentes Níveis de Concentrado. *Revista Brasileira de Zootecnia*, 29(1), 225-235. doi: 0.1590/S1516-35982000000100030
- Bowles, S., & Demiate, I. M. (2006). Caracterização físico-química de okara e aplicação em pães do tipo Francês. *Ciência e Tecnologia de Alimentos*, 26(3), 652-659. doi: 0.1590/S0101-20612006000300026
- Calixto Junior, M., Jobim, C. C., Osmari, M. P., & Tres, T. T. (2017). Nutritional additives in high moisture corn silage. *Revista Brasileira de Ciências Agrárias*, 12(1), 105-111. doi: 10.5039/agraria.v12i1a5413
- Casali, A. O., Detmann, E., Valadares Filho, S. C., Pereira, J. C., Henriques, L. T., Freitas, S. G., & Paulino, M. F. (2008). Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos in situ. *Revista Brasileira de Zootecnia*, 37(2), 335-342. doi: 10.1590/S1516-35982008000200021
- Cecava, M. J., Merchen, N. R., Gay, L. C., & Berger, L. L. (1990). Composition of ruminal bacteria harvested from steers as influenced by dietary energy level, feeding frequency, and isolation techniques. *Journal of Dairy Science*, 73(9), 2480-2488. doi:10.3168/jds.S0022-0302(90)78933-3
- Chaney, A. L., & Marbach, E. P. (1962). Modified reagents for determination of urea and ammonia. *Clinical Chemistry*, 8, 130-137.
- Geweke, J. (1992). Evaluating the accuracy of sampling- based approaches to the calculation of posterior moments. In J. M. Bernardo, J. O. Berger, A. P. Dawid, & A. F. M. Smith (Orgs.), *Bayesian Statistics 4*. Oxford, UK: Oxford University Press.
- Harthan, L. B., & Cherney, D. J. R. (2017). Okara as a protein supplement affects feed intake and milk composition of ewes and growth performance of lambs. *Animal Nutrition*, 3(2), 171-174. doi: 10.1016/j.aninu.2017.04.001
- Heidelberger, P., & Welch, P. (1983). Simulation run length control in the presence of an initial transient. *Operations Research*, 31(6), 1109-44. doi: 10.1287/opre.31.6.1109
- Hoffman, P. C., Esser, N. M., Shaver, R. D., Coblenz, W. K., Scott, M. P., Bodnar, A. L., ... Charley, R. C. (2011). Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. *Journal of Dairy Science*, 94, 465-2474. doi: 10.1287/opre.31.6.1109
- Hoover, W. H., & Stokes, S. R. (1991). Balancing carbohydrates and proteins for optimum rumen microbial yield. *Journal of Dairy Science*, 74(10), 3630-3644. doi: 10.3168/jds.S0022-0302(91)78553-6
- Jobim, C. C., Branco, A. F., Gai, V. F., Calixto Junior, M., & Santos, G. T. (2010). Qualidade da silagem de grãos de milho com adição de soja crua e parâmetros de digestibilidade parcial e total em bovinos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 62(1), 107-115. doi: 10.1590/S0102-09352010000100015
- Kung J. L., Shaver, R. D., Grant, R. J., & Schmidt, R. J. (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science*, 101(5), 4020-4033. doi: 10.3168/jds.2017-13909
- Mehrez, A. Z., & Orskov, E. R. (1977). A study of the artificial fiber bag technique for determining the digestibility feeds in the rumen. *Journal of Agricultural Science*, (88), 645-650. doi: 10.1017/S0021859600037321
- Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC International*, 85(6), 1217-1240.
- Mirzaei-Aghsaghli, A., & Maheri-Sis, N. (2008). Nutritive value of some agroindustrial by-products for ruminants: a review. *World Journal of Zoology*, 3, 40-46.
- National Research Council [NRC]. (2000). *Nutrient requirements of fish and shrimp*. Washington, DC: The National Academies Press.
- Orskov, E. R., & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agriculture Science*, 92(2), 499-503. doi: 10.1017/S0021859600063048

- Powell, J. M., Wattiaux, M. A., & Broderick, G. A. (2011). Short communication: Evaluation of Milk urea nitrogen as a management tool to reduce ammonia emissions from dairy farms. *Journal of Dairy Science*, 94(9), 4690-4694. doi: 10.3168/jds.2011-4476
- Passini, R., Silveira, A. C., Rodrigues, P. H. M., Castro, A. L., Titto, E. A. L., Arrigoni, M. B., & Costa, C. (2002). Digestibilidade de dietas a base de grão úmido de milho ou de sorgo ensilados. *Acta Scientiarum. Animal Sciences*, 24, 1147-1154. doi:10.4025/actascianimsci.v24i0.2604
- R Development Core Team. (2015). *R: A language and environment for statistical computing*. Vienna, AU: R Foundation for Statistical Computing.
- Redondo-Cuenca, R. A., Suárez, M. J. V., & Aparicio, I. M. (2008). Soybean seeds and its by-product okara as sources of dietary fibre. Measurement by AOAC and Englyst methods. *Food Chemistry*, 108(1), 1099-1105. doi: 10.1016/j.foodchem.2007.11.061
- Reis, R. A., Jobim, C. C.; Macedo, F. A., Martins, E. N., Cecato, U. & Silveira, A. (2001). Desempenho de cordeiros terminados em confinamento, consumindo silagens de milho de grãos com alta umidade ou grãos de milho hidratados em substituição aos grãos de milho seco da dieta. *Revista Brasileira de Zootecnia*, 30, 596-603, 2001. doi: 10.1590/S1516-35982001000200040
- Rodríguez, R., Sosa, A., & Rodríguez, Y. (2007). Microbial protein synthesis in rumen and its importance to ruminants. *Cuban Journal of Agricultural Science*, 41(4), 287-294.
- Rossi, R. M. (2011). *Introdução aos métodos Bayesianos na análise de dados zootécnicos com uso do WinBUGS e R*. Maringá, PR: Eduem.
- Satter, L. D., & Slyter, L. L. (1974). Effect of ammonia concentration on rumen microbial production *in vitro*. *British Journal of Nutrition*, 32(2), 199-208. doi: 10.1079/BJN19740073
- Silva, J. S., Borges, A. L. C. C., Lopes, F. C. F., Silva, R. R., Vieira, A. R., Duque, A. C. A., ... Gonçalves, L. C. (2014). Degradabilidade ruminal *in situ* do sorgo grão em diferentes formas de reconstituições. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 66(6), 1822-1830. doi: 10.1590/1678-6731
- Silva, M. R. H., Jobim, C. C., Neumann, M., & Osmari, M. P. (2018). Corn grain processing improves chemical composition and fermentative profile of rehydrated silage. *Acta Scientiarum. Animal Sciences*, 40, e42564. doi: 10.4025/actascianimsci.v40i1.42564
- Silva, D. J., & Queiroz, A. C. (2002). *Análise de alimentos: métodos químicos e biológicos* (3a ed.). Viçosa, MG: UFV.
- Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., & Russel, 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. doi: 10.2527/1992.70113562x
- Swenson, M. J., & Reece, W. (1996). *Dukes fisiologia dos animais domésticos* (11a ed.). Rio de Janeiro, RJ: Guanabara.
- Ushida, K., Lassalas, B., & Jouany, J. P. (1985). Determination of assay parameters for RNA analysis in bacterial and duodenal samples by spectrophotometry: Influence of sample treatment and preservation. *Reproduction Nutrition Developmen*, 25(6), 1037-1046. doi: 10.1051/rnd:19850804
- Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. *Journal of Dairy Science*, 74(10), 3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2
- Vargas Junior, F. M., Sanchez, L. M. B., Wechsler, F. S., Bianchini, W., Oliveira, M. V. M., & Schmidt, P. (2011). Influence of corn processing provided in the diet on the ruminal dynamics of dairy steer. *Revista Brasileira de Zootecnia*, 40(2), 433-440. doi:10.1590/S1516-35982011000200027