Research Article

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Nutrient content and nutrient availability of sorghum wet distiller's grain in comparison with the parental grain for ruminants

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Abstract

BACKGROUND: The present study aimed to compare wet sorghum distiller's grain (WSDG) with sorghum grain (SG) in terms of: (i) chemical composition; (ii) *in situ* rumen degradation kinetics of organic matter (OM) and neutral detergent fiber (NDF); (iii) crude protein (CP) sub-fractions; (iv) *in situ* disappearance at 12 and 48 h; and (v) energy values. The WSDG intestinal digestibility (ID) of undegradable crude protein (UCP) was compared to soybean meal (SBM).

RESULTS: Compared to SG, WSDG exhibited: (i) lower ($P < 0.01$) dry matter and non-fiber carbohydrate content, whereas the other chemical components were higher ($P < 0.01$); (ii) higher ($P < 0.01$) degradation rates of OM and NDF and lower ($P < 0.01$) degradable fraction of OM and NDF; (iii) lower ($P < 0.05$) contents of CP sub-fractions A, B1 and B2, and higher ($P < 0.05$) contents of B3 and C; (iv) lower ($P < 0.05$) protein disappearance at 12 and 48 h and higher UCP; and (v) lower ($P < 0.05$) energy content. The ID of UCP for WSDG was lower ($P < 0.05$) compared to SBM.

CONCLUSION: The WSDG as a supplement provides a good source of energy. To enable its use as a protein supplement, further studies should be performed.
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Keywords: sorghum grain; distiller's grain; nutritive value; degradation kinetic

INTRODUCTION

Sorghum grain is currently used as a renewable fuel source for ethanol production because it offers advantages in comparison with other cereals: its use is less competitive with human feeding[1] and it has a lower carbon footprint.[2] Consequently, the by-product sorghum distillers' grain is available for livestock feeding. Furthermore, distillers' grain comprises a relatively inexpensive supplement that provides a good source of energy and protein for cattle in pasture-based production systems.[3,4]

The main concern regarding distillers' grain is its highly variable nutritional value. The nutritional value depends on factors associated with the parental grain (i.e. crop, variety, management) and the technological process (i.e. grinding, temperature, extent of starch fermentation) used to obtain ethanol.[5] This variability has been extensively documented in corn distiller' grain.[6] However, information concerning WSDG as a ruminant feed is scarce. Therefore, it is necessary to investigate further with the aim of obtaining reliable values of nutrient content and nutrient availability. These values are essential for farmers and nutritionists so that they can accurately assess the economic implications of using WSDG as supplement in pasture-based production systems. The present study aimed to compare WSDG with SG in terms of: (i) chemical composition; (ii) *in situ* rumen degradation kinetics of organic matter (OM) and neutral detergent fiber (NDF); (iii) crude protein (CP) sub-fractions; (iv) *in situ* disappearance at 12 and 48 h; and (v) total digestible nutrients (TDN), digestible energy (DE) and metabolizable energy (ME) values for ruminants. In addition, the WSDG intestinal digestibility (ID) of undegradable crude protein (UCP) is compared with soybean meal (SBM).

MATERIALS AND METHODS

Three different batches of SG and their related batches of WSDG without solubles were collected from the bioethanol plant located in Paysandú, Uruguay. Animals were handled according to the guide of good practices for the Use of Animals in Research, Testing and Teaching of the Universidad de la Republica of Uruguay.

Chemical analysis

All samples (feedstuffs and *in situ* residues) for chemical analysis were ground through a 1-mm screen. Dry matter (DM) (AOAC[7] 967.03), ash (AOAC[7] 942.05), ether extract (EE) (AOAC[7] 920.39 A) and nitrogen (N) (AOAC[7] 984.13) contents were determined according to the procedure of the AOAC.[7] The OM was calculated as DM minus ash and the CP was calculated as $N \times 6.25$. The NDF was determined without sodium sulfite and with heat stable amylase and expressed as ash free; acid detergent fiber (ADF)

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also was expressed as ash free and lignin (LIG) was determined by solubilization of cellulose with sulfuric acid. All fibers were determined according to Van Soest *et al.*[8] using an ANKOM200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA). The acid (ADICP) and neutral detergent insoluble N (NDICP) values were determined by measuring N in the ADF and NDF residue, respectively. The non-protein nitrogen content and total soluble crude protein (SCP) were determined according to Licitra *et al.*[9] The protein sub-fractions were determined according to the Cornell Net Carbohydrate and Protein System.[10] Non-structural carbohydrates were calculated by difference between DM and the remaining fractions as described by the NRC.[11] The content of tannins was determined using the modified vanillin hydrochloric acid of Maxson and Rooney.[12] A quantitative analysis of zearalenone was performed in WSDG and SG by a competitive direct enzyme-linked immunosorbent assay (Veratox® test; Laboratorio Analitico Agro Industrial, Paysandú, Uruguay).

In situ ruminal degradation procedure and *in vitro* intestinal digestion procedure

Two dry multiparous Holstein cows (711 ± 65 kg of body weight) fitted with rumen cannula (KEHL®; Industria e Comercio LTDA ME, São Carlos, Brasil) were used to determine OM and NDF degradation kinetics using the nylon bag technique.[13] Fistulated cows grazed on a mixed pasture were supplemented with 2 kg of SG. The cows were adapted to the diet for 2 weeks prior to the incubation period. Briefly, 6 g DM of each feedstuff were weighed into a bag (10 × 20 cm) made of N-free monofilament polyester screen printing fabrics (PET 1000 120-34-W; mean pore size of 45 ± 3 μm; Sefar Inc., Heiden,Switzerland). Four bags of each batch of feedstuff were incubated in the rumen of the two cows (two bags per cow per incubation time) for 2, 4, 8, 12, 24 and 48 h. For 72 h, three bags per cow were incubated because of a low recovery of incubated residue. Bags were introduced simultaneously in the rumen immediately after the SG supplementation and removed sequentially. Before rumen incubation, bags were submerged (15 min) in warm water (39 °C) and, after collection from the rumen, were soaked in cold water, and stored at −20 °C. Four bags per batch of feedstuff were not incubated in the rumen, and handled similarly to the incubated ones to obtain the zero-time incubation (t0). Once thawed, bags were washed (three times) in an automatic machine (Mueller Pop Tank; capacity of 49 L, 30 bags per wash cycle, 3-min with program wash soft without centrifugation; Mueller Eletrodomésticos Ltda, Santa Catarina, Brazil), dried in a forced air oven (60 °C for 48 h) and weighed. Dry matter losses were computed as the difference in weight of the pre- and post-incubated bags, and expressed as proportion of initial weight. Residues of replicates per time within cows were pooled prior to chemical analysis.

Data regarding CP disappearance after 12 and 48 h ruminal incubation were obtained using an *in situ* procedure[13] as described previously for SG and WSDG.

Estimation of *in vitro* intestinal disappearance of UCP for WSDG was determined using a modified three-step method[14]. Ruminal degradation residues after 12 h of incubation of WSDG and a composite sample of SBM (503 CP g kg^{-1} DM), used as a standard protein feed, were performed using the previous *in situ* procedure. After washing the bags, the ruminal residues were pooled by animal. Approximately 0.5 g of the residue of WSDG and SBM was weighted into filter bags (made of N-free monofilament polyester screen printing fabrics; PET 1000 120-34-W; mean pore size of 45 ± 3 μm; Sefar Inc.). Eighteen bags were incubated in a bottle containing 2 L of 0.1 N pre-warmed HCl solution adjusted to pH 1.9 with 1 g L^{-1} of pepsin (P-7000; Sigma, St Louis, MO, USA) for 1 h with constant rotation in shaker at 39 °C using a Daisy incubator (Ankom, Fairpot, NY, USA). Three empty bags were used as blanks. After rinsing with cold tap water, bags were reintroduced into the incubation bottle containing 2 L of pre-warmed pancreatin solution (0.5 M KH_2PO_4 buffer adjusted to pH 7.75, containing 50 ppm of thymol and 3 g L^{-1} of pancreatin (P-7545; Sigma), and incubated for 24 h with constant rotation at 39 °C. After incubation, the bags were rinsed with tap water until the run-off was clear and dried at 60 °C for 48 h for determining DM and CP disappearance.

Energy values

Based on the values of heat combustion and truly digestible nutrients, the energy contents as TDN, DE and ME at maintenance level (ME: DE × 0.82) were determined using the summative equation with *in situ* coefficients of digestibility of nutrients (disappearance at 48 h of ruminal incubation) as described by Nuez-Ortin and Yu.[15]

Statistical analysis

Ruminal kinetics parameters of OM and NDF were estimated using a non-linear procedure of SAS[16] (PROC NLIN iterative Marquardt method).

The disappearance of OM and NDF were fitted with exponential models described by McDonald[17] (including *Lag* time, model 1) or by Ørskov and McDonald[18] (without *Lag* time, model 2), according to the best fit:

$$\text{Model 1: } Y(t) = a + b\left[1 - e^{-c(t-Lag)}\right] \quad \text{for } t > Lag;$$

$$\text{Model 2: } Y(t) = a + b\left(1 - e^{-ct}\right), t \geq 0$$

where Y is ruminal disappearance at time t, a is the soluble fraction; b is the potentially degradable fraction; c is the fractional disappearance rate constant at which b is degraded; *Lag* is the lag time (h) and t is the time of incubation (h).

Effective rumen degradability (ED) of OM and NDF was estimated using the equation of Orskov and McDonald[18] or McDonald[17], $ED = a + bc/(c + k)$, $ED = a + [bc/(c + k)]e^{-c(Lag)}$, respectively, where k is the fractional passage rate that was assumed to be 0.02/h according to the NRC[11].

All data were analyzed as a completely randomized design using the Mixed procedure of SAS[16]. The model used for the analysis was: $Yij = \mu + F_i + B_j + e_{ij}$, where, Y_{ij} is an observation of the dependent variable ij; μ is the population mean for the variable; F_i is the effect of feedstuffs (WSDG/SG), B_j is the batch effect and e_{ij} is the error associated with the observation ij. The fixed effect is the feedstuff and the random effect is the batch. When a significant difference was found, comparisons among means were carried out using the Tukey procedure[19]. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Chemical composition

Compared to the original grain, the DM content and non-fiber carbohydrate content for WSDG were lower, whereas the contents of EE, NDF, ADF, CP, NDICP, ADICP, LIG and tannins were considerably higher ($P < 0.01$) than for SG (Table 1). Most of the fermentable carbohydrates present in SG were removed from the grain during fermentation process, whereas the remaining nutrients in the WSDG

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Table 1. Chemical composition of SG and WSDG

Item	SG	WSDG	SEM
Dry matter (DM) (g kg^{-1})	894 ± 4.1 a	333 ± 2.6 b	5.61
Organic matter (OM) (g kg^{-1} DM)	986 ± 0.1 a	987 ± 1.7 a	0.73
Ether extract (EE) (g kg^{-1} DM)	34 ± 2.7 b	110 ± 6.8 a	2.97
Neutral detergent fiber (NDF) (g kg^{-1} DM)	130 ± 4.3 b	702 ± 22 a	7.69
Acid detergent fiber (ADF) (g kg^{-1} DM)	42 ± 1.8 b	283 ± 2.8 a	1.29
Crude protein (CP) (g kg^{-1} DM)	68 ± 2.3 b	314 ± 6.9 a	2.95
Neutral detergent insoluble CP (NDICP) (g kg^{-1} DM)	24 ± 0.7 b	193 ± 11 a	4.51
Acid detergent insoluble CP (ADICP) (g kg^{-1} DM)	15 ± 3.8 b	106 ± 7.2 a	3.33
Non-fiber carbohydrates (NFC) (g kg^{-1} DM)	776 ± 7.9 a	53 ± 21 b	8.20
Lignin (LIG) (g kg^{-1} DM)	14 ± 2.1 b	109 ± 2.4 a	1.29
Tannins (g kg^{-1} DM)	2.4 ± 0.6 b	14.2 ± 2.9 a	1.17

Data are the mean ± SD.
Means in the same row with different lowercase letters are significantly different ($P < 0.05$).

were concentrated. When the results were expressed as WSDG fold increase over SG, the contents of EE, NDF, ADF, CP, NDICP, ADICP, LIG and tannins had the values 3.27, 5.40, 6.67, 4.58, 7.99, 7.00, 7.95 and 5.40, respectively. The content of non-fiber carbohydrates in WSDG indicates that the completeness of carbohydrate fermentation in the process of obtaining ethanol was not achieved. Some of the starch can be associated with yeast or microbial contamination depending on the efficiency with which starch is fermented to ethanol;[20] however, our estimated non-fiber carbohydrate value is consistent with the low levels of starch observed in distillers' grains in general.[6]

We studied WSDG susceptibility to spoilage given the high moisture content and high nutrients concentration characteristics of this type of byproduct.[21] We found that WSDG is highly susceptible to rapid spoilage as a result of microbial growth (e.g. the zearalenone concentration was approximately two times greater for WSDG than for SG; data not shown) and this is a limitation to be addressed in the use of this product.

The average nutrients content for WSDG are similar to the values reported in the literature, with the exception of NDF. The NDF in the present study showed a value greater than in WSDG plus solubles.[21–24] However, our NDF value was closer to values of dried sorghum distiller's grain obtained previously from the same ethanol plant (608 g kg^{-1} DM).[25] In addition, the NDICP and ADIPC in the present study were also in agreement with data obtained by Marichal *et al.*[25] (59% and 38% of CP, respectively) and with the values of NDICP in distiller's grain from other grains,[10,20] reinforcing the idea that most of CP is bound to the cell wall in these agro industrial byproducts.

Rumen degradation kinetics and sub-fractions of CP

Compared to SG, the *a* and *b* fractions of OM for WSDG were lower ($P < 0.05$), whereas the *c* fraction was higher ($P < 0.05$). In addition, the OM degradation for WSDG showed a *Lag* time that was not detected for SG (Table 2). The WSDG NDF kinetic degradation followed a similar pattern as that for OM degradation. The NDF degradation of SG presented neither *a* fraction, nor *Lag* time, whereas NDF degradation of WSDG presented a low value of *a* fraction and *Lag* time was fitted (Table 2). The *b* fraction of NDF was lower ($P < 0.05$) and the *c* fraction was higher ($P < 0.05$) for WSDG than for the parental grain. The reduction in ruminal degradation kinetic parameters of OM and NDF for WSDG versus the original grain is a result of the removal of the highly fermentable carbohydrates during ethanol fermentation. The degradation kinetic pattern of the OM for SG corresponds to the kinetics of its main component (non-fiber carbohydrates), whereas the degradation pattern of OM for WSDG reflects the combined degradation kinetics of the cell wall and the CP.

Table 2. *In situ* ruminal kinetic parameters[a] of OM, NDF and ED[b] of OM and NDF for SG and WSDG

Item	SG	WSDG	SEM
OM			
a	227 a	79 b	7.41
b	739 a	550 b	27.18
c (h^{-1})	0.025 b	0.048 a	0.004
Lag (h)	M2	3.03	0.168
ED ($k = 0.02$)	698 a	413 b	5.13
NDF			
a	0.0	57 a	2.68
b	793 a	695 b	30.23
c (h^{-1})	0.035 b	0.063 a	0.005
Lag (h)	M2	2.78	0.37
ED ($k = 0.02$)	503	500	9.75

[a] Estimated by Model 1: $Y(t) = a + b[1 - e^{-c(t-Lag)}]$ or by Model 2: $Y(t) = a + b(1 - e^{-ct})$, $t \geq 0$.
a, soluble fraction; *b*, slowly degradable fraction; *c*, degradation rate constant of *b*; *t*, incubation time; *Lag*, lag time.
M2, model 2 (not *Lag* time included).
[b] $ED = a + [bc/(c + k)]$ or $ED = a + [bc/(c + k)]e^{-c(Lag)}$ for $k = 0.02$ h^{-1}.
Means in the same row with different lowercase letters are significantly different ($P < 0.05$).

The NDF for the WSDG has good potential for degradation despite increases in the most refractory fractions included in the cell wall. No studies for NDF degradation kinetic parameters of sorghum distillers' grain appear to be available for comparison with our results. The ruminal kinetics parameters from the present study are in agreement with the kinetics parameters of wheat distiller's grains reported by Mustafa *et al.*[26] and Nuez-Ortin and Yu.[27] The ED of NDF values were also comparable with their reported values. Accordingly, this byproduct is characterized as a readily degradable fiber source.

The protein chemical profile (Table 3) showed that SCP for WSDG and the A, B1 and B2 fractions was lower ($P < 0.05$) than for SG, whereas that for the B3 and C fractions was higher ($P < 0.05$). In SG, the predominant fraction of CP was the B2 fraction, whereas, in WSDG, the CP was distributed almost equally between B2, B3 and C fraction (Table 3). Nuez-Ortín and Yu[28] demonstrate that chemical sub-fraction values of CP of distillers' grains are affected by the type of cereal grain used as the fermentation substrate. Particularly, SG contains prolamins known as kafirins, which are proteins similar to zeins. Prolamins have a variable percentage of monomeric proteins and proteins highly cross-linked by disulfide bonds.[29] A moderate to high content of proteins linked by disulfide bonds may explain the lowest value of soluble protein of SG in comparison with other cereals grains;[28,30] therefore, the

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Table 3. Protein sub-fractions, disappearance of CP after 12 and 48 h of ruminal incubation for SG and WSDG and ID of UCP of WSDG

Item	SG	WSDG	SEM
Crude protein (CP) (g kg^{-1} DM)	68 ± 23 b	314 ± 6.9 a	1.91
Soluble CP (g kg^{-1} CP)[a]	41 ± 2.0 a	23 ± 1.8 b	1.51
Protein sub-fractions associated with rumen degradation (g kg^{-1} CP)[b]			
Fraction A (infinitely degradable)	13 ± 0.8 a	7 ± 1.7 b	0.77
Fraction B1 (rapidly degradable protein)	28 ± 2.0 a	16 ± 1.8 b	1.12
Fraction B2 (intermediately degradable protein)	606 ± 3.9 a	363 ± 28.8 b	11.8
Fraction B3 (slowly degradable protein)	131 ± 58.9 b	277 ± 27.9 a	26.6
Fraction C (undegradable protein)	221 ± 58.6 b	337 ± 15.9 a	24.8
Ruminal disappearance of CP (g kg^{-1} CP)			
12 h of incubation	335 ± 16.8 a	124 ± 31.9 b	13.0
48 h of incubation	558 ± 48.0 a	208 ± 19.3 b	19.1
ID of UCP (g kg^{-1} UCP)[c]	ND	520 ± 36	11.2

Data are the mean ± SD.
[a] Soluble CP, buffer soluble CP.
[b] Protein sub-fractions according to Cornell Net Carbohydrate and Protein System.[10]
[c] ID, intestinal digestibility of undegradable protein using a modified three-step method.[16]
ND, not determined.
Means in the same row with different lowercase letters are significantly different ($P < 0.05$).

Table 4. Total digestible nutrients, DE and ME values of SG and WSDG

Item	SG	WSDG	SEM
Total digestible nutrient at maintenance level			
TDN (g kg^{-1} DM)	886 ± 11.3 a	711 ± 28.3 b	12.4
Predicted energy values at maintenance level			
DE (MJ kg^{-1} DM)	15.49 ± 0.20 a	10.45 ± 0.52 b	0.23
ME (MJ kg^{-1} DM)	12.71 ± 0.16 a	8.57 ± 0.53 b	0.18

Data are the mean ± SD.
ME = DE × 0.82.
Means in the same row with different superscript letters are significantly different ($P < 0.05$).

low SCP in WSDG is expected. Changes in the content of CP sub-fractions in distillers' grains may be caused by the addition of nitrogen components[31] and/or temperature[32] during the ethanol production process. The WSDG from the ALUR (Alcoholes del Uruguay SA) ethanol plant does not contain additional nitrogen sources or distiller's solubles; therefore, SCP, A and B1 fractions were reduced compared to SG. Transformations in the structure of CP during the process of ethanol production can explain the differences observed in the proportions of B2, B3 and C fractions for WSDG compared to SG. The heat applied during the process increased the susceptibility to Maillard reaction as a result of interactions of sorghum protein with polyphenols, lipids and cell wall components.[33,34] In addition, heating may cause changes in the protein molecular structure.[35]

The *in situ* data in the present study showed a lower CP disappearance following 12 and 48 h of ruminal incubation for WSDG than for SG ($P < 0.05$) (Table 3) and presented a very high value of UCP for WSDG (792 g kg^{-1} DM). This value was higher than the UCP of dried sorghum distiller´s grain obtained by Marichal *et al.*[25] but is in accordance with the high value of C fraction. Most likely, the CP of SG was modified by the process of ethanol production causing changes in chemical characteristics, as well as in protein molecular structure determining a low CP ruminal utilization of WSDG.

Intestinal digestibility of UCP

The ID of UCP for WSDG (Table 3) obtained in the present study was lower ($P < 0.05$) than the ID of UCP of SBM used as a standard protein feed (98.2%; data not shown).

To the best of our knowledge, no values for ID of UCP of sorghum wet distiller's grains have been reported. Higher variability and greater values of ID of UCP were observed in corn dried distiller's grain plus solubles (59–77%) by Kleinschmit *et al.*[35] and in corn, wheat and triticale dried distiller's grain plus solubles (79.6–95.4) by Cherevoká *et al.*[30] The high value of ADICP content for WSDG in the present study can be explained by the heat applied during the ethanol process: this causes the formation of complexes between kafirins and other components, reducing the protein digestibility.[33,36] The effect of ADICP on ID of ruminal UCP of distillers' grains has been controversial. Although several studies[10,37] have suggested that ADICP is entirely indigestible and does not contribute to the animal's metabolizable protein pool, other studies[38] have shown that ADICP of dried distillers' grains is partially digestible. The CP availability of the WSDG obtained in the present study shows a low to moderate utilization at the intestinal level.

Energy content

Estimations of TDN, DE and ME energy contents for WSDG in the present study were lower ($P < 0.05$) than the energy values of SG (Table 4). The high-energy value of distillers' grains can be attributed to the fat and digestible fiber contents.[28] The comparison of the energy values for distiller's grains with their parental grains is controversial. Some studies have reported that the energy content of distillers' grain reaches or is higher than the energy content of the parental grain,[28,39] whereas other studies show opposite results.[30] These differences can be a result of the different industrial production processes and the method used to estimate energy. The energy value of feedstuffs can be calculated from the chemical composition.[11,37] However, it is questionable whether the chemical approach can accurately estimate energy values of distillers' grains. For this reason, biological approaches including *in situ*/*in vitro* digestibility coefficients are considered better predictors for energy content.[40] The high-energy value for WSDG estimated by the this approach is probably a result of both the high content of degradable NDF and by the high content of fat. This coincides with the data reported by Nuez-Ortin and Yu [16,28] for other distillers' grains.

CONCLUSIONS

The results of the present study demonstrate that WSDG is characterized by a high level of degradable fiber and fat. Consequently, it has a high content of energy, although it does not reach the

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parental SG energy value. In addition, WSDG presents higher levels of CP, higher UCP and low to moderate ID UCP as a result of the CP characteristics of the parental grain and the modifications occurred during the ethanol production. Our data suggest that the WSDG can be used as a supplement providing a good source of energy; however, further studies are needed to assess its utilization as a protein supplement because inherent variations in the parental grain and in the industrial process.

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