A comparison of processed sorghum grain using different digestion techniques

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ABSTRACT
This study compares in situ, in vitro (DaisyII and gas production) and in vivo techniques to estimate the degradation of dry matter (DM), organic matter (OM), and N of sorghum grain. We used whole dried sorghum (WDS), dry cracked sorghum (DCS), the reconstituted whole sorghum silage (WSS) and reconstituted cracked sorghum silage (CSS). The residues obtained from the ruminal digestion in vitro (DaisyII) and in situ were analysed for their intestinal digestion (pepsin–pancreatin). OM was similar (981.32 ± 0.52) in all treatments, WSS showed the highest (P < .001) crude protein (CP) concentration compared with the other treatments, whereas CSS showed the highest amount of starch (P < .001) compared to other treatments. The apparent degraded substrate (ADS) was higher (P < .038) for whole sorghums, rumen degradable protein (RDP) was higher for WDS and WSS (P = .003), while protein digestible in the intestine (PDI) was higher for sorghums silage (P < .001) compared with dry sorghums. ADS was higher (P < .022) using the in sacco technique compared with the other methods, while for the RDP and PDI methods in sacco and in vitro (DaisyII) showed the better degradation compared with in vivo. The reconstituted ensiling sorghum grains had a favourable response in the availability of nutrients, compared with dried sorghums.

1. Introduction
There are areas where limited rainfall or unfavourable soil conditions make corn production uncertain. Because of its drought tolerance and high forage production, sorghum is an alternative crop (Andewakun et al. 1989; Gurbuz 2009). The major potential limitation of sorghum grain is the lower digestibility due to the dense proteinaceous matrix in the peripheral endosperm layer of the kernel (Gutierrez et al. 1982), which renders starch granules inaccessible to digestion in the rumen. However, rolling, steam flaking, reconstituted grains and high-moisture grains silage can overcome this limitation. Studies related to the effects of processing on the alteration of starch and protein in cereals and their use can be classified into three categories: performance and efficiency in feed utilization, in vitro measurements in structural starch changes, rates of ruminal microbial fermentation or enzyme degradation, and ruminal and post-ruminal in vivo determinations (DePeters et al. 2003; Abdelhadi and Santini 2006). There are numerous laboratory tests to estimate ruminal and intestinal digestion of protein (Koenig and Rhode 2001; Gargallo et al. 2006) using several enzymatic procedures, which are simple and affordable compared to in vivo methods (Danesh Mesgaran et al. 2005). The in vitro gas production technique is a method for determining the extent and kinetics of degradation of the food through the volume of gas produced during the fermentation process (Theodorou et al. 1994). An advantage of this procedure is that the course of the fermentation and the role of the soluble components of the substrate can be quantified (Pell et al. 1997; Calabrò et al. 2006). The main purpose of the in sacco method is to provide estimates of the rate and dynamics of the degradation of food constituents subject to the effect of rumen environment (Olivera 2001). The system DaisyII (ANKOM Corp., Fairport, NY, USA) is used as an alternative method to calculate the in vitro degradation of food in the rumen and intestine under laboratory conditions. Moreover, the rate of digestion of nutrients in a food varies inversely with the particle size; this effect is most obvious in low-rumen-degradability grains, such as sorghum and maize, since depending on the type of processing and accompanying diet can change the site of digestion of grains (Ramos et al. 2009; Al-Rabadi et al. 2011).

In this study, we examined whether the ensiling process can improve sorghum protein and starch digestibility in the small intestine to allow a complete substitution of sorghum grain with sorghum whole or cracked reconstituted, in a high concentrate diet using growing bulls as the study model.

2. Material and methods
Sorghum samples were obtained from a commercial lot (Jilotepec, Mexico), which contained whole grains and negligible

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quantities (<5 wt.%) of broken kernels. In the present study, we used whole dried sorghum (WDS); reconstituted whole sorghum silage (WSS), dry cracked sorghum (DCS) and reconstituted cracked sorghum silage (CSS). The grain was reconstituted by adding water to the whole grain to raise the humidity to 35% (Huck et al. 1999) and divided into two samples: the first was ensiled during 42 days using WSS and 21 days using CSS based on Simpson et al. (1985) and Balogun et al. (2005). After the time of silage, 1000 g of samples were taken directly from the silos in triplicates and frozen at −20°C for further analysis.

2.1. Particle size

To determine particle size, a stirrer was used WS 8570 TYLER (Model RX-812) with sieves (mm) at 3.36, 2.83, 2.00, 0.84, 0.59 and 0.42 mm, with a stirring time of 10 minutes per treatment (Ensor 1970).

2.2. In vivo evaluation

We used four Angus x Holstein calves (body weight, BW, 300 ± 50 kg) of 6 months age, fitted with rumen and duodenal cannula, which were fed one of four treatments, WDS, WSS, DCS and CSS in a 4 × 4 Latin square design. The animals were fed at 08:00 and 20:00 h at 2% live weight, with free access to drinking water. Each experimental period lasted 20 d, the first 15 d for diet adaptation and 5 d for sample collection. The diets were used (Table 1) with 73% inclusion in each of dry diets were used (Table 1) with 73% inclusion in each of dry

2.3. In situ evaluation

We used three growing Angus x Holstein bulls (300 ± 50 kg BW), 6 months age, with permanent rumen cannula to determine the digestibility of DM and crude protein (CP) according to Ørskov and McDonald (1979). The animals were fed ad libitum (08:00 and 16:00 h), with a diet containing 60% oat hay and 40% concentrate based on sorghum grain and soybeans (15% CP, 12.56 MJ ME/kg DM). Five gram DM of each sample was placed in nylon bags (Ankom R510, 5 × 10 cm, with a pore size of 50 μm), to be subsequently subjected to ruminal incubation for 24 h in triplicate, and at the end of the incubation the bags were washed with water, until drained, cleaned and dried in a forced air oven at 60°C for 72 h for further analysis.

2.4. In vitro ruminal digestibility

The grain samples were incubated in vitro, a culture medium was prepared according to the methodology described by Daisy (Ankom Technology 2008). The culture medium was performed in a ratio of 4:1 (medium: rumen fluid) at 39°C, stirring the solution to allow a uniform mixture. The rumen fluid was obtained from three growing Angus x Holstein bulls (300 ± 50 kg BW) fed with the same diet (60% oat hay and 40% concentrate). 45 nylon bags were used (Ankom R510, 5 × 10 cm, pore size 50 μm) weighting 5 g DM of each treatment. The bags were incubated for 24 h, deposited five bags per jar, making three replicates per treatment according to Bagolun et al. (2005) who did not find differences in the degradation after 24 h of incubation. At the end of the incubation, the bags were washed with tap water until the water drained clean, and then dried in a forced air oven at 60°C for 72 h for further analysis.

2.5. Intestinal digestibility in vitro

The residues obtained from the in vitro digestion (Daisy) and in situ were subjected to enzymatic digestion (pepsin–pancreat) for protein digestible in the intestine (PDI) according to Gargallo et al. (2006). One gram DM of each residual sample was placed in nylon bags (Ankom R510, 5 × 10 cm, with a pore size of 50 μm), to be subsequently subjected to incubation for 24 h in triplicates. In each jar, randomly placed eight replicates plus two blanks were incubated in order to generate the correction factor. Each jar contained a solution of 2 L of 0.1 N HCl adjusted to pH 1.9 with 1 g/L of pepsin (P-700), remaining 1 h at a constant rotation at

### Table 1. Chemical composition of sorghum grain processing using different methods (g/kg DM).

| Item       | WDS | DCS | WSS | CSS | SEM | Tx  | D vs. S | W vs. C |
|------------|-----|-----|-----|-----|-----|-----|---------|---------|
| DM         | 977 | 985 | 659 | 646 | 1.65 | 0.001 | 0.001   | 0.654   |
| OM         | 981 | 981 | 981 | 981 | 0.14 | 0.155 | 0.536   | 0.631   |
| CP         | 101 | 98  | 110 | 98  | 0.5  | 0.001 | 0.001   | 0.001   |
| Starch     | 721 | 730 | 736 | 784 | 1.43 | 0.001 | 0.001   | 0.001   |
| NDF        | 92  | 65  | 95  | 71  | 0.57 | 0.001 | 0.026   | 0.001   |
| GE, MJ/kg DM | 18.0 | 18.0 | 19.6 | 20.4 | 0.21 | 0.001 | 0.001   | 0.510   |

Abbreviations: WDS, Whole dried sorghum; DCS, dry cracked sorghum; WSS, whole sorghum silage; CSS, cracked sorghum silage; OM, organic matter; NDF, neutral detergent fiber.

Means with different literals within the same row are different (P < .0001).
39°C. After 24 h the incubation bags were washed with running water manually squeezed (three times), and were deposited in jars which contained 2 L of pancreatic solution (with KH₂PO₄ buffer solution 0.5 M adjusted to pH 7.75) containing 50 ppm of thymol and 3 g/L of pancreatic (P-7545, Sigma), and remained in a circular rotation at 39°C, 24 h; subsequently washed with tap water, drained and dried in a forced air oven at 60°C, 72 h for further analysis.

2.6. In vitro gas production

Rumen inoculum was collected from three growing Angus × Holstein bulls (300 ± 50 kg BW) fitted with permanent rumen cannula and fed ad libitum with the same diet (60% oat hay and 40% concentrate). Ruminal contents from each bull was obtained before the morning feeding, mixed and strained through four layers of cheesecloth into a flask with O₂ free headspace. Samples of each feed (0.800 g DM) were weighed into 120 mL serum bottles. Consequently, 10 mL of particle-free ruminal fluid was added to each bottle followed by 90 mL of the buffer solution (Theodorou et al. 1994). A total of 36 bottles (three bottles of each triplicate sample for each of the four treatments in three runs in different weeks plus three bottles as blanks (i.e. rumen fluid only)) were incubated for 24 h. Once all bottles were filled, they were immediately closed with rubber stoppers, shaken and placed in the incubator at 39°C. The volume of gas produced was recorded at 3, 6, 9, 12, 18 and 24 h of incubation using the pressure reading technique (Streeter et al. 1993) (Pressure transducer, HD 8804, DELTA OMS, Casselle di Selvazzano, Italy) of Theodorou et al. (1994). At the end of incubation (i.e. 24 h), pH was measured using a potentiometer (Conductronic pH15, Puebla, Mexico), contents of each bottle were then filtered to get the non-fermented residue for the determination of apparent degraded substrate (ADS, g/100 g) and the contents of each serum bottle were filtered under vacuum through glass crucibles with a sintered filter (coarse porosity no. 1, pore size 100–160 µm, Pyrex, Stone, UK). Fermentation residues were dried at 105°C overnight to estimate potential DM disappearance. Loss in weight after drying was indicative of undegradable DM. The DM disappearance at 24 h of incubation (i.e. ADS; g/100 g DM) was calculated as the difference between DM content of substrate and its undegradable DM. Five millilitre of fluid content of each bottle was taken for the determination of volatile fatty acids (VFA) by the method proposed by Jouany (1982) using 4-methylvaleric as the internal marker, and 10 mL to which were added 3.5 mL of HCl to obtain N–NH₃ (Weatherburn 1967). The gas production at 24 h was correlated with the ADS to obtain relative gas production (RGP mL⁻¹ gas g ADS) (Guzman Cedillo et al. 2016). The residue obtained from the samples used in the tests of ruminal incubation and pepsin–pancreatin and gas production were analysed for DM (60°C, 48 h) and N (%954.01, AOAC 1997). PDI was analysed according to the technique described by Gargallo et al. (2006), and it was not determined in the residue of gas production due to insufficient residue.

2.8. Calculations

The particle size was determined using following the equation:

\[ d_{gw} = \log^{-1} \left( \sum (W_i \log d_i) / \sum W \right) \]

\[ S_{gw} = \log^{-1} \left[ \left( \sum W_i (\log d_i - \log d_{gw})^2 \right)^{1/2} / \sum W \right] \]

where \( d_i \) is the diameter of the opening of the \( i \)th mesh sieve, \( d_i + 1 \) the diameter of the next screen size that is the \( i \)th screen (just above the group), \( d_{gw} \), the geometric mean diameter, \( d_i \), the diameter geometric particle in \( i \)th sieve, with sieve \( = \left| X d_i d_i + 1 \right|^{1/2} \) and \( S_{gw} \), geometric standard deviation.

2.9. In vivo digestibility

Digestion of N was estimated according to Faichney (1975):

\[ \text{Ruminal nutrient digestibility} = 100 - \left[ \left( \frac{\% \text{ marker in feed}}{\% \text{ marker in duodenum}} \right) \times \left( \frac{\% \text{ of duodenal nutrient}}{\% \text{ nutrient in feed}} \right) \right] \]

2.10. In vivo, in vitro (Daisy™) and in sacco digestibility

Nutrient digestibility and ADS were calculated by the following equation:

\[ \text{Nutrient digestibility (g/100 g DM)} = \left( \frac{\text{Nutrient intake} - \text{Nutrient in faeces}}{\text{Nutrient intake}} \right) \times 100 \]

\[ \text{ADS} (\text{mg/100 mg}) = \left( \frac{\text{Initial weight} - \text{Final residue weight}}{\text{Initial weight}} \right) \times 100. \]
2.11. Intestinal digestibility

2.11.1. In vitro
The pepsin–pancreatin digestion of N was calculated as (Gar-gallo et al. 2006):

\[
\text{% N disappeared} = \frac{\left(\text{Initial N in the sample} - \text{N remaining after incubation pepsin - pancreatin}\right)}{\text{Initial N in the sample}} \times 100. \tag{6}
\]

2.11.2. In vivo
N intestinal digestion was calculated following the equation:

\[
\text{Nutrient digestibility (g/100 g)} = \frac{\left(\text{Nutrient enters the intestine (g/day)} - \text{Nutrient in feces (g/day)}\right)}{\text{Nutrient enters the intestine (g/day)}} \times 100. \tag{7}
\]

2.11.3. In vitro gas production
Gas production was estimated in mL/gas per hour, so it was adjusted according to the model proposed by France et al. (1993):

\[
Y = a[1 - \exp(-b^{(t-T)} - c\times\sqrt{t^2 + T})], \tag{8}
\]

where \(Y\) represents the cumulative gas production (mL), \(t\) is the incubation time (h), \(a\) is the asymptote of the curve (total gas production, mL), \(b\) (h\(^{-1}\)) and \(c\) (h\(^{-1/2}\)) are the initial and later gas production rate constants and \(T\) represents the lag time (h), which is the time when the food begins to be degraded by microorganisms in the rumen.

2.12. Statistical analysis
The data from in vitro and in sacco tests were separately adjusted to an analysis of variance using a completely randomized design:

\[
Y_{ij} = \mu + T_i + e_{ij},
\]

where \(\mu\) is the overall mean, \(T_i\) is the effect due to cereal treatment and \(e_{ij}\) is the experimental error.

In the analysis of variance, the sorghum treatment was included \((n = 4)\) and replicated (three sets of incubation). The corresponding analysis of variance was done using the ANOVA procedure of the SAS program (2002). Means were compared using the Tukey test (Steel and Torrie 1997). The effect of treatments was performed using orthogonal contrasts, comparing dry sorghum (DS) vs. silage sorghum (SS), and whole sorghum (WS) vs. cracked sorghum (CS).

3. Results and discussion

3.1. Chemical composition
The chemical composition is presented in Table 1. The DM content of silage was lower \((P < .01)\) compared to the rest of the treatments used; likewise, had a similar OM content between them \((P = .155)\). Total N was higher \((P < .001)\) for WSS than the rest of the treatments, the reconstitution and silage of the grain increased the availability of starch in CSS and WSS, the neutral detergent fiber (NDF) content was different \((P < .001)\) between treatments, being DCS showing the lowest content. The DM content in this study was lower for grain silage \((P < .05)\) compared to Rodríguez (2005) and Abdelhadi and Santini (2006), who found an average DM content of 881 g/kg DM using DS grain and 402 g/kg using sorghum silage. The content of N in the present study was higher for WDS and WSS compared to Baker et al. (2010) and Abdelhadi and Santini (2006) who found an N content of 16.6 g/kg DM for sorghum cooked and 10.7 g/kg DM using sorghum silage, respectively. However, the results obtained in the present study are lower than that of Hamid et al. (2007) using corn grain with 18.8 g/kg DM. The starch content was higher compared to CSS and compared to DePeters et al. (2003) and Lanzas et al. (2007) with a starch content of 741 g/kg DM in steam flaking corn and 696 g/kg DM different varieties of sorghum. Finally, the NDF content of CSS was not different from that of Lanzas et al. (2007) with 78 g/kg DM.

3.2. Particle size
The effect of processing treatments used and their physical characteristics are given in Table 2. The 3.36 mm particle size was higher \((P < .001)\) for WDS followed by WSS, being lower for DCS and CSS; however when it sieved between 2.8 and 3.36 mm, it is observed that the highest percentage was for WDS and WSS (65 ± 0.4%) and the lowest percentage was for DCS and CSS (6 ± 0.4%), when analysing the particle size of 0.8 to 2.0 mm, cracked sorghums (DCS and CSS) showed the higher percentage \((P < .001)\) (56 ± 0.4%) compared with the WSS. The geometric mean diameter value of processed

\[
\text{Y}_{ij} = \mu + P_i + A_j + T_k + e_{ijk},
\]

where \(\mu\) is the overall mean, \(P_i\) is the effect of period, \(A_j\) is the effect of animal, \(T_k\) is the effect due to treatment and \(e_{ijk}\) is the random error.

The data obtained between techniques \((\text{in vivo, in vitro and in sacco})\) were adjusted to an analysis of variance using a completely randomized design:

\[
Y = \mu + T_i + e_{ij},
\]

where \(\mu\) is the overall mean, \(T_i\) is the effect due to cereal and \(e_{ij}\) is the experimental error.

In the analysis of variance, the technique was included \((n = 3)\) and replicated (three sets of incubation). The corresponding analysis of variance was done using the ANOVA procedure of the SAS program (2002). Means were compared using the Tukey test (Steel and Torrie 1997). The effect of treatments was performed using orthogonal contrasts, comparing dry sorghum (DS) vs. silage sorghum (SS), and whole sorghum (WS) vs. cracked sorghum (CS).
sorghums was lower \( (P < .001) \) for CSS (1028.3 ± 13.9 microns), compared with WDS and WSS (3250 ± 0.4). The number of particles was higher \( (P < .001) \) for CSS, followed by for DCS. The surface area \( (cm^2/g) \) was higher \( (P < .001) \) for CSS and DCS (78 ± 0.4), and the lower surface was for WDS and WSS (17 ± 0.4). Although the CSS are not commonly used in the food industry, the potential value of this practice to control the digestive process has been well documented, so that in the future, the development of this practice cannot be excluded, especially when the concentrate is produced in the same production industry, the potential value of this practice to control the digestion of dry matter (g/100 g DM) as ADS, RDP, and PDI of different treatments in sorghum grain.

### 3.3. Protein digestibility

Sorghum grains are covered by a protein matrix within the endosperm, which varies in quantity and solubility of the protein within the same, avoiding the availability of nutrients, and its rupture is required to be released and digested by the rumen microorganisms. Moreover, the digestion of nutrients is also affected by other factors such as the processing method, type of grain and conservation, and the type of endosperm (Oba et al. 2003). Table 3 presents the digestibility per treatment and technique. According to the ADS and RGP, whole grains were higher \( (P = .001) \) than cracked, the rumen undegradable protein (RUP) on the other hand was lower \( (P = .001) \) for WS compared with CS, the PDI was higher for CSS \( (P = .012, 11.96\%) \) followed by WSS \( (P = .012, 9.83\%) \) compared with DCS and WDS. Endogenous enzymes synthesized during germination hydrolyse the protein matrix and protein bodies in the grain endosperm making the starch more digestible in cattle (Rooney and Pflugfelder 1986). The initiation of germination during reconstitution processing is thought to be responsible for the increased digestibility of reconstituted sorghum grain (Hibberd et al. 1986; Pflugfelder and Rooney 1986) and increases N solubility (Bagolun et al. 2005). The DM digestibility of the sorghums was higher than that of Abdelhadi and Santini (2006), Bagolun et al. (2006) and Lema et al. (2000), who obtained a digestibility of 51.5 g/100 g DM using sorghum silage, and 58 g/100 g MS using different varieties of sorghum silage; however, the ADS for WS was lower than that of Mabjeesh et al. (2000). When contrasted by treatment, the ADS, the rumen degradable protein (RDP) and PDI were higher for the silages sorghums \( (P = .001) \) compared with the dried sorghums. When compared by treatment, CSS show higher \( (P = .038) \) ADS than the WSS, founding no differences \( (P > .1) \) in the RDP, the RUP and PDI was higher \( (P = .001) \) for WSs compared with the CSS. In the comparison of the different techniques used to determine the digestion in sorghum grain, the \( in sacco \) technique had the better DM digestibility \( (P = .001, 2.96\%) \) compared with the other techniques. The PDI was higher \( (P = .001, 4.44\%) \) for the \( in vitro \) \( Daisy2 \) and \( in sacco \) technique than the \( in vivo \) and \( in vitro \) gas production technique. The PDI was lower \( (P = .001, 3.90\%) \) for the \( in vivo \) method compared to the \( in sacco \) and \( in vitro \) Daisy methods. When contrasted by treatment, the ADS, RDP and PDI were higher \( (P = .001) \) for the dried sorghums than the WSS, while the RUP

### Table 3. Digestibility of dry matter (g/100 g DM) as ADS, RDP, and PDI of different treatments in sorghum grain.

| Treatment | ADS   | RDP  | RUP  | PDI  |
|-----------|-------|------|------|------|
| WDS       | 68.02 | 46.58 | 53.42 | 48.42 |
| DCS       | 65.74 | 42.49 | 57.50 | 45.56 |
| WSS       | 67.73 | 46.55 | 53.44 | 51.59 |
| CSS       | 65.05 | 43.87 | 56.12 | 52.59 |
| SEM       | 0.23  | 0.21  | 0.46  | 0.24 |
| Method    |       |      |      |      |
| \( In vivo \) | 65.35 | 44.23 | 55.77 | 64.97 |
| \( In sacco \) | 69.11 | 46.20 | 57.39 | 67.52 |
| \( In vitro \) (Daisy1) | 68.28 | 45.30 | 54.49 | 67.49 |
| \( In vitro \) (gas production) | 66.26 | 43.57 | 56.42 |      |
| SEM       | 0.25  | 0.27  | 0.39  | 0.54 |
| P value   | 0.02  | 0.03  | 0.01  | 0.01 |
| W vs. C   | 0.001 | 0.001 | 0.001 | 0.001 |
| Abbreviations: WDS, Whole dried sorghum; DCS, dry cracked sorghum; WSS, whole sorghum silage; CSS, cracked sorghum silage; D, dry sorghum; S, sorghum silage; W, grain whole sorghum; C, cracked sorghum.

**Different literals in the same row differ \( P < .01 \).
was higher ($P=.001$) for silage compared with the dried sorghums. When compared by the processing method, the ADS was higher ($P=.040$) for the CSS, while there were no differences compared with the rest of the treatments ($P=.972$). RDP, RUP and PDI were higher ($P=.006$) for WSS compared with CSS. Ortégaa et al. (1998) found a ruminal digestibility (in sacco) of the CP (32.94 g/100 g DM) in sorghum treated with formaldehyde being lower than the present study, Zinna et al. (2008) found a ruminal digestibility of CP (49–52.3 g/kg DM using steam flaking sorghum) higher than the present study, and also intestinal digestion of the protein that reaches the small intestine (72–74 g/kg DM digestibility) was lower than CSS. Bakera et al. (2010) found a protein digestibility of 21.8 g/kg DM in untreated sorghum; this was because the fermentation process increases the digestibility of the protein due to the presence of endogenous enzymes (Correia et al. 2010). The CP digestion was higher ($P<.001$) in WSS and WDS than Bakera et al. (2010) who showed a protein digestibility of 37.0 g/kg DM in cooked sorghum using an enzymatic digestion and in cooked sorghum, Coreira et al. (2011) using an enzymatic digestion, found a decrease in protein digestion subjecting sorghum grain to high pressure before being cooked. The values of in sacco ADS obtained in the present study were higher than Molina et al. (2000) who showed an ADS (60 g/100 g DM) with low tannin sorghums, while Ramos et al. (2000) showed a DM digestibility of 44 g/100 g DM. Ortégaa et al. (1998) showed a DM digestibility of 53.9–54.8 g/kg DM using sorghum sprayed and soaked with formaldehyde, respectively, using the in sacco method during 24 h incubation. The ADS was lower ($P<.019$) in the present study than Mabjeesha et al. (2000) (77 g/kg DM) using the traditional DaisyII, evaluating different foods used in the livestock industry including sorghum, but higher than Defoor et al. (2000) (50.6 g/kg MS) using an in vitro enzymatic digestion to assess the nutritional value of sorghum varieties. The in vivo ADS in the present study were higher than Bárceanaa et al. (2002). The RDP values are higher than Ortega et al. (1998) (33 g/100 g DM) in sorghum treated with formaldehyde in order to avoid degradation of the protein in the rumen. The digestibility of the protein was similar to Duodua et al. (2002) (65 and 67 g/100 g MS) using untreated sorghum, diminishing the protein digestibility (44 g/100 g DM) in cooked sorghum, being lower than the present study. The results obtained in SSS are higher than Coreira et al. (2011) who found a decrease in protein digestion using an in vitro digestion in sorghums with pepsin at high-pressure treatments before cooking them to reduce the effects of the protein digestibility in the grain.

### 3.4. In vitro gas production and fermentation characteristics

Table 4 presents the parameters of gas production (ml gas/g DM incubated) of the different treatments used in this study, fraction $a$ was lower ($P<.001$) for WSS compared with WDS; $b$ fraction was lower ($P>.01$) for CSS and WDS, followed by WSS, being superior to DCS. Fraction $c$ was higher ($P<.01$) for WSS followed by DCS, and lag time was higher for WDS and WSS ($P<.01$) than CSS and DCS. RGP was higher ($P<.01$) for CSS, followed by WDS $>$ DCS $>$ WSS. When compared between treatments, fraction $a$ was higher ($P=.001$) for dried sorghums compared with SSS, there were no differences ($P>.05$) between treatments for fraction $b$, $c$ and lag time, ADS was higher ($P=.001$) for DSS than SSS, on the contrary previous studies showed increased digestibility of reconstituted sorghum (Schake et al. 1983; Hibberd et al. 1985, 1986); while the RGP was higher ($P=.001$) for SSS compared with DSS. With respect to the processing method, the fraction $a$ was higher ($P=.001$) for whole grains sorghum vs CSS, and there were no differences ($P>.05$) for fraction $b$, $c$ and lag time. The fermentation of grain mainly determines the nutritional value of these grains for ruminant’s intake. This affects the site starch digestion (since most degrade after the first 6 h of incubation and is complete after 24 h) and the microbial protein supplement, having a significant effect on the ruminal environment from its relationship with pH, the VFA production and cellulolytic activity (Chai et al. 2004; Lanzas et al. 2007). Also Calabro et al. (2005) studied impacts of sample preparation on gas production in 10 silages incubating the forages either as fed or oven dried; they found some differences in fermentation characteristics probably due to more rapid colonization by rumen microorganism of fresh samples in contrast to the dried samples. The increased fermentation and degradability in the reconstituted sorghum silage can be attributed to the combination of the endogenous enzyme activity in the grain during the aerobic phase and exogenous enzymes of microorganisms during the anaerobic phase (Balogun et al. 2005). The ADS at 24 h was higher for CSS and WSS (79% and 77%, respectively) compared with the rest of the treatments, being higher than Rodríguez (2005) who reported 66% DM, but differs from Ortega et al. (1998) who reported 87% DM in vitro digestion using sorghum treated with formaldehyde. The low digestibility of DM in the diet could be due to the different sources or rapidly fermentable carbohydrates (Mertens et al. 1980), which can be achieved with processing methods applied to the grains, improving modifications on their nutritional quality (Correia et al. 2010).

### Table 4. In vitro gas production (ml gas/g DM) of different sorghum grain treatments.

| Item     | WDS  | DCS  | WSS  | CSS  | SEM  | Tx  | W vs. S | W vs. C |
|----------|------|------|------|------|------|-----|---------|---------|
| $a$      | 106$^a$ | 95$^a$ | 89$^a$ | 111$^g$ | 0.23 | 0.001 | 0.001 | 0.001 |
| $b$      | 0.015$^c$ | 0.018$^d$ | 0.021$^g$ | 0.015$^a$ | 0.02 | 0.001 | 0.387 | 0.341 |
| $c$      | $-0.022^c$ | $-0.031^d$ | $-0.038^g$ | $-0.023^e$ | 0.02 | 0.001 | 0.283 | 0.499 |
| Lag time | 1.69$^g$ | 0.74$^d$ | 1.42$^g$ | 0.74$^d$ | 0.07 | 0.001 | 0.650 | 0.001 |

Abbreviations: WDS, Whole dried sorghum; DCS, dry cracked sorghum; WSS, whole sorghum silage; CSS, cracked sorghum silage; Tx, treatment; D, dry sorghum; S, sorghum silage; W, whole grain sorghum; C, cracked sorghum grain; $a$, total gas production (ml gas/g DM); $b$, index fermentation ($h^{-1}$); $c$, fermentation rate ($h^{-1/2}$), lag time (h); ADS, apparent degrade substrate (mg/100 mg DM); RGP (ml gas 24 h/g ADS24 h).

$^a$Different literals in the same row $P<.05$. 

$^c$Different letters in the same row $P<.05$. 

$^g$Different letters in the same row $P<.01$. 

$^d$Different letters in the same row $P<.001$.
Table 5. Influence of sorghum grain processing on pH, production of NH₃-N and VFA (mmol/100 mol) and methane (mmol/100 mol)

| Item         | WDS | DCS | WSS | CSS | SEM  | Tx  | D vs. S | W vs. C |
|--------------|-----|-----|-----|-----|------|-----|---------|---------|
| pH           | 7.0 | 7.0 | 7.0 | 7.0 | 0.01 | 0.225 | 0.703   | 0.596   |
| N–NH₃, (mg/dl) | 9.98⁹ | 9.73⁹ | 12.26⁹ | 11.03⁹ | 0.21 | 0.001 | 0.062   | 0.446   |
| VFA (mol/100 mol) | 58.78c | 61.94a | 54.94b | 53.39b | 0.44 | 0.001 | 0.001   | 0.051   |
|            | 32.82c | 28.46a | 36.82b | 36.39b | 0.24 | 0.001 | 0.065   | 0.001   |
| Propionic (P) | 8.23c | 9.59b | 7.72c | 10.30b | 0.22 | 0.018 | 0.883   | 0.001   |
| Butyric      | 1.79⁹ | 2.17d | 1.53c | 1.86b | 0.02 | 0.001 | 0.037   | 0.001   |
| A/P ratio    | 0.47c | 0.52² | 0.45c | 0.41b | 0.03 | 0.001 | 0.023   | 0.001   |
| Methane      | 0.23a | 0.16b | 0.20a | 0.16b | 0.03 | 0.001 | 0.023   | 0.001   |

Abbreviations: WDS, Whole dried sorghum; DCS, dry cracked sorghum; WSS, whole sorghum silage; CSS, cracked sorghum silage; Tx, treatment; D, dry sorghum; S, sorghum silage; W, whole grain sorghum; C, cracked grain sorghum.

abcdDifferent literals in line P < .05.

The in vitro pH values (Table 5) were not different (P = .225) between treatments which differ from Azkar et al. (2006) with a pH of 4.9–5.6, in lambs fed diets based on whole grain barley-supplemented protein. Moreover, Corona et al. (2005) found a pH of 6.0–6.6 in beef cattle diets containing 75% of corn with different processing methods (steam flaking, whole, rolled dried and ground); Galyean et al. (1979) reported that the pH is not affected by the particle size. N–NH₃ production (mg/dl) was lower (P = .001) for sorghum silage compared with dried sorghums. The concentration of N–NH₃ in WSS was not different from Azkar et al. (2011) using whole barley grain in the diet. In vitro VFA proportions (mmol/100 mol) shows that the dry grains have the highest acetic acid production compared with the silage grains; the propionic acid was higher (P < .001) for silage grains than dried grains. Previous studies (Bagolun et al. 2005, 2006) showed a strong and positive correlation between the amount of starch fermented and the total VFA production or propionic acid from sorghum grain silage. The acetic: propionic ratio was higher (P = .001) for DCS, and lower for WSS. The highest propionic acid production was for silage grains compared with the dry grains, while the higher (P = .01) acetic acid production was for dried grains. Acetic: propionic ratio and methane production were higher (P < .001) for CSS compared with SS. Koenig et al. (2003) found the acetic acid concentration (58 mol/100 mol) higher than the present study. Propionic acid from sorghum silage differed slightly from Azkar et al. (2006) (40 mol/100 mol) in diets based on whole barley grain but similar to Azkar et al. (2011) and Zinn et al. (2008) (36 mol/100 mol) using the same diets with sodium bicarbonate.

4. Conclusions

Appropriate processing of the grains increases the digestibility of nutrients in the digestive tract of the ruminants; the in vitro studies indicate that rumen dry matter digestion can be more efficient if the grain is processed properly. In our study, reconstituted ensiling sorghum grains had a favourable response in the availability of nutrients, compared with dried sorghums.

Disclosure statement

No potential conflict of interest was reported by the authors.

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