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In vitro digestibility and nutritional characterization of distillers dried grains with solubles according to the Cornell Net Carbohydrate and Protein System

Digestibilidade *in vitro* e caracterização nutricional de grão seco de destilaria com solúveis segundo o sistema Cornell

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Abstract

We evaluated the fractions of protein and carbohydrates in distillers dried grains with solubles (DDGS), corn grain (CG), soybean meal (SM), and corn silage (CS), as well as the *in vitro* digestibility (*IV*D) of DDGS, CG, SM, CS, rations containing 0.0, 8.0, 16.0, and 24.0% DDGS, and *in vitro* fermentation parameters after 24 h of incubation. DDGS were obtained following microbial fermentation for ethanol production from a sugar and alcohol distillery located in the state of Mato Grosso – Brazil. The Cornell Net Carbohydrate and Protein System (CNCPS) was used to determine the protein and carbohydrate fractions of experimental diets. For the *in vitro* nutrient digestion assay using the experimental foods and experimental diets, two sheep with an average body weight of 26 kg were used as inoculum donors. The *in vitro* digestibility of food and feed was assayed in three replicates. Fraction A of DDGS CP was 88, 71, and 37% lower in relation to fraction A of SM, CG, and CS, respectively. Fraction B2 of DDGS protein contained 21% CP, which represents 78.84% of DDGS protein in fraction B2, and is higher than the SM, which was 70.44%. The B3 fraction of CP, which is partly released during ruminal fermentation, was 18% lower for SM compared to DDGS, and is expressed in %CP. For carbohydrate fractionation, the DDGS presented 8.64% for the A + B1 fraction on a DM basis, which was 62, 86, and 74% lower compared to those obtained for SM, CG. and CS, respectively. The hemicellulose and cellulose contents of DDGS were higher than those of SM, as verified in fraction B2, with a value of 46.92%, expressed in DM. The *in vitro* digestibility coefficients (*IV*DC) of the DDGS nutrients did not differ ($p > 0.05$) in relation to those of the other experimental foods. The inclusion of DDGS in rations formulated for sheep did not change ($p > 0.05$) the *IV*DC of DM, OM, CP. NDF, or ADF, with mean values of 70.93, 70.64, 59.58, 52.83, and 43.40%, respectively. Therefore, DDGS comprise a protein-rich food containing more than 70% CP in fraction B2, with a large amount of carbohydrates bound to the cell wall. In addition, DDGS possess a similar digestibility coefficient to corn grain and soybean meal; however, up to 24% can be included in feed formulations for ruminants without changing the *in vitro* digestibility coefficient of nutrients.

Key words: Rumen degradable protein. Ruminal fermentation. Sheep. Soybean meal.

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Resumo

Foram avaliadas as frações da proteína e dos carboidratos de grãos secos de destilaria com solúveis (GSDS), grão de milho (GM), farelo de soja (FS), e silagem de milho (SM), e a digestibilidade *in vitro* (CD*IV*) do GSDS, GM, FS, SM e de rações contendo a inclusão de 0,0%; 8,0%; 16,0% e 24,0% de GSDS, bem como os parâmetros de fermentação *in vitro* após 24 horas de incubação. O GSDS foi obtido após processo de fermentação microbiana para a produção do etanol, de uma destilaria de flex de açúcar e álcool localizada no estado de Mato Grosso – Brasil. Para determinação das frações proteicas e de carboidratos dos alimentos experimentais foi utilizado Cornell Net Carbohydrate and Protein System – CNCPS. Para o ensaio de digestão *in vitro* dos nutrientes dos alimentos experimentais e das rações experimentais foram utilizados dois ovinos com peso corporal médio de 26 kg, como doadores de inóculo. O ensaio de digestibilidade *in vitro* dos alimentos e das rações foi conduzido com três repetições de campo. A fração A da PB do GSDS apresentou valores 88%, 71% e 37% menores em relação a fração A do FS, GM e SM, respectivamente. A fração B2 da proteína do GSDS apresentou um teor de 21% da PB, o que representa 78,84% da proteína do GSDS na fração B2, valor superior ao do FS, que foi de 70,44%. A fração B3 da PB, a qual parte escapa da fermentação ruminal foi 18% menor para o FS em relação ao GSDS, expresso em % da PB. Para o fracionamento de carboidratos, o GSDS apresentou um valor para a fração A+ B1 de 8,64% na MS resultado inferior em 62%; 86% e 74% aos obtidos para o FS, GM e SM. O GSDS apresentou teores de hemicelulose e celulose superior ao FS, como verificado na fração B2, com valor de 46,92 % expresso na MS. Os coeficientes de digestibilidade *in vitro* (CD*IV*) dos nutrientes do GSDS não diferiu ($p > 0,05$) em relação aos demais alimentos experimentais. A inclusão do GSDS em rações formuladas para ovinos não alterou ($p > 0,05$) o CD*IV* da MS; MO; PB; FDN e FDA, com valores médios de 70,93%; 70,64%; 59,58%; 52,83% e 43,40%, respectivamente. Desta maneira, conclui-se que o grão seco de destilaria com solúveis apresenta-se como alimento proteico com mais de 70% da PB na fração B2 e possui levada quantidade de carboidratos ligados à parede celular. Além disso, o grão seco de destilaria com solúveis apresenta um coeficiente de digestibilidade semelhante ao do grão de milho e ao farelo de soja, contudo, este pode ser incluído em até 24% nas formulações de rações para ruminantes sem alterar o coeficiente de digestibilidade *in vitro* dos nutrientes.

Palavras-chave: Farelo de soja. Fermentação ruminal. Proteína degradável no rúmen. Ovinos.

Introduction

In recent years, several countries have intensified research into alternative energy sources that may replace petroleum, due to its high price and its detrimental effect on the environment. Therefore, agricultural production has diversified toward the production of ethanol and biodiesel. This has led to two major implications for animal production: first, consumers of ethanol and biodiesel compete directly with livestock production for energy sources (grains and oilseeds) and, second, the production of ethanol and biodiesel generates waste, which can be used in several processes, including animal feed. Examples of residues from ethanol production include sugarcane bagasse and distillers dried grains with solubles (DDGS), which contain around 31% crude protein – CP (PENZ JÚNIOR; GIANFELICE, 2008).

Evidence from the literature suggests that the production of 1.0 L of ethanol requires the fermentation of 2.5 kg of corn grain, and that the fermentation of this volume of grains can generate 0.3 kg of carbon dioxide and 0.8 kg of DDGS (LIM; YILDIRIM-AKSOY, 2008; BOTELHO, 2015). According to COASUL (2014), in 2014, the central-western region of Brazil processed 900 thousand tons of sugar cane and 185 thousand tons of corn grain for the generation of ethanol and sugar.

Following corn fermentation, DDGS is produced as a co-product of ethanol production, which allows the flex ethanol distilleries to remain sustainable during the production of ethanol from corn in relation to the use of sugarcane. The literature reports that DDGS can be used in animal feed, because it has protein characteristics, which can replace soybean meal, in order to reduce the cost

of animal feed (SPIEHS et al., 2002; McKEOWN et al., 2009).

According to the Association of Soya and Maize Producers of Mato Grosso, (APROSOJA), the bromatological composition of DDGS produced in Brazil contains 96% dry matter (DM), 36% CP, 9% ether extract (EE), and 10% crude fiber (CF) (APROSOJA, 2012). According to Valadares Filho et al. (2010), distillery grain has 32% CP content, 30% acid detergent fiber (ADF), and 18% acid-detergent insoluble nitrogen (ADIN).

According to Spiehs et al. (2002), DDGS produced in different ethanol distilleries in the South Dakota region of the United States showed 87 to 93% variation in DM, 28 to 32% variation in CP, 8 to 12% variation of EE, and 35 to 49% variation of NDF, as well as 3.661 to 3.838 Mcal kg^{-1} of metabolizable energy, and 70% of total digestible nutrients (TDN). According to these authors, the variation in the bromatological and, consequently, nutritional composition of DDGS can occur due to differences in the content of each nutrient that make up this co-product. This can result from the quality of the corn grain and the method of processing used in the ethanol distilleries. The three main factors that affect variability of the raw material (corn grain) of the DDGS are the bromatological and nutritional composition of the grain used by the distilleries, variation in the mixing ratio of cereal grains to ethanol production, and different drying times and temperatures for DDGS used by ethanol distilleries.

In view of the large-scale production, there is a need to generate technical and scientific information on the use of DDGS in ruminant feed in the state of Mato Grosso – Brazil, which will enable ethanol distilleries and cattle farmers to make better use of this co-product of the national ethanol industry.

To obtain technical knowledge of food to integrate a system of dietary calculations, the chemical and bromatological composition, and the proportion of food digested and used by the animal, should be evaluated (PEREIRA et al., 2010). Many methods have been developed over the years to carry out these analyses, with the objective of more accurately predicting the nutritional values of foods, so that they meet the energetic and protein demands generated by the productive and/or the reproductive functions of the animals (SOUZA et al., 2011). Among the different methods used to analyze food, special attention has been given to the Cornell Net Carbohydrate and Protein System (CNCPS), which was developed from mechanistic concepts, mainly regarding the nutrient dynamics in the gastrointestinal tract of ruminants (CARVALHO, 2012).

To evaluate the digestibility of food, *in vitro* techniques present advantages in their rapidity, physicochemical uniformity of the fermentation site, and convenience to maintain few fistulated animals (ALCALDE et al., 2001). In addition, they permit *in vitro* digestibility to be estimated for a large number of samples simultaneously with the use of an artificial incubator (SANTOS et al., 2000).

The objective of this study were to evaluate the protein and carbohydrate fractionation of DDGS, corn grain, soybean meal, and corn silage through CNCPS, and to evaluate the *in vitro* digestibility coefficient of nutrients and ruminal parameters of experimental foods and diets containing 0.0, 10.0, 20.0, and 30.0% DDGS.

Material and Methods

The study was conducted at the Pontes e Lacerda University *Campus* of the Universidade do Estado de Mato Grosso – UNEMAT, Brazil. DDGS was obtained from a sugar ethanol distillery.

To evaluate the *in vitro* digestibility (*IV*D) of the experimental foods (DDGS, CG, SM, and CS; Table 1) and experimental diets containing different levels of DDGS (0.0, 10.0, 20.0, and 30.0% based on DM; Table 2), two male, castrated, mixed-breed male sheep, with an average body weight of 26 kg and 10 months of age, were used as the inoculum

donors (bacteria). The animals received a total mixed ration to meet their requirements following the recommendations of the NRC (2007) with a 65:35 forage-to-concentrate ratio.

Inoculum donor lambs were kept in indoor metabolism cages with free access to water and were fed twice daily (0800 and 1600) with basal feed containing 0.0% DDGS.

The *IV*D of the nutrients was determined in triplicate, using the one-stage technique described by Silva and Queiroz (2002) and adapted to a 24-h fermentation stage as described by Smith et al. (2010).

Table 1. Bromatological composition of experimental foods used in the formulation of experimental rations containing the inclusion of distillers dried grains with solubles (DDGS) evaluated in the determination of the *in vitro* digestibility coefficient (*IV*DC) of nutrients.

Foods	Nutrients (% DM)								
	DM	OM	CP	EE	NDF	ADF	TC	NFC	TDN[1]
CS	29.33	93.36	7.54	3.59	54.38	28.58	82.23	27.85	61.30
CG	90.99	97.38	9.20	5.85	14.05	6.47	82.33	68.28	86.03
SM	91.29	92.69	49.26	2.19	15.37	10.97	41.24	25.87	80.73
DDGS	90.04	98.00	34.41	4.47	48.80	15.37	59.11	10.31	78.50

CS: corn silage; CG: corn grain; SM: soybean meal; DDGS: distillers dried grain with solubles; DM: dry matter; OM: organic matter; CP: crude protein; EE: ethereal extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; TC: total carbohydrate; NFC: non-fibrous carbohydrates and [1]TDN: total digestible nutrients – obtained from the literature (VALADARES FILHO et al., 2010 and SPHIES et al., 2002).

Table 2. Ingredients and bromatological composition of rations containing inclusion levels of distillers dried grains with solubles (DDGS).

Ingredients	Levels of inclusion of the DDGS			
	0.0%	8.0%	16.0%	24.0%
Corn silage (CS)	65.0	65.0	65.0	65.0
Corn grain (CG)	21.5	18.0	14.5	11.0
Soybean meal (SM)	13.5	9.0	4.5	0.0
Distillers dried grains with solubles (DDGS)	0.0	8.0	16.0	24.0
Bromatological composition of rations (% of DM)				
dry matter (DM)	50.95	50.86	50.77	50.69
organic matter (OM)	94.13	94.39	94.66	94.92
crude protein (CP)	13.53	13.74	13.96	14.17
ethereal extract (EE)	3.89	3.94	4.00	4.05
neutral detergent fiber (NDF)	40.44	43.16	45.88	48.61
acid detergent fiber (ADF)	21.45	21.96	22.47	22.98
total carbohydrate (TC)	76.72	76.71	76.70	76.69
non-fibrous carbohydrates (NFC)	36.27	33.55	30.82	28.09
total digestible nutrients (TDN)	69.24	68.88	68.51	68.15

Sheep ruminal fluid was collected by vacuum suction pump in the morning, 2 h after the morning feed in order to obtain a suitable microbial population and a minimum number of food particles. The ruminal liquid was conditioned in a thermos flask at 39°C.

After ruminal liquid was collected, the pH was measured and then mixed with artificial saliva, maintained under anaerobic conditions at a temperature of 39°C until it was added to the digestibility tubes containing samples of different foods and experimental rations.

Three incubations were performed to reduce the effect of the collection day. Each sample (0.5 g) and 50 mL of the incubation solution (37.5 mL artificial saliva and 12.5 mL ruminal liquid) were added to each tube, which were then purged with CO_2. The tubes were then sealed with corks fitted with Bunsen valves and placed in a water bath with constant stirring at 39°C. Every 2 h, the tubes were gently shaken manually to aid the release of formed gases. Three tubes containing only incubation solution were incubated in each run to serve as controls, and three tubes containing only Tifton 85 hay (*Cynodon*) as index forage (SMITH et al., 2010).

After the 24-h incubation, fermentation was stopped by placing the tubes in a container containing crushed ice for 10 min. The contents of the tubes were filtered on quantitative filter paper (black strip, 15-cm diameter for rapid filtration for thick and gelatinous precipitates), and the fermented content retained in the filters was placed in an oven at 105°C where they remained for 24 h. After this period, the filters were weighed and the liquid that passed through the filter was stored for further analysis of ammoniacal nitrogen content ($N\text{-}NH_3$).

The *in vitro* digestibility coefficient (*IV*DC) was determined by the following formula (SILVA; QUEIROZ, 2002):

$$\text{sample weight (g of nutrient)} - [\text{weight of residue (g nutrient)} - \text{control weight (g of nutrient)}] / \text{sample weight (g of nutrient)} \times 100$$

Immediately after the *in vitro* fermentation process (24 h), the pH of the fermented contents of the tubes was measured before filtration, using a digital pH meter. An aliquot (20 mL) of the fermented content after filtration was mixed with 0.2 mL of 1: 1 sulfuric acid to acidify the medium and stop fermentation. These samples were used to determine the concentration of ammoniacal nitrogen ($N\text{-}NH_3$) of the fermented content after 24 h of *in vitro* incubation. The concentrations of $N\text{-}NH_3$ in the fermented samples were determined by distillation with potassium hydroxide 2 mol L^{-1} KOH, according to a technique described by Preston (1995).

Samples of food, feed, and food after in vitro digestion were processed in a Willey mill equipped with a 1-mm mesh sieve and stored in plastic pots previously labeled for further analysis.

The determination of dry matter (DM), organic matter (OM), crude protein (CP), and ethereal extract (EE) followed the recommendations of AOAC (2007). The neutral detergent fiber (NDF), acid detergent fiber (ADF), and permanganate lignin (PEL) contents of the food samples followed the recommendations of Van Soest et al. (1991). Neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN), insoluble nitrogen in trichloroacetic acid (NI – TCA), and nitrogen insoluble in borate – phosphate buffer (NI – BPB) were estimated as described by Geron et al. (2007) and, subsequently, the fractions of proteins and carbohydrates were determined by the method described by Cornell (SNIFFEN et al., 1992).

Protein and carbohydrate fractionation of DDGS, SM, CG, and CS were analyzed according to CNCPS, and the following fractions were obtained: A, B1, B2, B3, and C. Fraction A was determined as described by Krishnamoorthy et al. (1983). The values of fractions B1, B2, and B3 were determined as described by Sniffen et al. (1992). Fraction C was determined by ADIN, according to the method described by Silva and Queiroz (2002), after obtaining the ADF, according to Van Soest et al. (1991).

The carbohydrate fractions of the experimental foods (DDGS, SM, CG, and CS) were obtained from the following equations, reported by Sniffen et al. (1992): total carbohydrates (TC) = organic matter (OM) – [ethereal extract (EE) + (CP)]. Fraction B2 was obtained by the equation: B2 = 100 × (NDF (% DM) – NDIP (% CP) × 0.01 × CP (% DM)) NDF (% DM) × 0.01 × lignin NDF) × 2.4)) / TC (% DM), where NDF is the neutral detergent fiber and NDIP is the neutral detergent insoluble protein. Carbohydrate fractions A and C were determined according to the formulas described by Sniffen et al. (1992).

The statistical model used to evaluate the *IV*D of nutrients, the pH value, and ammoniacal nitrogen concentration of the four diets with different DDGS levels was a completely randomized experimental design. To evaluate the fractionation of carbohydrates and proteins of the individual foods, no statistical analyses were performed, since the foods presented different characteristics and classifications.

Statistical analyses of the variables studied for the nutrient *IV*D assay were interpreted in the Statistics and Genetic Analysis System (SAEG) through analysis of variance (UFV, 2007). For the differences obtained, Tukey's test was performed at 5% significance for foods and, for the diets containing DDGS, regression was performed at 5% probability.

Results and Discussion

The *IV*DC of the DDGS nutrients did not differ compared to those of the other foods used in the formulations of experimental diets (Table 3). However, the DM *IV*DC of DDGS (63.34%) was 14% lower than the SM (73.84%), possibly due to the higher contents of the B3 and C fractions of PB and the B2 fraction of TC, expressed in DM, for DDGS in relation to SM. Similarly, the CP *IV*DC of the DDGS was 14% lower than the SM; it is possible that higher protein contents in fractions B2 and C contributed to these results.

Table 3. *In vitro* digestibility coefficient (*IV*DC) and *in vitro* fermentation parameters of distillers dried grain with soluble (DDGS) and other foods (corn silage – SM, corn grain – CG and soybean meal – SM) used in the formulation of experimental rations.

Variables	Experimental foods				CV
	CS	CG	SM	DDGS	(%)
In vitro digestibility coefficient (*IV*DC)					
*IV*DC DM	64.78 a	74.78 a	73.84 a	63.34 a	13.96
*IV*DC OM	61.22 a	70.07 a	67.14 a	64.50 a	15.39
*IV*DC CP	74.51 a	79.01 a	82.10 a	70.66 a	13.05
*IV*DC NDF	60.37 a	61.17 a	41.50 a	52.60 a	21.27
*IV*DC ADF	51.96 a	42.73 a	37.13 a	46.12 a	35.29
In vitro fermentation parameters					
pH of FC *in vitro*	7.33 a	7.27 a	7.57 a	7.51 a	1.98
N-NH_3 of FC *in vitro*	40.60 a	37.10 a	57.49 a	41.48 a	57.87

DM: dry matter; OM: organic matter; CP: crude protein; EE: ethereal extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; FC: fermented content *in vitro e* N-NH_3: Ammoniacal nitrogen from ruminal fluid and CV: coefficient of variation.

According to Thulasiraman et al. (2015), the chemical composition and *in vitro* digestibility of the DDGS obtained using the method described by Tilley and Terry (1963), demonstrated that the DM *IV*DC was 52.0%, which is lower than that obtained in the present study (63.34%). This difference may be associated with the bromatological composition of DDGS. According to these authors, DDGS presented CP of 47%, crude fiber of 1.1%, and EE of 1.2% of EE, which differ from the values observed in the present study (Table 1).

According to Kelzer et al. (2010), DDGS presented 92.0% total CP digestibility using the mobile bag technique as described by Kononoff et al. (2007). This value for the DC of CP was higher than the value of 70.6% observed in the present study (Table 3), and this variation may be associated with the methodology used, since the mobile bag method utilizes a cannulated animal, and study *in vitro* the environment is closed and controlled. In addition, variation in the bromatological composition of the DDGS used in the different studies may be

consistent with the amplitude observed for the DC of PB. According to these authors, DDGS presented a DM content of 86.7%, CP of 26.0%, NDF of 30.2%, and ADF of 13.0%, which were lower than the values observed in the present study (Table 1).

The NDF and ADF *IV*DC of the DDGS were 21 and 19% respectively, which were higher than the SM. This may be due to the higher levels of the B2 and C fractions present in the TC for DDGS, when compared to the SM.

The pH and ammoniacal nitrogen ($N\text{-}NH_3$) concentration of the fermented contents for the different foods evaluated following incubation *in vitro* for 24 hours did not differ, with mean values of 7.41 and 44.17 mg $N\text{-}NH_3$ per 100 mL^{-1} of ruminal fluid, respectively.

Similarly, Pecka-Kiełb et al. (2015) evaluated the *in vitro* fermentation of DDGS and corn derivative, and demonstrated that DDGS did not alter methanogenesis or the ammonia concentration during *in vitro* fermentation for 8 and 24 h, with an average value of 12.3 mg $N\text{-}NH_3$ per 100 mL^{-1} of the fermented content at 24 hours, and an average pH value of 6.65 for the content fermented with DDGS.

Geron et al. (2014, 2015) evaluated the DM *IV*D and fermentative parameters of diets with increasing levels of concentrate and different forages, and showed that the mean pH was 7.30, regardless of the level of concentrate (20, 40, 60, and 80%) and forage evaluated. Normally, in *in vivo* studies on ruminal parameters, the ruminal liquid has a lower pH in relation to that observed *in vitro*; this may be due to the absorption dynamics of short-chain fatty acids and ammoniacal nitrogen in the rumen, which does not occur in *in vitro* studies.

The inclusion of 0.0, 8.0, 16.0, and 24.0% of DDGS in rations formulated for sheep containing 13.5% of CP, 68.5% of TDN, and a forage-to-concentrate ratio of 65:35 did not change the *IV*DC of DM, OM, CP, NDF, and ADF, with mean values of 70.93, 70.64, 59.58, 52.83, and 43.40%, respectively (Table 4).

Table 4. *In vitro* digestibility coefficient (*IV*DC) and *in vitro* fermentation parameters of rations for sheep containing levels of 0.0%; 8.0%; 16.0% and 24.0% inclusion of distillers dried grain with soluble (DDGS).

Variables	Rations with inclusion levels of DDGS				Regres.	CV (%)
	0.0%	8.0%	16.0%	24.0%		
In vitro digestibility coefficient (*IV*DC)						
*IV*DC DM	71.64	68.97	71.16	71.91	Y = 70.93	12.05
*IV*DC OM	70.83	68.89	71.89	70.94	Y = 70.64	14.47
*IV*DC CP	63.78	58.40	57.41	58.73	Y = 59.58	36.08
*IV*DC NDF	54.46	51.88	49.10	55.89	Y = 52.83	14.57
*IV*DC ADF	41.05	45.27	40.02	47.25	Y = 43.40	27.19
In vitro fermentation parameters						
pH of FC *in vitro*	7.39	7.34	7.50	7.43	Y = 7.41	2.16
$N\text{-}NH_3$ of FC *in vitro*	36.14	37.36	47.21	30.01	Y = 37.68	47.51

DM: dry matter; OM: organic matter; CP: crude protein; EE: ethereal extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; FC: fermented content *in vitro e* $N\text{-}NH_3$: Ammoniacal nitrogen from ruminal fluid and CV: coefficient of variation. Regres.: regression equations.

The CP *IV*DC was 7.9% lower for the diet with 24% DDGS (58.73%) when compared to the diet with 0% DDGS (63.78%). This variation may be associated with the rate of CP degradability of the DDGS, which was 47.04% of effective degradability at a passage rate of 0.05 h^{-1} according to Teixeira (2014), while the SM shows effective degradability of 74.23% at 0.05 h^{-1} (TEIXEIRA, 2014). Thus, the lower availability of CP from the DDGS may have contributed to the variation in CP *IV*D observed between the experimental diets.

The CP fraction A of DDGS presented values that were 88, 71, and 37% lower than those of SM, CG, and CS, respectively (Table 5). The fermentation process for ethanol production occurs under conditions (temperature, time, hydrolysis process, etc.) that probably result in the solubilization of a large proportion of the N. This hypothesis is corroborated by the difference between the CP fraction A of DDGS (0.39), CS (0.69%), and CG (1.32%). The content 34.41%, the PB of the DDGS (Table 1) was 30% lower than that of the SM with 49.26% of CP, which may have contributed to the 88% variation observed in the protein A fraction of these foods. Similarly, the B1 fraction of the DDGS protein was 66% lower in relation to the SM, but was close to that of the ground corn grain (CG).

Table 5. Nitrogen fractions and carbohydrates from foods experimental.

Variables	Foods			
	CS	CG	DDGS	SM
Nitrogen fractions (% DM)				
A	0.62	1.32	0.39	3.29
B1	2.50	1.39	1.70	5.04
B2	2.75	4.06	27.13	34.70
B3	1.00	1.88	3.49	4.32
C	0.66	0.74	1.70	1.92
Nitrogen fractions (% CP)				
A	8.28	12.27	1.12	6.68
B1	33.18	15.15	4.93	10.23
B2	36.51	44.12	78.84	70.44
B3	13.33	20.40	10.16	8.76
C	8.69	8.06	4.95	3.89
Carbohydrates fractions (% DM)				
A+B1	33.34	62.29	8.64	22.83
B2	43.63	19.72	46.92	17.99
C	5.17	0.32	3.55	0.41
Carbohydrates fractions (% TC)				
A+B1	40.66	75.66	14.62	55.36
B2	53.06	23.95	79.38	43.64
C	6.29	0.39	6.00	1.01

CS: corn silage; CG: corn grain; SM: soybean meal; DDGS: distillers dried grain with solubles. DM: dry matter; CP: crude protein; TC total carbohydrates.

DDGS DM presented 21% true protein (B2 fraction), which means that 78.84% of the DDGS protein is composed of true protein (fraction B2 expressed in %CP). This is higher than that found for SM, which was 70.44% (Table 5) and is available for ruminal degradation. Corn silage (CS) and corn grain (CG), which correspond to forage and basal concentrate, respectively, presented a nitrogen content 44% lower in fraction B2 of the protein.

The CP of SM had a lower protein content (18%), some of which escapes ruminal fermentation (fraction B3) in relation to DDGS, expressed in %CP. The variation in the B3 fraction of CP obtained between the DDGS and SM can be related to the fermentation process during the production of ethanol, which may have altered the characteristics of the nitrogenous fractions of the food, that is, transformed the B3 fraction into B2, through the action of bacteria and enzymes (JAKOBSEN et al., 2015; SILVA et al., 2016).

The CP of DDGS contained a 21% higher proportion of fraction C in relation to soybean meal;

however, the fraction C content of DDGS was 43 and 39% lower than CS and CG, respectively (Table 5).

Considering that the A + B1 fractions indicate a higher supply of non-protein nitrogen and degradable peptides in the rumen, the SM provided higher levels of these fractions (16.91% of CP) than DDGS (6.05% of CP). Conversely, the substitution of soybean meal by DDGS could reduce the ruminal degradation of the protein in the rations. This would increase the passage of dietary protein to the intestine, making it available for animal metabolism, since the B3 fraction was 21% higher for DDGS (10.16% of CP) compared to SM (8.76% of CP).

However, in a study conducted by Yu et al. (2010), who evaluated the molecular structure of the DDGS protein, they used the protein fractionation of the DDGS, and presented a CP content of 10.5% for fraction A, 0.0% CP for fraction B1, 55.0% CP for fraction B2, 30.7% CP for fraction B3, and 3.9% CP for fraction C. The results of the present study differ from those reported previously in the literature (YU et al., 2010). This variation in the bromatological composition, and in the protein fractionation profile, may be associated with the grain varieties used by the ethanol distilleries, in addition to the fermentation methods used in Brazilian distilleries to produce ethanol from cereal grains (corn). According to Jakobsen et al. (2015), the use of different enzymatic complexes during ethanol production can alter the profile of the fibrous carbohydrates as well as the content of the passage protein. Silva et al. (2016) noted that the routes of ethanol production in the distilleries are divided into three processes depending on the raw material used, where the sugarcane passes through the extraction by pressure or diffusion, but the cereal grains are crushed and pass through enzymatic hydrolysis (JAKOBSEN et al., 2015). In addition, the use of sugarcane bagasse as a raw material for the production of ethanol is crushed and is subjected to acid hydrolysis. In this way, these processes alter the bromatological and nutritional characteristics of DDGS.

Kelzer et al. (2010) evaluated the protein fractionation of corn and its co-products (distillers wet grains with solubles, DDGS, and high protein distillery dry grain) and corroborated the data reported above. This indicates that the grain processing method used to obtain ethanol (JAKOBSEN et al., 2015), as well as the different enzymatic complexes used during the processing of the cereal grains, alter the profile of the protein fractions of the dry distillery grain. Those authors observed that the protein fractionation expressed in % CP for the different types of distillers wet grains with solubles varied from 7.4 to 18.6% for fraction A, 0.6 to 15.9% for fraction B1, 53.1 to 82.4% for fraction B2, 4.8 to 11.0% for fraction B3, and 0.9 to 14.9% for fraction C.

For the carbohydrate fractionation, the DDGS presented 8.64% in DM for the easily fermentable carbohydrates, sugars and starch (fraction A + B1; Table 3), which is 62; 86, and 74% lower than the carbohydrates obtained for SM, CG, and CS, respectively. For the values of the fractionation of carbohydrates of easy fermentation (fraction A + B1) expressed in %TC, the DDGS was 14.62%, while the CG and the CS were 75.66 and 40.66%, respectively. This may be due to the fermentation process, such that both CS and DDGS passed in relation to the corn grain. In addition, the CS contains easily fermented carbohydrates (starch) and fibrous carbohydrates (NDF and ADF; Table 1), which may have contributed to these results.

The potentially degradable B2 fraction of carbohydrates contained 79.38% of the TC for the DDGS, which was higher than that for CG and CS at 23.95 and 53.06%, respectively. It is likely that the TC content of 59.11% (Table 1) for DDGS, compared to the CG and CS TC content of 82.33 and 82.23%, respectively, may have corroborated this result. Another factor that may have contributed to the variation in the carbohydrate fraction B2 was

the fermentation process applied to corn grain during ethanol production, in which the microorganisms and the enzymatic hydrolysis (JAKOBSEN et al., 2015) used most of the soluble carbohydrate fraction (fraction A + B1), increasing the proportion of carbohydrates with lower solubility in fraction B2.

DDGS had higher hemicellulose and cellulose contents than SM, as verified in fraction B2, with a value of 46.92% expressed in DM. In the same way, DDGS presented a mean value of 79.38% TC for the B2 fraction in the form of cell walls, and only 43.64% SM expressed as %TC. These results are also confirmed by the higher values of NDF (% in DM) for DDGS, which were 48.80% in relation to 15.37% SM (Table 1).

Fraction C of the carbohydrates (indigestible fiber) expressed in %TC of the DDGS was 94 and 83% higher than the CG and SM, respectively, which may have been influenced by the fermentation process of the corn grain during ethanol production. This increased the concentration of structural and complex carbohydrates for DDGS. However, DDGS and CS showed similar values for the C fraction of the carbohydrates at 6.00 and 6.29% of the TC, respectively (Table 3). This may be associated with the TC profile of the CS, since this forage is made using the entire corn plant and probably contributed to the high content of fraction C.

A study conducted by Lanzas et al. (2007) to evaluate carbohydrate fractionation, showed that DDGS produced in the United States presented 15.7% DM for fraction A + B1, 30.0% DM for fraction B2, and 11.1% DM for fraction C. Variety in grains and in the processing methods used by distilleries, especially the use of different enzymatic complexes (JAKOBSEN et al., 2015), may have influenced the profile of the soluble and insoluble carbohydrates present in DDGS, resulting in variations for the different fractions of DDGS carbohydrates being obtained between studies.

Conclusions

DDGS present a lower *in vitro* digestibility coefficient than soybean meal; however, up to 24% can be included in feed formulations without changing the *in vitro* digestibility coefficient of the nutrients.

DDGS present protein food with more than 70% of the CP in fraction B2 (true protein), as well as presenting a high amount of carbohydrates bound to the cell wall.

In vivo studies of DDGS should be performed to confirm the results obtained in the in vitro studies, and to measure animal performance with the inclusion of this alternative source of protein in the ruminant diet.

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