

# Partial replacement of soybean meal by other protein sources on nutritional characteristics of beef cattle fed a whole corn grain diet

## Substituição parcial do farelo de soja por outras fontes protéicas sobre as características nutricionais de bovinos de corte alimentados com dieta de milho grão inteiro

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### Highlights

The inclusion of DDG increased the dry matter intake.  
Protein sources did not affect the efficiency on N utilization.  
The grain-use efficiency was 96.6% for whole corn grain diets.  
Protein sources do not modify animal behavior.

### Abstract

Diets composed of whole flint corn grain (WCG) without any roughage source are often used in South American countries. The primary source of protein in these diets is soybean meal. We hypothesized

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that the combination of different protein sources improves ruminal fermentation and nutrient digestibility. This study was carried out to assess the impacts of replacing 50% of the soybean meal by other protein sources on voluntary dry matter intake (DMI), digestibility, efficiency of N utilization, efficiency of grain use, metabolic characteristics, and ingestive behavior of Nelore cattle fed WCG diets. Five rumen-cannulated Nelore Bulls (average BW = 651.6 ± 31.83 kg) were used in a 5 x 5 Latin square design, randomly assigned to five diets consisting of 85% of WCG and 15% of pellet supplement with combination of different protein sources: SM - pellet supplement with soybean meal only; CM - soybean meal and cottonseed meal; SFM - soybean meal and sunflower meal; DDG - soybean meal and DDG; DDGS - soybean meal and DDGS. The replacement of 50% of soybean meal by DDG increased ( $P < 0.05$ ) DMI and organic matter intake (OMI), both DMI and OMI were lower with CM. Digestibility, ruminal pH (mean = 5.7), efficiency of N utilization, and diurnal ingestive behavior were not affected ( $P > 0.05$ ) by protein sources. Protein sources also did not affect the grain-use efficiency ( $P > 0.05$ ), with an average value of 96.6% and only 3.4% of the corn grains recovered in the feces. The concentration of GGT enzyme was greatest ( $P < 0.05$ ) for CM and DDGS. The inclusion of DDG increased the DMI but did not improve the nutrients' digestibility or N metabolism. The results suggest that 50% of the soybean meal can be replaced by sunflower meal, DDG, and DDGS without affecting the nutritional and metabolic parameters of Nelore cattle fed whole flint corn grain diets, providing opportunities for reducing costs in feedlot systems.

**Key words:** Cattle. Feedlots. Whole flint corn.

## Resumo

Dietas com milho grãos inteiros (MGI) e sem volumoso são frequentemente usadas em países da América do Sul. A principal fonte de proteína nessas dietas é o farelo de soja. Nossa hipótese é que a combinação de diferentes fontes de proteína melhora o padrão de fermentação ruminal e a digestibilidade dos nutrientes. Este estudo foi realizado para avaliar os impactos da substituição de 50% do farelo de soja por outras fontes proteicas sobre o consumo voluntário, digestibilidade, eficiência de utilização do N, eficiência de utilização de grãos, características metabólicas e comportamento de bovinos Nelores alimentados com dietas de MGI. Cinco bovinos Nelores, machos não-castrados e com cânula ruminal (Peso corporal médio = 651,6 ± 31,83 kg) foram utilizados em delineamento quadrado latino 5 x 5, distribuídos aleatoriamente em cinco dietas com 85% de MGI e 15% de um suplemento peletizado com combinação de diferentes fontes proteicas, a saber: FS - núcleo somente com farelo de soja; FA - núcleo com farelo de soja e farelo de algodão; FG - núcleo com farelo de soja e farelo de girassol; DDG - núcleo com farelo de soja e DDG; DDGS - núcleo com farelo de soja e DDGS. A substituição de 50% do farelo de soja por DDG aumentou ( $P < 0,05$ ) o consumo de matéria seca (CMS) e o consumo de matéria orgânica (CMO). Tanto o CMS quanto o CMO foram menores com FA. A digestibilidade dos nutrientes, pH ruminal (média = 5,7), eficiência de utilização do N e comportamento não foram afetados ( $P > 0,05$ ) pelas fontes proteicas. A eficiência de utilização dos grãos também não modificou ( $P > 0,05$ ) com a combinação das fontes proteicas, com valor médio de 96,6% e cerca de apenas 3,4% dos grãos de milho recuperados nas fezes. A concentração da enzima gama aminotransferase foi maior para CM e DDGS ( $P > 0,05$ ). A inclusão de DDG aumentou o CMS, mas não melhorou a digestibilidade dos nutrientes da dieta ou o metabolismo do N. Os resultados sugerem que 50% do farelo de soja pode ser substituído por farelo

de girassol, DDG ou DDGS sem afetar os parâmetros nutricionais e metabólicos de bovinos Nelore alimentados com dietas de milho grão inteiro, fornecendo uma oportunidade para reduzir os custos.

**Palavras-chave:** Bovinos. Confinamento. Milho inteiro flint.

## Introduction

Whole grain diets have been used in the USA since 1970, while in Brazil, it only started around 2005. Whole grain diets are gaining popularity particularly in regions with abundant grain (e.g.: corn and sorghum) supplies which are widely utilized in feedlots. Diets with lower levels of roughage enhance feed efficiency, increase greater carcass yield, and lower feed costs in feedlots by reducing operational management and production costs (Carvalho et al., 2016; Contadini et al., 2017; Neumann et al., 2018).

Whole grain diets without roughage are typically supplemented with concentrate, such that 85% of the feed is whole grain, and 15% is a pelleted concentrate containing a blend of proteins, minerals, vitamins, and other feed additives, designed to prevent possible metabolic disorders that might occur from the low inclusion of fiber carbohydrates in the overall diet (Lemos et al., 2016; Marques et al., 2016; Michels et al., 2018).

Protein is one of limiting nutrients for ruminants, and is typically the most expensive dietary component (Suriyapha et al., 2022). Soybean meal is ubiquitously the main protein source used for ruminants fed on pelleted supplements (Michels et al., 2018; Neumann et al., 2018), and its costs vary seasonally (Porsh et al., 2018).

Alternative protein sources used in feedlots include cottonseed meal,

sunflower meal, and coproducts from ethanol production (e.g. Dried Distillers Grains - DDG, and Dried Distillers Grains with Solubles - DDGS). Any increase in soybean meal price increases beef production costs, so alternative protein sources are hugely important for intensification in the livestock sector (Araújo et al., 2020).

The combination of different protein sources in the feeding of ruminants may also enhance the extent and rate of ruminal degradability of the protein, synchronizing the availability of nitrogen (N) and energy in the rumen, thereby promoting efficient microbial growth (Stern et al., 1979), leading to a more favorable amino acid profile ingested and improved rumen undegradable protein supply (Cowley et al., 2019).

The objective of the present study was to evaluate the effects of partial replacement of soybean meal with other protein sources on intake, digestibility, efficiency of use of grain, N metabolism, and ingestive behavior of *Bos indicus* cattle fed with whole flint corn grain diets.

## Material and Methods

The study was conducted in the experimental feedlot cattle facilities and Laboratory of Animal Nutrition at the Federal University of Mato Grosso (UFMT), Cuiabá, Brazil. The experimental protocol complied with the Ethical Principles for Animal Research, and was approved by the

institutional Committee for Ethics in the Use of Animals (CEUA, protocol number 23108.008091/2019-47).

### *Animals, experimental design, and treatments*

Five rumen-cannulated Nellore bulls (BW = 651.6 ± 31.83) were used in a 5 x 5 Latin square design incorporating whole corn grain diets with five distinct protein sources.

Animals were allocated to individual pens (30.5 m<sup>2</sup>) equipped with waterers, covered feed bunks, and concrete floors. The experiment lasted 95 days, split into periods of 19 days, being 14 days for adaptation (Machado et al., 2016), and 5 days for sample collections.

Prior to the study, animals were acclimatized to the experimental conditions and diet. Acclimatization to the diet was carried out with a supply of whole grains in individual paddocks, each composed of 0.25 ha of *Urochloa brizantha* cv. Marandu, and equipped with waterers and individual bunks. The offer of whole grain started with 0.75% of BW, increasing by 0.25% every three days based on the study by Parra et al. (2019). Animals were allocated into individual pens when intake reached 1.8% of BW (dry matter, DM), and the study started when the intake stabilized.

The treatments involved replacing 50% of the soybean meal with other protein sources as follows: SM - 85% whole flint corn grain (WCG), 15% pelleted supplement with soybean meal; CM - 85% WCG, 15% pelleted supplement with cottonseed meal plus soybean meal; SFM - 85% WCG 15% pelleted supplement with sunflower meal plus soybean meal; DDG - 85% of WCG, 15% pelleted supplement with Distillers Dried Grains plus soybean meal; DDGS - 85% of a WCG, 15% pelleted supplement with Distillers Dried Grains with Solubles plus soybean meal (Table 1).

Pelleted supplements in the experimental diets were manufactured at the Agrocria Nutrição Animal & Sementes feed mill (Cuiabá, Mato Grosso, Brazil) using the same set of ingredients (soybean hulls, soybean meal, urea, and minerals), along with the appropriate protein source for each treatment.

Feed management was performed twice daily, at 07h30 and 16h30, and DM intake (DMI) was recorded daily by calculating the difference in weight between the amount offered and the orts from the previous day. DMI was recorded as a percentage of the average BW. Intake adjustment was performed daily, aiming at *ad libitum* intake, with orts at 5% of the previous day's DMI.

**Table 1**  
**Ingredients and chemical composition of experimental diets**

Item	Treatments <sup>1</sup>				
	SM	CM	SFM	DDG	DDGS
<i>Ingredient</i>					
Whole corn	85	85	85	85	85
Pelleted supplement <sup>2</sup>	15	15	15	15	15
<i>Chemical composition, DM basis</i>					
DM, %	89.07	89.31	88.27	88.88	88.48
Ash, %	5.08	5.61	6.02	6.03	6.14
CP, %	13.41	14.51	14.53	14.5	15.47
NDF, %	15.69	16.67	18.85	17.08	16.31
RDP <sup>2</sup> , %	9.10	9.29	9.36	8.51	9.24
TDN <sup>2</sup> , %	81.08	80.52	81.31	81.23	81.81

<sup>1</sup>Treatments: SM - 85% of whole corn grain with 15% of a pelleted supplement of soybean meal, CM - 85% of whole corn grain with 15% of a pelleted supplement cottonseed of meal plus soybean meal, SFM - 85% of whole corn grain with 15% of a pelleted supplement of sunflower meal plus soybean meal, DDG - 85% of whole corn grain with 15% of a pelleted supplement of Distillers Dried Grains plus soybean meal, DDGS - 85% of a whole corn grain with 15% of pelleted supplement of Distillers Dried Grains with Soluble plus soybean meal. SEM: standard error of the mean.

<sup>2</sup>The pelleted supplement contained minerals (4% Ca, 0.6% P, 9.7 g kg<sup>-1</sup> Na, 15 g kg<sup>-1</sup> K, 3 g kg<sup>-1</sup> Mg, 4.5 g kg<sup>-1</sup> S, 5 mg kg<sup>-1</sup> Co, 195 mg kg<sup>-1</sup> Cu, 1.4 mg kg<sup>-1</sup> Cr, 5 mg kg<sup>-1</sup> I, 180 mg kg<sup>-1</sup> Mn, 0.35 mg kg<sup>-1</sup> Mb, 0.3 mg kg<sup>-1</sup> Ni, 1.8 mg kg<sup>-1</sup> Se, and 420 mg kg<sup>-1</sup> Zn), feed additives (Virginiamycin 150 mg kg<sup>-1</sup>) and vitamins (21,000 IU vitamin A, 3,000 IU vitamin D, and 138 IU vitamin E per kilogram of pelleted concentrate).

<sup>2</sup>Based on National Research Council [NRC] (1996) nutrient analysis of each dietary ingredient.

### *Sample collection, laboratory analyses, and measurements*

Animals were weighed at the beginning and end of each experimental period. Voluntary intake was measured between days 15 and 19 of each experimental period. Samples of the offered feeds and orts were weighed and taken each day to be frozen (-20 °C) for later chemical analyses.

Feces were sampled on day's 15-18, beginning at 06h00. The total fecal production was collected after each spontaneous defecation, and packed in polyethylene containers. Animals were

monitored 24h00 a day to avoid fecal sample cross-contamination.

After each day, feces were homogenized and weighed, and representative samples (250 g) were taken and dried in a forced-air oven at 55 °C for 72h00. At the end of each period, the dried samples were proportionally homogenized to obtain a pooled fecal sample per animal and per period, and then frozen (-20 °C) for later chemical analyses.

The number of whole corn grains in offered feed and orts, and those that appeared in feces, were counted in representative samples of 10% of the total

weight. When the orts were less than 1 kg, all corn grains in the refused feed were counted (Gorocica-Buenfil & Loerch, 2005).

A pooled fecal sample (1000 g) was wet-sieved, and grain particles retained on a 1.59 mm screen were collected. The extent of damage to grains was visually assessed, and particles were classified into one of four categories: whole (grains with no visible damage to the pericarp), damaged (pericarp damaged but grain intact), broken (grain fractured into pieces), or empty (fractured pieces of the pericarp containing no endosperm). Each fraction was dried at 105 °C to a constant weight, and the percentage weight of each fraction was determined (Beauchemin et al., 1994).

A second pooled sub-sample (1000 g) was wet-sieved (NASCO®) for particle size distribution, and the mean particle size was determined according to the methodology adapted by Yu et al. (1998), in which it is assumed that corn held on a 4.76 mm screen has a mean particle size of 6 mm, which is multiplied by the percentage retained on this screen. The percentage of corn retained by smaller screens is multiplied by a mean value calculated by adding the screen just above the value of the smaller screen, and dividing by 2  $[(4.76 \text{ mm} + 2.38 \text{ mm}) / 2 = 3.57 \text{ mm}]$ . The residue retained in the pan was multiplied by 0.12 mm. The multiplicands for all screen sizes were summed to obtain a mean particle size.

The samples of feeds, orts, and feces were processed in a knife mill (Willye Technal TE-650) through a 1-mm screen sieve. Chemical analyses were performed according to the Association of Official Analytical Chemists [AOAC] (2000) for DM

content (method 930.15), ash (method 942.05), crude protein (CP; method 984.13), ether extract (EE; method 920.39), and neutral detergent fiber (NDFap, using a heat-stable  $\alpha$ -amylase; method Van Soest et al. (1991).

To evaluate urine and urea daily excretions, spot urine samples were collected twice daily on days 16 and 17. The samples were filtered, then an aliquot of 50 mL (concentrated) was stored, and a second aliquot of 10 mL was diluted with 40 mL of H<sub>2</sub>SO<sub>4</sub> (0.036 N), and frozen at -20°C (Teobaldo et al., 2020).

Daily excretion of urinary urea N was analyzed according to the methodology described by Rennó et al. (2000), and urinary creatinine and urea concentrations were determined via colorimetry with commercial kits (Wiener metrolab 350®).

Daily urine volume was calculated as the relationship between creatinine excretion and BW, and creatinine concentration detected in the spot urine samples, using the equation proposed by Costa e Silva et al. (2012). N balance was calculated as the difference between the total N ingested and the total N excreted in the feces and urine.

Blood samples were collected at 03h00 and 06h00 after supplementation on day 19 of each experimental period, via a puncture of coccygeal vessels, for analyses of total protein, albumin, urea N, and the liver enzymes gamma glutamyltransferase (GGT) and aspartate aminotransferase (AST). Blood samples were immediately placed on ice and transported to the laboratory to separate plasma and serum from whole blood by centrifuging at 4000 g for 15 min. All plasma and serum samples were stored frozen

at -20 °C until colorimetry analysis using commercial kits (Wiener metrolab 350®).

Rumen contents samples were manually collected at the liquid-solid interface of the rumen mat before supplementation (00h00), and 03h00, 06h00, and 09h00 after the first feeding on day 19 of each experimental period. Samples were filtered through three layers of cheesecloth and subjected to a pH measurement (Potentiometer TEC 2, Tecnal, Piracicaba, SP, Brazil).

### *Animal behavior*

The diurnal ingestive behavior activities of feedlot cattle were recorded on days 13 and 14 of each experimental period. All animals were monitored continuously for 12h00 (06h00 to 18h00) during daylight by a human observer (Paula et al., 2019).

The observers were at a sufficient distance from the pens that their presence did not interfere with the animal's behavior, making the annotations of the start and end times of the behavioral activities (Araújo et al., 2020).

Individual cattle were observed during behavioral activities of leisure, rumination, water and feed intake, self-grooming, and non-nutritious oral behavior (NNOB). The time that each animal changed activity was recorded, and the duration of each activity was subsequently computed (Paula et al., 2019).

### *Statistical analysis*

All variables were analyzed using the MIXED procedure of SAS (version 9.4). Data

were analyzed as a 5 x 5 Latin square design with fixed effects of treatment. Period and cattle were treated as random effects for all analyses. A Kenward-Rogers denominator degrees of freedom adjustment was used. Measures of pH and ruminal ammonia N were analyzed as repeated measures in time, with treatment, time and the treatment x time interaction as fixed effects, and cattle and experimental period as random effects. According to the Bayesian information criterion, the data had an "unstructured" covariance structure. When the interaction between treatment and time was significant, effects of time within each treatment were investigated as linear, quadratic, and cubic effects. Differences between individual means for each treatment were identified using Fisher's Least Significant Difference (LSD) method. Differences were declared significant at  $P \leq 0.05$ .

## **Results and Discussion**

Soybean meal is the primary protein concentrate used in pelleted supplements of WCG diets, and also is one of most expensive dietary components. This study evaluated the effects of partial replacement of soybean meal with cottonseed meal, sunflower meal, DDG and DDGS in WCG diets.

Dry matter intake ( $\text{kg day}^{-1}$  and % BW) and organic matter intake (OMI,  $\text{kg day}^{-1}$ ) were greatest ( $P < 0.01$ ) for DDG, intermediate for SM, SFM, and DDGS, and lowest for CM (Table 2). Neither fecal output nor digestibility were affected ( $P > 0.05$ ) by the protein source (Table 2).

**Table 2**  
**Intake and digestibility of whole corn grain diets (no-roughage) and different protein source in Nellore cattle**

Item	Treatments <sup>1</sup>					SEM	P - value
	SM	CM	SFM	DDG	DDGS		
<i>Intake (% BW)</i>							
DM	1.435b	1.299c	1.401bc	1.576a	1.319bc	0.065	0.0032
<i>Intake (kg day<sup>-1</sup>)</i>							
DM	9.303b	8.281c	9.356b	10.423a	8.616bc	0.564	0.0016
OM	8.776b	7.696c	8.850b	9.855a	8.093bc	0.564	0.0024
CP	1.334	1.309	1.337	1.431	1.172	0.090	0.0664
NDF	1.589	1.369	1.532	1.794	1.232	0.176	0.1515
<i>Fecal output (kg day<sup>-1</sup>)</i>							
DM	0.801	0.713	0.903	0.914	0.726	0.284	0.4049
<i>Digestibility (%)</i>							
DM	92.39	91.58	90.87	91.42	91.95	2.288	0.6890
OM	93.90	93.52	92.65	92.69	93.08	2.340	0.5714
CP	91.18	90.49	88.71	90.07	90.42	2.291	0.3889
NDF	92.39	90.89	87.62	91.70	90.53	2.485	0.4674

Means with a different letter in the same row are significantly different ( $P < 0.05$ ). 1Treatments: SM - 85% of whole corn grain with 15% of a pelleted supplement of soybean meal, CM - 85% of whole corn grain with 15% of a pelleted supplement cottonseed of meal plus soybean meal, SFM - 85% of whole corn grain with 15% of a pelleted supplement of sunflower meal plus soybean meal, DDG - 85% of whole corn grain with 15% of a pelleted supplement of Distillers Dried Grains plus soybean meal, DDGS - 85% of a whole corn grain with 15% of pelleted supplement of Distillers Dried Grains with Soluble plus soybean meal. SEM: standard error of the mean.

Animals fed with whole corn grain diets frequently have lower DMI than traditional diets (with ground corn and silage), because of the greater metabolizable energy content of the whole corn diets (Carvalho et al., 2016). However, the average DMI (expressed as % BW) observed in the present study was considerably lower than the values reported in others studies with WCG (Carvalho et al., 2016; Marques et al., 2016; Lemos et al., 2016), possibly because of greater BW of the bulls in this study.

The partial replacement of the protein source, in addition to differences related to the amino acid profile, modifies the physical

characteristics of the pellets. Throughout the study, we observed that the pellets with DDG had less integrity than the other treatments (data not shown), and this may have affected the DMI. Buchanan et al. (2010) noted that the inclusion of by-product meals, such as DDG, has been shown to decrease pellet quality. Trials assessing the effects of protein sources on cattle fed with whole corn grain in feedlots are scarce.

Lack of difference in nutrient digestibility between treatments might have been due to a similar rumen fermentation among protein sources. Another potential factor is the balance of rumen degradable



protein (RDP) and total digestible nutrients (TDN) in the diets, allowing good synchrony between carbohydrate fermentation and protein source degradation (Russel et al., 1992). Despite the absence of differences in digestibility, we found high values of digestibility (91.64% for DM and 90.17% for CP). These values are much higher than those reported by Lemos et al. (2016) of 74% for DM and 62.3% for CP. The higher digestibility values found in our study might have been related to a higher retention time of the grain in the rumen; WCG diets are thought to increase retention time and reduce passage rate (Gorocica-Buenfil & Loerch, 2005; Contadini et al., 2017). Low values obtained in the collection of total feces production support this interpretation.

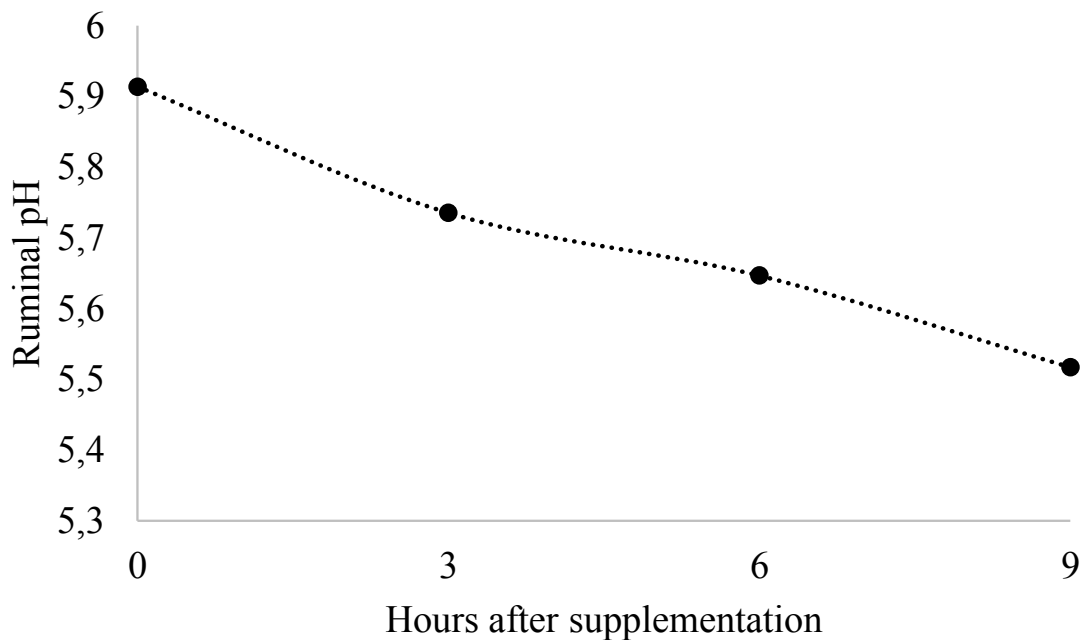
There was no treatment x time interaction ( $P > 0.05$ ) for ruminal pH (Table 3). However, there was a linear decrease over time ( $P < 0.05$ ; Figure 1). According to Carvalho et al. (2016), bulls fed WCG spend less time ruminating and eating, and probably produced less saliva and, hence, have lower ruminal pH compared with forage-based diets. Beauchemin et al. (2001) suggested a ruminal pH of 5.8 as the threshold for subclinical acidosis; Owens et al. (1998) used values of 5.6 and 5.2 as benchmarks for chronic and acute acidosis, respectively. In the present study, the mean ruminal pH was 5.7, and the optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0 (Bach et al., 2005). The pH fluctuation did not affect the digestibility of the diets.

**Table 3**  
**Ruminal pH on Nelore cattle fed whole corn grain diet with different protein sources**

Item	Treatments <sup>1</sup>					SEM <sup>2</sup>	P - value		
	SM	CM	SFM	DDG	DDGS		treat.	time	treat. * time
Ruminal pH	5.66	5.78	5.48	5.86	5.73	0.158	0.321	<0.001	0.536

Means with a different letter in the same row are significantly different ( $P < 0.05$ ). <sup>1</sup>Treatments: SM - 85% of whole corn grain with 15% of a pelleted supplement of soybean meal, CM - 85% of whole corn grain with 15% of a pelleted supplement of cottonseed meal plus soybean meal, SFM - 85% of whole corn grain with 15% of a pelleted supplement of sunflower meal plus soybean meal, DDG - 85% of whole corn grain with 15% of a pelleted supplement of Distillers Dried Grains plus soybean meal, DDGS - 85% of a whole corn grain with 15% of pelleted supplement of Distillers Dried Grains With Soluble plus soybean meal.

<sup>2</sup>SEM: standard error of the mean.



**Figure 1.** Time effect on ruminal pH (linear decrease).

There was also no effect of protein source on ruminal pH, a factor which is largely dependent on the diet's composition, especially in terms of carbohydrates with high ruminal and structural degradability. The lack of any relationship with the protein source might, therefore, be attributed to the similar composition of the diets in terms of non-structural carbohydrates, as the amount of corn grain in each diet remained constant. Diets containing a higher level of RDP could potentially result in more ruminal fermentation and greater pH changes, but this did not occur, possibly due to similar levels of RDP in all diets.

Intake, excretion, and disappearance of corn grains did not differ ( $P>0.05$ ) between treatments (Table 4). There was

no effect ( $P>0.05$ ) of the protein sources on characteristics of grain particles isolated from the feces (Table 5), grain damage evaluation (Table 5), or particle size distribution (Table 6). One of the biggest factors that limits the use of whole corn in feedlot cattle diets is the visual presence of whole corn grains in feces. In the present experiment, the number of corn grains ingested and excreted did not differ between treatments. Although the cattle ate an average of 30914 grains of corn per day, they excreted only an average of 1165 grains per day. This equates to a grain-use efficiency of 96.6% for the WCG diet, without roughage inclusion, and independent of protein source; only 3.4% of the corn grains ingested whole were recovered in feces, even using "flint" corn.

**Table 4**  
**Effect of protein source on whole corn grain excretion and disappearance on feces of Nelore cattle**

Item	Treatments <sup>1</sup>					SEM <sup>2</sup>	P - value
	SM	CM	SFM	DDG	DDGS		
<i>Whole corn grains</i>							
Intake, grains per day	30897	30969	32016	30295	30393	2151.20	0.845
Excretion, grains per day	1256.14	905.69	1412.06	1271.13	980.35	864.26	0.671
Disappearance, %	96.56	97.21	96.29	96.32	96.74	2.30	0.828

Means with a different letter in the same row are significantly different (P < 0.05). <sup>1</sup>Treatments: SM - 85% of whole corn grain with 15% of a pelleted supplement of soybean meal, CM - 85% of whole corn grain with 15% of a pelleted supplement cottonseed of meal plus soybean meal, SFM - 85% of whole corn grain with 15% of a pelleted supplement of sunflower meal plus soybean meal, DDG - 85% of whole corn grain with 15% of a pelleted supplement of Distillers Dried Grains plus soybean meal, DDGS - 85% of a whole corn grain with 15% of pelleted supplement of Distillers Dried Grains with Soluble plus soybean meal. <sup>2</sup>SEM: standard error of the mean.

**Table 5**  
**Characteristics of grain particles isolated from the feces of cattle fed whole corn grains**

Item	Treatments <sup>1</sup>					SEM <sup>2</sup>	P - value
	SM	CM	SFM	DDG	DDGS		
DMR	29.68	33.75	42.07	40.59	36.12	8.42	0.519
<i>% of DM retained on 1.59-mm screen</i>							
<i>Proportion<sup>3</sup></i>							
Whole	26.55	25.32	28.57	30.61	27.80	7.74	0.915
Damaged	12.01	13.24	11.08	14.10	12.51	3.07	0.882
Broken	10.06	9.81	9.15	11.96	10.13	2.72	0.844
Empty	51.38	51.63	51.20	43.33	49.56	11.93	0.863

Means with a different letter in the same row are significantly different (P < 0.05). <sup>1</sup>Treatments: SM - 85% of whole corn grain with 15% of a pelleted supplement of soybean meal, CM - 85% of whole corn grain with 15% of a pelleted supplement of cottonseed meal plus soybean meal, SFM - 85% of whole corn grain with 15% of a pelleted supplement of sunflower meal plus soybean meal, DDG - 85% of whole corn grain with 15% of a pelleted supplement of Distillers Dried Grains plus soybean meal, DDGS - 85% of a whole corn grain with 15% of pelleted supplement of Distillers Dried Grains With Soluble plus soybean meal. <sup>2</sup>SEM: standard error of the mean. DMR: DM retained on 1.59-mm screen, % of fecal DM. <sup>3</sup>Fractions described as whole (kernels with no visible damage to the pericarp). Damaged (pericarp damaged but kernel intact). Broken (kernel fractured into pieces). Empty (fractured pieces of the pericarp containing no endosperm).

**Table 6**  
**Particle size distribution on feces of cattle fed whole corn grains**

Item	Treatments <sup>1</sup>					SEM <sup>2</sup>	P - value
	SM	CM	SFM	DDG	DDGS		
<i>Screen mesh size</i>							
4.76 mm	21.61	26.05	31.38	29.02	27.99	7.627	0.715
2.38 mm	4.09	5.04	4.76	4.34	4.07	0.808	0.838
1.59 mm	4.46	4.23	6.20	4.62	3.58	1.045	0.292
0.12 mm <sup>3</sup>	69.84	64.68	57.66	62.02	64.36	7.360	0.548
MPS4, mm	1.615	1.904	2.244	2.062	1.973	0.441	0.659

Means with a different letter in the same row are significantly different ( $P < 0.05$ ). <sup>1</sup>Treatments: SM - 85% of whole corn grain with 15% of a pelleted supplement of soybean meal, CM - 85% of whole corn grain with 15% of a pelleted supplement of cottonseed meal plus soybean meal, SFM - 85% of whole corn grain with 15% of a pelleted supplement of sunflower meal plus soybean meal, DDG - 85% of whole corn grain with 15% of a pelleted supplement of Distillers Dried Grains plus soybean meal, DDGS - 85% of a whole corn grain with 15% of pelleted supplement of Distillers Dried Grains With Soluble plus soybean meal. <sup>2</sup>SEM: standard error of the mean. <sup>3</sup>The residue retained in the pan was multiplied by 0.12 mm. 4MPS - Mean Particle Size.

Gorocica-Buenfil & Loerch (2005) have reported pooled corn grain disappearance for cattle of different ages fed at 92%. According to these authors, the concentration of starch (and to a lesser extent protein) was numerically lower in corn grains recovered from feces than in those originally present in the feed. Despite their high visibility, only a small percentage of grains had actually escaped digestion.

Between 29% and 42% of DM excreted in feces was retained on a 1.59 mm screen, and the physical damage to grains recovered in feces was similar between treatments. This damage is caused by mastication and rumination (Beauchemin et al., 1994), allowing bacteria to gain access to and to colonize the nutrient-rich tissues of the endosperm—a process that is essential for the digestion of cereal grains in the rumen (McAllister et al., 1990).

The mean particle size (1.96 mm) isolated from feces did not differ between treatments. The constant composition of the diet (85% whole corn grain, 15% concentrate) yielded similar granulometry between treatments. This, coupled with the lack of effect of protein source on digestibility, resulted in similar particle sizes in feces. Considerable variation in grain damage nonetheless occurs among animals, mainly those that consume grain more rapidly and with different frequencies of mastication, and this is reflected in particle size at excretion (Beauchemin et al., 1994).

There was no difference between protein sources ( $P > 0.05$ ) for any variable related to nitrogen balance (Table 7). The mean N intake across all treatments in the present study was 210.6 g day<sup>-1</sup>, and the mean N excretion in feces was 21.8 g day<sup>-1</sup>. The balance of RDP in diets, coupled with the lack of effect of protein source on CP digestibility, may support these results.

**Table 7**  
**Nitrogen balance on cattle fed whole corn grain diets with different protein sources**

Item	Treatments <sup>1</sup>					SEM <sup>2</sup>	P - value
	SM	CM	SFM	DDG	DDGS		
Intake N, g day <sup>-1</sup>	213.37	209.36	213.84	229.01	187.43	14.41	0,066
Fecal N, g day <sup>-1</sup>	20.74	20.82	24.92	23.08	19.60	6.70	0.373
Urinary N, g day <sup>-1</sup>	33.15	27.12	35.27	29.14	46.36	15,30	0,872
Urea-N, g day <sup>-1</sup>	6.20	8.71	9.37	11.19	26.35	5,62	0.203
N Absorbed, g day <sup>-1</sup>	192.63	188.54	188.92	205.93	167.04	9.65	0.079
N Retained, g day <sup>-1</sup>	159.48	161.41	153.64	176.78	122.87	16,00	0.298
<i>Efficiency of N utilization (g g<sup>-1</sup>)</i>							
N absorbed per N intake	0.912	0.905	0.887	0.901	0.904	0.022	0.392
N retained per N intake	0.774	0.783	0.711	0.781	0.651	0.082	0.573
N retained per N absorbed	0.843	0.864	0.802	0.858	0.722	0.079	0.663

Means with a different letter in the same row are significantly different ( $P < 0.05$ ). <sup>1</sup>Treatments: SM - 85% of whole corn grain with 15% of a pelleted supplement of soybean meal, CM - 85% of whole corn grain with 15% of a pelleted supplement of cottonseed meal plus soybean meal, SFM - 85% of whole corn grain with 15% of a pelleted supplement of sunflower meal plus soybean meal, DDG - 85% of whole corn grain with 15% of a pelleted supplement of Distillers Dried Grains plus soybean meal, DDGS - 85% of a whole corn grain with 15% of pelleted supplement of Distillers Dried Grains With Soluble plus soybean meal. <sup>2</sup>SEM: standard error of the mean.

N excretion in urine was not affected by the protein source, probably due to the high starch contents in all diets, providing enough energy for the efficient use of  $\text{NH}_3$ , which is produced by the degradation of protein, thereby avoiding excessive losses of N (mainly in the form of N urea). The means of N and N urea excretions across all the treatments were  $34.2 \text{ g day}^{-1}$  and  $12.6 \text{ g day}^{-1}$ , respectively.

Excess  $\text{NH}_3$  in the rumen is absorbed by the ruminal wall into the bloodstream, and is converted to urea in the liver before being released into the blood. Blood urea can be recycled, returning to the rumen via saliva, or be excreted in the kidney as urine (Bach et al., 2005; Salazar et al., 2008). Changes

in N excretion in feces and urine generally occur when N intake is increased (Hoffman et al., 2001), though this did not occur in the present study.

Knaus et al. (2001), evaluating high-concentrate diets fed to Holsten steers with 70 to 80% of cracked corn, observed that supplementation with protein sources rich in digestible rumen-undegradable protein (RUP), and balanced in amino acids, did not increase digestibility, balance, or efficiency of use of N. Devant et al. (2015), investigating a high-moisture whole corn grain diet, found CP digestibility of 86.9%, with N retention of  $61.4 \text{ g day}^{-1}$ , and N intake of 39.5%. This is an inferior N retention to that observed in the present study.

Concentrations of total protein, albumin, and creatinine in blood were not affected ( $P > 0.05$ ) by protein source (Table 8). The concentration of GGT enzyme

was greatest ( $P < 0.05$ ) for DDGS and CM, intermediate for SFM, and lowest for DDG and SM.

**Table 8**  
**Effect of protein source on blood metabolites and concentration of serum enzymes on Nelore cattle fed whole corn grains**

Item	Treatments <sup>1</sup>					SEM <sup>2</sup>	P - value
	SM	CM	SFM	DDG	DDGS		
Total protein, g dL <sup>-1</sup>	8.33	8.35	8.08	7.95	8.26	0.208	0.486
Albumin, g dL <sup>-1</sup>	3.42	3.39	3.27	3.35	3.33	0.149	0.466
Creatinine, mg dL <sup>-1</sup>	2.88	2.90	2.88	2.70	2.82	0.265	0.754
AST, IU L <sup>-1</sup>	79.40	81.90	85.70	62.22	94.50	27.090	0.075
GGT, IU L <sup>-1</sup>	27.00b	31.90a	30.30ab	27.20b	33.60a	2.507	0.012

Means with a different letter in the same row are significantly different ( $P < 0.05$ ). <sup>1</sup>Treatments: SM - 85% of whole corn grain with 15% of a pelleted supplement of soybean meal, CM - 85% of whole corn grain with 15% of a pelleted supplement of cottonseed meal plus soybean meal, SFM - 85% of whole corn grain with 15% of a pelleted supplement of sunflower meal plus soybean meal, DDG - 85% of whole corn grain with 15% of a pelleted supplement of Distillers Dried Grains plus soybean meal, DDGS - 85% of a whole corn grain with 15% of pelleted supplement of Distillers Dried Grains With Soluble plus soybean meal. <sup>2</sup>SEM: standard error of the mean.

The values of blood metabolites and enzyme activities observed in the present study fell within the typical range for cattle. The liver, being the primary organ responsible for the synthesis and storage of total protein and albumin; showed no significant differences in serum parameters between treatments in the

present study suggests that protein sources do not affect protein synthesis in the liver of cattle. The activities of the enzymes AST and GGT suggested normal liver function in cattle in the present study (Jiang et al., 2015).

The behavior of cattle was not affected ( $P > 0.05$ ) by the protein source (Table 9).

**Table 9**  
**Diurnal behavior (minutes) of Nellore cattle fed with whole corn grain diets, confined in individual pens**

Item	Treatments <sup>1</sup>					SEM <sup>2</sup>	P - value
	SM	CM	SFM	DDG	DDGS		
Resting while standing	320.4	335.2	357.2	346.4	346.8	35.134	0.923
Resting while lying	242.2	244.2	230.8	228.2	251.2	37.064	0.988
Ruminating while standing	6.0	4.2	8.8	4.0	12.2	3.937	0.379
Ruminating while lying	6.2	18.4	7.5	3.9	3.8	6.108	0.281
Eating at feed bunk	98.8	92.6	92.4	112.2	83.2	14.689	0.616
Ingesting water	10.4	8.8	8.5	8.1	6.8	3.167	0.867
Self-grooming	9.2	2.4	5.5	1.5	4.6	3.203	0.408
NNOB <sup>3</sup>	29.6	19.2	5.5	13.7	12.2	9.537	0.280

Means with a different letter in the same row are significantly different ( $P < 0.05$ ). <sup>1</sup>Treatments: SM - 85% of whole corn grain with 15% of a pelleted supplement of soybean meal, CM - 85% of whole corn grain with 15% of a pelleted supplement of cottonseed meal plus soybean meal, SFM - 85% of whole corn grain with 15% of a pelleted supplement of sunflower meal plus soybean meal, DDG - 85% of whole corn grain with 15% of a pelleted supplement of Distillers Dried Grains plus soybean meal, DDGS - 85% of a whole corn grain with 15% of pelleted supplement of Distillers Dried Grains With Soluble plus soybean meal. <sup>2</sup>SEM: standard error of the mean. <sup>3</sup>NNOB: Non-nutritious Oral behavior.

In general, the protein source had no discernible impact on the behavior of the test subjects. The diets offered to the animals were highly similar in terms of particle size, energy, and protein composition. The average times spent on each activity were as follows: feed intake (96 min day<sup>-1</sup>), water intake (8.5 min day<sup>-1</sup>), and rumination (15 min day<sup>-1</sup> - standing and lying). These values are less than those reported by Neumann et al. (2018), who also assessed the behavior of animals fed WCG diets, recording a rumination time of 67.8 min day<sup>-1</sup>. Rumination time was also notably shorter than that observed by Carvalho et al. (2016). According to Loerch and Gorocica-Buenfil (2006), younger cattle exhibit much greater chewing capacity than older cattle. This fact may partially explain the differences in rumination time between studies. Mechanical damage of the pericarp

of the grain and the decrease of particle size during eating and rumination time are crucial to enable ruminal bacteria access to the starch and avoid the presence of whole kernels in the feces (Beauchemin et al., 1994; McAllister et al., 1994).

## Conclusion

The results of the present study indicate that 50% of the soybean meal fed to Nellore cattle on a whole grain flint corn diet can be replaced by cottonseed meal, sunflower meal, DDG and DDGS without affecting the nutritional and metabolic parameters of Nellore bulls fed WCG diets. The inclusion of DDG increased the DMI, but did not improve digestibility of dietary nutrients or N metabolism.

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## Declarations

### *Ethics Approval*

The trial was performed at the Faculdade de Agronomia e Zootecnia, Universidade Federal de Mato Grosso (UFMT; Cuiabá, Mato Grosso, Brazil), and it followed humane animal care and handling procedures based on UFMT guidelines (protocol number 23108.008091/2019-47).

## Conflict of interest

The authors declare that they have no conflict of interest.

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