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Total nutrient digestibility and small intestine starch digestion in Nellore and Angus young bulls fed a whole shelled corn diet

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Abstract

Eighteen Nellore and 18 Angus young bulls with BW of 381 ± 12 kg were randomly assigned into two feeding groups (whole shelled corn [WSC] or ground corn with silage [GC]) to evaluate the interaction of breed and diet on total nutrient digestibility, pancreatic α-amylase, and maltase activity and *SLC5A1* expression in the small intestine. Experimental diets (DM basis) included (a) a diet containing 30% corn silage and 70% GC and soya bean meal-based concentrate and (b) a diet containing 85% WSC and 15% of a soya bean meal- and mineral-based pelleted supplement. The treatments were Nellore fed GC diet; Nellore fed WSC diet; Angus fed GC diet; and Angus fed WSC diet. Total faecal collection for the digestibility trial occurred from day 48 until day 50 of the experimental period. Feeding the WSC diet reduced DM and NDF intake ($p < 0.01$). Angus had greater DM and nutrient intake in kg/day ($p < 0.01$). However, there was no breed effect on DM and nutrient intakes based on percentage of BW ($p > 0.19$). Angus had greater starch digestibility ($p = 0.03$) than Nellore. Cattle fed the WSC diet had greater DM, NDF and starch digestibility ($p < 0.01$) compared with those fed the GC diet. The activity of pancreatic α-amylase (U/g of protein) was greater in Nellore ($p < 0.01$) and was not affected by diet ($p = 0.52$). In duodenum, maltase activity (U/g of protein) was greater in bulls fed GC diet ($p = 0.02$). Expression of the gene *SLC5A1* was not affected by breed or diet ($p > 0.05$). In conclusion, Nellore had less capacity to digest starch. However, they did not have less pancreatic α-amylase and duodenal maltase activity compared to Angus. The use of the WSC diet increases DM and total nutrient digestibility.

KEYWORDS
amylase, *bos indicus*, maltase, post-rumen digestibility, SGLT1

1 | INTRODUCTION

Starch is an important nutrient in feedlot diets, providing large amount of digestible energy to cattle. Therefore, the evaluation of its use in the gastrointestinal tract of beef cattle is important to achieve high animal performance. According to Kreikemeier, Harmon, Brandt, Avery, and Johnson (1991), α-amylase action may limit small intestinal starch digestion in ruminants. However, it is unknown whether this potential limitation occurs similarly in *Bos taurus* and *Bos indicus* animals. Olbrich (1996) observed greater faecal

starch excretion in *B. indicus* animals feeding high grain diets. Some possible explanations for the lesser starch digestion in *B. indicus* cattle are differences in the gastrointestinal tract size, ruminal microbiome and post-ruminal enzymatic activity (Caetano et al., 2015).

Moreover, post-ruminal starch digestion is affected by the diet. Swanson, Matthews, Woods, and Harmon (2002) evaluated the effect of abomasal infusion of partially hydrolysed starch and reported that starch tended to decrease expression of the gene that encodes pancreatic α-amylase and also decreased the enzyme activity. In addition, differences in maltase activity may also be a factor that influences starch utilization (Bauer, Harmon, Bohnert, Branco, & Huntington, 2001; Guimaraes et al., 2007; Liao et al., 2010; Rodriguez et al., 2004).

Therefore, since diet is an important factor influencing small intestinal starch digestion, it is necessary to evaluate how the use of whole shelled corn (WSC) without forage affects starch digestion in finishing cattle. The use of WSC diets has increased in the last decade in Brazil and other South American countries in order to reduce land used to produce silage, and according to Oliveira and Millen (2014), 9.4% of the large feedlots in Brazil use WSC in their diets. In addition, this practice has also been used in small feedlots. Therefore, a comparison between the effect of a no forage diet and a conventional diet used in Brazilian feedlots is essential.

Our hypothesis was that Nellore bulls would have limited capacity to digest starch compared to Angus bulls because they have reduced pancreatic α-amylase and small intestine maltase activity and lesser *SLC5A1* mRNA expression which encodes the sodium-dependent glucose transporter 1 (SGLT1). In addition, we hypothesize that the lack of corn processing and forage in a diet with WSC would decrease starch digestibility in both breeds. Therefore, the objectives of this study were to test the interaction of breed, Nellore or Angus, and diet, WSC or ground corn with silage (GC), on nutrient digestibility, pancreatic α-amylase and small intestinal maltase activities, and duodenal *SLC5A1* mRNA expression.

2 | MATERIAL AND METHODS

The Ethics and Animal Welfare Committee of the Federal University of Lavras (Universidade Federal de Lavras; protocol 002/2013) approved the experimental procedures. The experiment was carried out in the Beef Cattle facility of the Animal Science Department of the Federal University of Lavras.

2.1 | Experimental design, animals and diets

Eighteen Nellore and 18 Angus young bulls ranging in age from 18 to 22 months and with BW of 381 ± 12 kg were housed in individual pens with individual feeders and automatic waterers. Treatments were arranged as a 2 × 2 factorial (two diets and two breeds). Experimental diets (DM basis) consisted of (a) a diet containing 30% corn silage and 70% GC and soya bean meal-based concentrate and (b) a diet containing 85% WSC and 15% of a soya bean meal- and mineral-based pelleted supplement. Flint corn was used in both diets, differing only by the processing (whole or ground). The treatments were Nellore fed GC diet (n = 9); Nellore fed WSC diet (n = 9); Angus fed GC diet (n = 9); and Angus fed WSC diet (n = 9). Bulls had a period of 28 days for adaptation to the facilities and the diets, and the total experimental period lasted for 81 days after adaptation. Performance data were published in Carvalho et al. (2016). The experimental diets were fed ad libitum, twice daily at 07:30 hr and 15:30 hr (Table 1).

2.2 | Digestibility trial

The digestibility trial occurred from 48 to 50 days of the experimental period, with total faecal collection performed for three consecutive days. Chemical analysis of the diets and faeces was carried out following the methods of the Association of Official Analytical Chemists (AOAC, 1990) (CP, AOAC 984.13; Ash, 942.05; EE, 920.39;

TABLE 1 Ingredients and chemical composition of experimental diets (DM basis)

	Ground corn diet	Whole shelled corn diet
Ingredient, % of DM		
Whole shelled corn[a]	–	85.0
Protein supplement[b]	–	15.0
Corn silage[a]	30.0	–
Ground corn[a]	58.0	–
Soya bean meal	10.0	–
Mineral supplement[c]	2.0	–
Chemical composition, % of DM		
Dry matter, % as is	57.3	87.8
Crude protein	12.7	14.6
Neutral detergent fibre (NDF)	24.0	11.1
NDF from forage	14.7	0.0
Non-fibre carbohydrate[d]	56.2	67.1
Starch	49.0	61.8
Ether extract	2.2	2.6
Metabolizable energy[e], Mcal/kg DM	2.59	2.97

[a]Flint corn. [b]Composition: corn, soya bean meal, cottonseed meal and soya bean hulls (concentrations not provided by the company); CP: 32.0%, TDN: 50.0%, Ca: 45 g/kg, Mg: 7.5 g/kg, P: 11 g/kg, Cu 104 mg/kg, Zn: 344 mg/kg, Se: 0.83 mg/kg, 30,500 IU/kg of vitamin A, 3,800 IU/kg of vitamin D_3, 134 IU/kg of vitamin E (Nutronbeef Grano Entero; Nutron Alimentos, Campinas, Brazil). [c]Assurance levels per kilogram of product: Ca: 170 g, P: 31 g, Na: 155 g, Zn: 2 mg, Cu: 396 mg, Mn: 515 mg, Co: 15 mg, I: 29 mg, Se: 5.4 mg, vitamin A: 111,000 IU, vitamin D3: 22,000 IU, vitamin E: 265 IU. [d]NFC calculated according to Sniffen et al. (1992). [e]ME = TDN (g/kg DM) × 4.4 × 0.82

Moisture, 934.01). Non-fibre carbohydrates were obtained according to Sniffen, O'Connor, Van Soest, Fox, and Russell (1992), NDF according to Van Soest, Robertson, and Lewis (1991), and starch was analysed according to Hall (2008). Total apparent digestibility of DM, OM, CP, ether extract (EE), non-fibre carbohydrates (NFC), NDF and starch were calculated as follows: (nutrient intake—nutrient excretion in faeces/nutrient intake) × 100. TDN was calculated using the following equation: TDN = %DCP + %DNDF + %DNFC + (2.25 × % DEE). Diet passage rate (Kp) was calculated according to the NRC (2001), using DMI (% of BW) and percentage of concentrate in the diet.

Faecal pH was analysed using a pH meter (Model HI 208—Splabor, Presidente Prudente, SP, Brazil) according to Turgeon, Brink, and Britton (1983), after adding 100 ml of distilled deionized water into 15 g of fresh faeces.

2.3 | Animal slaughter, tissue sample collection

At the end of the experiment, animals were slaughtered by cerebral concussion and exsanguination of the jugular vein followed by removal of the skin and evisceration. The small intestine was removed, and its total length (from pyloric valve to ileal-caecal junction) was measured. Samples from duodenum, jejunum and ileum were removed to analyse *SLC5A1* mRNA expression, the gene that encodes the sodium-dependent glucose transporter 1 (SGLT1), and to determine maltase activity and morphometry of intestinal villi. Samples of the pancreas were collected to measure α-amylase activity.

Intestinal samples were collected according to Soto-Navarro et al. (2004). Briefly, one-metre sections of duodenum (0.5–1.5 m distal to the pyloric junction), jejunum (middle of the first half non-duodenal small intestine) and ileum (middle of the second half non-duodenal small intestine) were taken after removing the digesta. All instruments used for the tissue collection were sterile. Samples were wrapped in aluminium foil, frozen and transported in liquid nitrogen and stored at −80°C.

For morphological analyses, the duodenum, jejunum and ileum samples were dehydrated in ethanol solutions (70, 80, 90, 95 and 100°GI), diaphanized in xylol PA (two baths of 30 min each), embedded in histological paraffin and cut into a 5-μm sections in a rotating manual microtome MRP-09 (LUPETEC—Lupe Industry of Laboratory Equipment Technology, São Carlos, SP, Brazil). The sections were stained with haematoxylin–eosin for determination of villus height and crypt depth in the different segments of the small intestine. Villus height and crypt depth were obtained by images captured with a digital camera coupled to a light microscope Olympus CX31 RT F (Olympus Corporation, Tokyo, Japan). The images were then analysed by Cell B Imaging software for life science microscopy (Olympus and Olympus soft imaging solutions GmbH, Muenster, Germany).

2.4 | Enzyme activity and gene expression analyses

Pancreatic tissue (100 mg) was collected and homogenized in 0.5 ml of the Amylase Assay Buffer, and duodenal and jejunal (300–600 mg) tissues were collected and homogenized in Maltase Assay Buffer in a 1:10 proportion (one part of sample to nine parts of buffer) with a T 25 basic ULTRA-TURRAX® (IKA®, Wilmington, NC, USA). The enzyme activity analyses were performed using commercial kits (MAK009 for amylase and MAK123 for maltase; Sigma-Aldrich, St. Louis, MO, USA) and measured with a Multiskan GO® spectrophotometer (Thermo Scientific, Waltham, MA, USA) according to manufacturer's specifications. Enzyme activity data were expressed as units per gram of protein, where protein concentrations were determined according to Lowry, Rosebrough, Farr, and Randall (1951).

The design of *SLC5A1* and reference gene primers (*β-actin* and *GAPDH*) were performed using sequences registered and published in the GenBank public data bank, a National Center for Biotechnology Information (NCBI) platform (Table 2). For the gene characterization, the open-reading frames (ORF) of the selected sequences were obtained using the ORFinder tool from NCBI and the sequences of the codified proteins were obtained using the translate tool from the ExPASy protein bank. The primers were designed using the OligoPerfect Designer software (Invitrogen, Karlsruhe, Germany) and synthesized by Invitrogen (Carlsbad, CA, USA).

Total RNA was extracted from the intestine samples using QIAzol (QIAGEN, Valencia, CA, USA) and treated with DNA-free DNase (Ambion, Austin, TX, USA) according to the manufacturer's instructions. For the analysis of the 28S and 18S bands of the rRNA, the total RNA was electrophoresed in a 1.0% (m/v) agarose gel, stained with GelRed nucleic acid gel stain (Biotium, Hayward, CA) and visualized with a UVItec FireReader XS D-77Ls-20M (UVItec, Cambridge, UK). The RNA quantity (ng/μl) and quality (260/280 and 260/230) were quantified using a spectrophotometer (DeNovix DS-11/DS-11 + Spectrophotometer). The cDNA synthesis was

TABLE 2 Sequences (5′–3′) and efficiencies of the primers used in quantitative real-time PCR

Symbol	Forward (F) and Reverse (R)	Access number	R2	Efficiency
SLC5A1[a]	F ACCGCCCTTTACACAATCAC	NM_001103222.1	0.987	99
	R AGGATGAAAGACCCCAGGAG			
β-actin	F GTCCACCTTCCAGCAGATGT	BC142413.1	0.996	100
	R CAGTCCGCCTAGAAGCATTT			
GAPD[b]	F CGACTTCAACAGCGACACTC	NM_001034034.1	0.998	101.42
	R TTGTCGTACCAGGAAATGAGC			

[a]Gene which encodes the sodium-dependent glucose transporter 1. [b]Glyceraldehyde-3-phosphate.

performed using the HighCapacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, and then, samples were stored at −20°C. Quantitative gene expression analysis was performed by reverse-transcription quantitative PCR (RT–qPCR) using the Mastercycler ep realplex (Eppendorf) with the SYBR Green detection system (Applied Biosystems). The RT–qPCR programme was as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. For each reaction, 1.0 μl cDNA (10 ng/μl), 0.3 μl of each primer (1.5 μM; forward and reverse) and 5.0 μl SYBR Green Master Mix were combined in a 10.0-μl/sample final volume in a 96-well MicroAmp Optical plate (Applied Biosystems). The RT–qPCR analyses for each studied gene were performed using cDNAs from nine biological replicates per treatment, with three technical replicates per biological replicate. The results were normalized against the reference genes *β-actin* and *GAPDH* using the threshold cycle (CT) method. The CT was determined by the total number of cycles using the comparative CT method. As one requisite of this method, a validation assay was performed to demonstrate that the amplification efficiencies of the target and reference genes were approximately the same. Standard curves were generated for the studied genes with the following dilutions: 1:625, 1:5, 1:315, 1:25 and 1:125. The relative expression levels were calculated according to the method described by Pfaffl (2001) which is based on Ct values that are corrected for the amplification efficiency for each primer pair.

2.5 | Statistical analyses

A Shapiro–Wilk test was performed to check the normality of the data. When the data did not have a normal distribution, they were transformed using PROC RANK from SAS 9.4 (SAS Inst., Cary, NC, USA).

The data were analysed as a completely randomized design, and animal was considered the experimental unit. Apparent digestibility, gene expression and enzyme activity were analysed using the MIXED procedure of SAS 9.4 with diet, breed and diet × breed interaction as fixed effects. The covariance structure was chosen according to the Bayesian information criterion, by comparing four covariance structures for each variable (compound symmetry, autoregressive order one, heterogeneous autoregressive order one and unstructured), and the structure that yielded the smallest Bayesian information criterion was used. The least squares means (LSMEANS) statement was used to calculate the adjusted means for treatments and, when there was significant interaction, Tukey's test were used to compare treatments. Differences were considered statistically significant when $p \leq 0.05$ and tendencies were discussed when $0.05 < p \leq 0.10$.

TABLE 3 Intake and digestibility of Nellore and Angus young bulls fed a ground corn diet (GC) or a whole shelled corn diet (WSC)

	Nellore		Angus			*P*-value		
Item	GC[a]	WSC[b]	GC	WSC	*SEM*	Breed	Diet	B × D
Intake, kg/day								
DM	11.0	7.0	13.0	9.6	0.65	<0.01	<0.01	0.53
NDF	2.6	0.8	3.1	1.1	0.19	<0.01	<0.01	0.69
NFC	6.2	5.0	7.4	6.9	0.39	<0.01	0.02	0.31
Starch	5.1 b	4.2 c	6.3 a	6.6 a	0.34	<0.01	0.28	0.05
Intake, %/BW								
DM	2.3	1.6	2.4	1.8	0.12	0.33	<0.01	0.59
NDF	0.6	0.2	0.6	0.2	0.03	0.67	<0.01	0.42
Starch	1.11	1.08	1.16	1.20	0.069	0.19	0.96	0.58
Apparent digestibility, %								
DM	72.5	82.5	72.3	83.7	1.90	0.77	<0.01	0.69
OM	73.6	83.4	73.3	84.3	1.87	0.87	<0.01	0.73
NDF	60.2	79.6	54.6	81.7	2.99	0.52	<0.01	0.16
NFC	86.8	89.8	88.7	89.8	1.38	0.45	0.12	0.44
CP	72.1	79.0	71.2	80.3	1.66	0.89	<0.01	0.47
EE	58.3	80.1	63.5	82.7	0.05	0.42	<0.01	0.78
Starch	86.3	92.3	90.5	93.3	1.28	0.03	<0.01	0.17
TDN[c]	72.0	81.9	71.6	82.8	1.79	0.88	<0.01	0.69

Note. Different online letters in the row indicate statistically significant differences according to Tukey's test.

[a]GC = 58% of ground corn, 30% corn silage, 10% of soya bean and 2% mineral supplement. [b]WSC = 85% whole shelled corn with 15% of a pelleted protein, mineral and vitamin supplement. [c]TDN was calculated using the following equation: TDN = %DCP + %DNDF + %DNFC + (2.25 × %DEE).

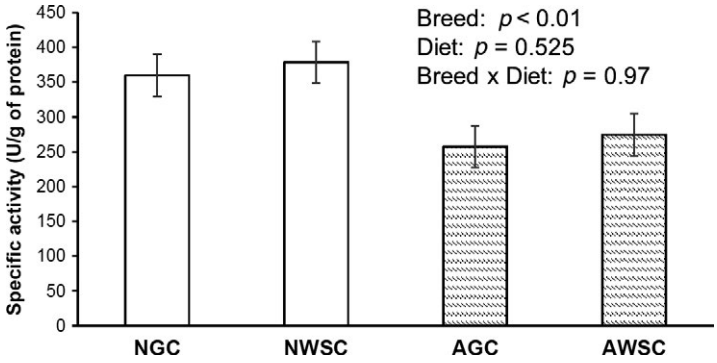

FIGURE 1 Specific activity (U/g of protein) of α-amylase pancreatic in Nellore (N) and Angus (A) young bulls fed a ground corn diet (GC; 58% ground corn, 30% corn silage 10% soya bean and 2% mineral) or a whole shelled corn diet (WSC; 85% whole shelled corn with 15% protein/mineral/vitamin supplement). *SEM* = 29.9

3 | RESULTS

Intake of DM, NDF and NFC and starch were greater in Angus compared to Nellore bulls (Table 3; $p < 0.01$). However, when DM and nutrient intake were analysed as percentage of live weight, there was no breed effect ($p > 0.05$). Bulls fed WSC diet ate less DM, NFC and NDF when analysed in kg/day or percentage of live weight. However, there was a breed × diet interaction for starch intake. In this case, Nellore bulls fed WSC diet ate less starch than Nellore bulls fed GC diets, while starch intake of Angus bulls was not affected by diet. Cattle fed the GC diet had a greater predicted passage rate ($p < 0.01$, 4.74%/hr vs. 3.20%/hr) compared to cattle fed the WSC diet. Digestibility of DM, OM, NDF, EE, CP, TDN and starch was greater in bulls fed the WSC diet ($p < 0.01$). Only starch digestibility was affect by breed ($p = 0.03$), being greater in Angus than in Nellore bulls, regardless of diet.

Despite the greater total starch digestibility in Angus bulls, their pancreatic α-amylase specific activity was lower compared to Nellore bulls (Figure 1). These data are presented in U/g of protein because there was no effect of breed on the average pancreas weight (0.372 g, $p = 0.47$). The duodenum of bulls fed the WSC diet had less specific activity of maltase compared to bulls fed the GC diet (Figure 2). On the other hand, in jejunum, there was no effect of diet and breed on maltase activity. However, there was greater maltase activity ($p < 0.001$) in the jejunum than in the duodenum, regardless of breed and diet. In addition, there was no effect of diet or breed on the abundance of *SLC5A* mRNA (Figure 3) in the duodenum or the jejunum of these animals.

Diets used in this study did not alter villus height or crypt depth in the different segments of the small intestine (Table 4). Furthermore, there was no effect of breed or diet on faecal starch concentration (Table 5). However, overall starch excretion (kg/day) was lesser ($p < 0.01$) when bulls were fed the WSC diet. Moreover, faecal pH was greater ($p < 0.01$) when bulls were fed the WSC diet.

4 | DISCUSSION

The greater intakes of DM, NDF, NFC and starch in Angus bulls were due to the greater body weight in the period that the digestibility assay was performed (538 vs. 449 kg for the Angus vs. the Nellore bulls, respectively, $p < 0.01$). With the greater starch intake by Angus bulls, it was expected that the amount of starch reaching the small intestine would be greater in this breed as compared to Nellore. Because the action of α-amylase may be limiting for intestinal use of starch (Kreikemeier et al., 1991), greater starch reaching the small

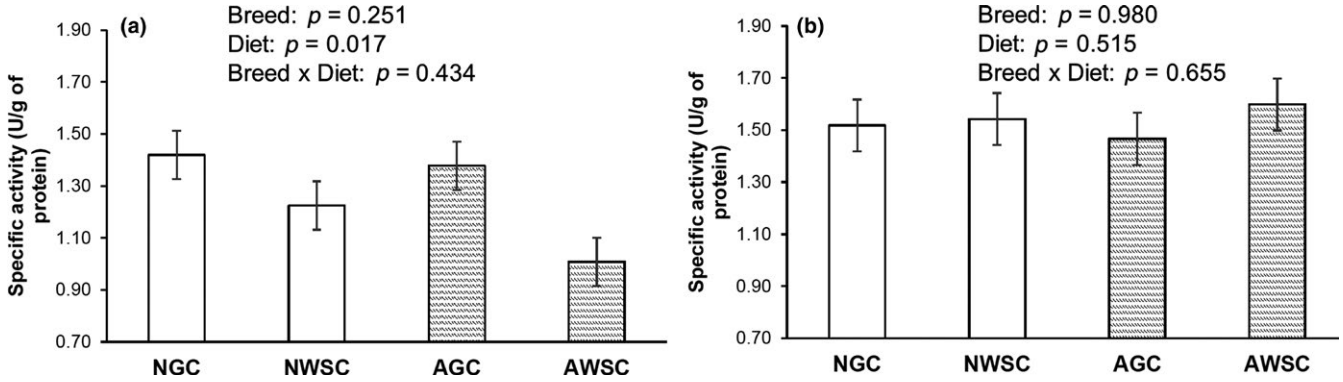

FIGURE 2 Specific activity (U/g of protein) of maltase in the duodenum (a) and jejunum (b) in Nellore (N) and Angus (A) young bulls fed a ground corn diet (GC; 58% ground corn, 30% corn silage, 10% soya bean and 2% mineral) or a whole shelled corn diet (WSC; 85% whole shelled corn with 15% protein/mineral/vitamin supplement). *SEM* (a) = 0.117; *SEM* (b) = 0.125

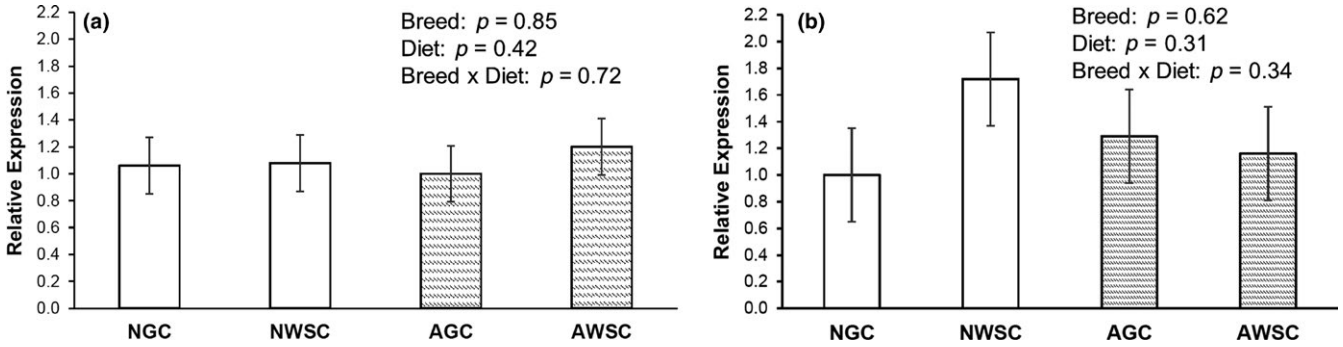

FIGURE 3 Relative expression *SLC5A1* gene in the duodenum (a) and jejunum (b) in Nellore (N) and Angus (A) young bulls fed a ground corn diet (GC; 58% ground corn, 30% corn silage, 10% soya bean and 2% mineral) or a whole shelled corn diet (WSC; 85% whole shelled corn with 15% protein/mineral/vitamin supplement). *SEM* (a) = 0.209; *SEM* (b) = 0.358

TABLE 4 Morphometry of intestinal villi and depth of intestinal crypt in different segments of the small intestine (duodenum, jejunum and ileum) of Nellore and Angus young bulls fed a ground corn diet (GC) or a whole shelled corn diet (WSC)

	Nellore		Angus			*P*-value		
Item	GC[a]	WSC[b]	GC	WSC	*SEM*	Breed	Diet	B × D
Height, μm								
Duodenum	952	961	929	941	28.3	0.42	0.71	0.95
Jejunum	972	983	992	978	19.5	0.72	0.90	0.53
Ileum	792	760	778	723	33.1	0.48	0.21	0.69
Crypt depth, μm								
Duodenum	669	677	676	686	23.1	0.81	0.78	0.90
Jejunum	625	627	630	650	15.0	0.44	0.55	0.50
Ileum	568	552	607	578	18.4	0.08	0.24	0.71

[a]GC = 58% of ground corn, 30% corn silage, 10% of soya bean and 2% mineral supplement. [b]WSC = 85% whole shelled corn with 15% of a pelleted protein, mineral and vitamin supplement.

TABLE 5 Starch in faeces, faecal output and faecal pH of Nellore and Angus young bulls fed a ground corn diet (GC) or a whole shelled corn diet (WSC)

	Nellore		Angus			*P*-value		
Item	GC[a]	WSC[b]	GC	WSC	*SEM*	Breed	Diet	B × D
Starch in faeces, %	23.7	24.0	18.4	23.7	0.02	0.21	0.21	0.27
Faecal output, kg/day	3.02	1.21	3.59	1.57	0.263	0.06	<0.01	0.65
Starch in faeces, kg/day	0.710	0.307	0.647	0.392	0.0763	0.87	<0.01	0.28
Faecal pH	5.15	5.88	5.24	5.97	0.095	0.35	<0.01	0.97

Note. Faecal output = faecal production.
[a]GC = 58% of ground corn, 30% corn silage, 10% of soya bean and 2% mineral supplement. [b]WSC = 85% whole shelled corn with 15% of a pelleted protein, mineral and vitamin supplement.

intestine would have a negative effect on efficiency of post-ruminal starch utilization.

Bulls fed the WSC diet had lower intake of DM, NDF and NFC, which can be explained by the greater nutrient digestibility and energy content in this diet. In this sense, the greater digestibility resulted in better utilization of dietary energy by the animals, which would allow them to meet their energy requirements with less feed. In other words, digestibility and, therefore, energy was likely regulating intake. According to Allen, Bradford, and Oba (2009), diets with high volatile fatty acids (VFA) production, especially propionate, increase ATP production in liver and activates the animal's satiety centre, reducing intake. Although VFA production was not analysed in this study, the lower ruminal pH values observed for animals fed the WSC diet (5.74 vs. 6.22; Carvalho et al., 2016) indicate

greater ruminal fermentation for the WSC diet. Moreover, according to Furlan et al. (2006), grain-based diets cause less tension on the rumen mechanoreceptors than forage-based diets, reducing ruminal motility and consequently intake and passage rate of DM and nutrients, which also explain the lower DM intake for bulls fed the WSC diet.

Goulart and Nussio (2011) recommended that the inclusion of physically effective NDF (peNDF) from forage in beef cattle diets should be between 10% and 18% to ensure the minimum requirements for rumen health. One role of forage in the rumen is to increase motility and rumination, which are important because of their direct correlation with saliva production and passage rate. Therefore, the greater intake of DM and nutrients in bulls fed the GC diet may also be a result of the long particles of corn silage increasing rumination and salivation by the animal (Nagaraja & Lechtenberg, 2007).

The greater digestibility of DM, NDF, CP and starch of the WSC diet can be explained by the possibility of greater retention time in the rumen for this diet, as previously discussed. Owens, Secrist, Hill, and Donald (1997) reported that starch digestibility and net energy were greater in WSC diet compared to rolled corn diets. The authors justified this difference due to the lower forage content in WSC diets when compared to diets with processed corn. Forage in the diet is important for rumination and gastrointestinal tract motility, and a low level of forage can decrease motility. The WSC diets with lower forage in the present study may have decreased motility and passage rate allowing for greater retention time in the rumen and increased time for fermentation. In addition, in the present study, the forage in the ground corn diet was the main source of peNDF, which likely increased motility and passage rate of this diet compared to the WSC diet.

Despite the greater total starch digestibility in Angus compared to Nellore bulls, the lower pancreatic α-amylase activity can be explained by the greater starch intake and possible greater flow to the small intestine in Angus bulls. According to Swanson et al. (2002), there may be an inverse relationship between starch flow and mRNA expression and activity of pancreatic α-amylase in ruminants. The authors evaluated the effect of abomasal infusion of partially hydrolysed starch and casein and found that animals receiving starch had less α-amylase mRNA expression and less pancreatic α-amylase activity. Therefore, it appears that the reduced starch digestion in *B. indicus*, reported in the literature (Olbrich, 1996), does not occur due to low pancreatic α-amylase activity.

Harmon (1992) reported that the main substrates in ruminants responsible for affecting insulin and glucagon concentrations in the blood are VFA, particularly butyrate and propionate. Therefore, the greater starch digestibility in Angus bulls could result in increased VFA production in the rumen, particularly propionate, which may lead to enhanced circulating insulin levels. In addition, the large amount of starch reaching the small intestine and resulting glucose absorption in Angus bulls would increase insulin secretion as well. It is assumed that circulating insulin levels may have a negative effect on pancreatic α-amylase activity, since the function of insulin is to maintain blood glucose levels within a normal range. According to O'Brien and Granner (1991), insulin is able to act in many body tissues and organs, including the pancreas. Hamden, Jaouadi, Carreau, Aouidet, and Elfeki (2011) observed that isoflavones enhanced insulin secretion and inhibited pancreatic α-amylase activity in diabetic rats. Söling and Unger (1972) also found that injections of insulin in diabetic rats may reduce pancreatic α-amylase activity. After insulin injection in these studies, pancreatic α-amylase activity decreased along with lipase, trypsinogen and chymotrypsinogen enzymatic activity.

Rodriguez et al. (2004) and Guimaraes et al. (2007) observed greater maltase activity in the jejunum compared to duodenum and ileum, which agrees with results found in this study, where maltase activity was greater in the jejunum than in the duodenum, regardless of breed or diet. In addition, Kreikemeier, Harmon, Brandt, Nagaraja, and Cochran (1990) commented that diet has little or no effect on maltase and isomaltase activity in the small intestine. In the present study, the GC diet likely had a positive effect on maltase activity in the duodenum due to a possible greater flow of starch to the small intestine, considering that passage rate may have been faster in bulls fed the GC diet. On the other hand, starch reaching the small intestine of bulls fed the WSC diet in the current study was probably more intact due to the use of flint corn. Rodriguez et al. (2004) observed greater maltase activity when steers received an abomasal infusion of starch and glucose, regardless of location in the intestine. In another study, Swanson et al. (2000) did not observe an effect of starch levels on maltase activity. The breed effect found for pancreatic α-amylase activity in the current study was not observed for maltase activity which could indicate that the greater digestion of starch by Angus bulls was also likely not due to differences in maltase activity.

According to Harmon and McLeod (2001), the small intestine of cattle fed high grain diets may change due to the considerable amounts of glucose reaching the lumen, while in cattle fed forage-based diets, the amount of glucose in gastrointestinal tract is low. In the present study, despite differences in starch and NDF content, diet did not influence villi height or crypt depth in the different segments of the small intestine, indicating that bulls fed either diet had the same surface and intestinal absorptive capacity.

The SGLT1 carrier protein has a high affinity for monosaccharides, especially glucose and galactose (Liao et al., 2010). These authors infused hydrolysed starch into the rumen or the abomasum of bovine and observed greater abundance of *SLC5A* mRNA in the duodenal epithelium when hydrolysed starch was infused into the rumen, and only the ileal epithelium responded to the infusion of hydrolysed starch in the abomasum. These results show that differences between Nellore and Angus bulls in total starch digestibility were not likely due to the capacity of glucose absorption by the intestine, because there was no effect of breed on the expression of *SLC5A* in intestinal villi.

Despite differences found in starch digestibility, there was no effect of breed or diet on faecal starch concentration, which has been used as a tool by feedlot nutritionists to evaluate starch digestion using equations outlined by Zinn, Barreras, Corona, Owens, and Ware equations (2007). However, according to the

current study, only measuring starch concentration in faeces may not be a good method to calculate starch digestibility when WSC diets are used.

5 | CONCLUSIONS

Results of the current study indicate that Nellore bulls have lesser capacity to digest starch than Angus bulls. However, Nellore cattle do not have less pancreatic α-amylase, duodenal maltase activity or *SLC5A1* mRNA expression. The use of a whole shelled flint corn diet without forage increases digestibility of DM and starch, compared to a diet with corn silage and a ground corn-based concentrate.

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CONFLICT OF INTEREST

No competing financial, personal or professional interests have influenced writing of this paper. This manuscript has not been submitted anywhere else for possible publication.

AUTHORS' CONTRIBUTIONS

M.M.L. and M.L.C. designed research and got the financial support; J.R.R.C., P.D.T and A.C.R. carried out the research; J.R.R.C., J.C.O.D., T.T.S.G. and S.F.C contributed in laboratory analyses; J.R.R.C., J.P.S. and M.M.L. analysed data; and J.R.R.C., J.P.S and M.M.L. wrote the manuscript.

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