R. Bras. Zootec., 49:e20200133, 2020
https://doi.org/10.37496/rbz4920200133

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Effects of corn processing on piglet performance and intestinal parameters

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ABSTRACT - The objective of the present study was to compare the effects of corn processing on performance and intestinal parameters of weanling piglets. To accomplish our goal, 42 piglets (21 days-old, 7.18±1.0 kg body weight) were randomly allocated (seven pens/treatment; three pigs/pen) to one of two treatments: ground corn – corn ground through a hammer mill with a 1.0-mm screen after being ground by a hammer mill with a 3.0-mm screen, and extruded corn – corn was wet extruded after being ground by a hammer mill with a 2.0-mm screen and, after extrusion, it was ground by a hammer mill with a 1.0-mm screen. In both methods, corn from the same batch was used. Results were considered statistically significant when P<0.05 and a tendency when P<0.10. Analyses were performed using the GLIMMIX procedure of SAS. Pigs fed ground or extruded corn diet had similar growth performance. Duodenum and jejunum of pigs fed extruded corn had greater villus height compared with those of pigs fed ground corn. Crypt depth was not influenced by corn processing. Duodenum and jejunum of pigs fed extruded corn had greater villus:crypt ratio compared with those of pigs fed ground corn. In the duodenum, the expression of zonula occludens-1 (ZO-1) was greater, and the expression of Occludin-1 tended to be greater in pigs fed the extruded corn diets. There was no effect of corn processing on ZO-1 and Occludin-1 expression in the jejunum, nor TGF-β1 expression in duodenum and jejunum. There was no effect of the type of corn processing on colonic total bacteria or *Enterobacteriaceae* and *Lactobacillus* abundance. Piglets fed diets with ground corn or extruded corn have similar growth performance. However, piglets fed diets with extruded corn present improved intestinal morphology and tight junction protein expression compared with those fed ground corn.

Keywords: extrusion, grinding, morphology, pigs

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Received: June 7, 2020
Accepted: October 18, 2020

How to cite: Scalco, T.; Valini, G. A. C.; Silva, C. B.; Calderano, A. A.; Rodrigues, G. A.; Campos, P. H. R. F.; Mantovani, H. C.; Sanglard, L. M. P. and Rocha, G. C. 2020. Effects of corn processing on piglet performance and intestinal parameters. Revista Brasileira de Zootecnia 49:e20200133. https://doi.org/10.37496/rbz4920200133

1. Introduction

The increase in post-weaning gut disorders is one of the main challenges for the pig industry following the ban on in-feed antibiotic growth promoters in the EU. At weaning, milk is replaced with diets containing cereals with complex carbohydrates that cannot be adequately digested by the immature digestive system of early age piglets (De Lange et al., 2010). Grinding and extrusion are processes that induce changes in feed ingredient structure and might facilitate digestion. Thus, these processes may improve gastrointestinal tract development and digestive capacity resulting in greater performance.

Grinding reduces particle size, increasing the specific surface area, thereby improving enzymatic degradation and digestion (Mavromichalis et al., 2000; De Jong et al., 2016). It has been suggested that a reduction in corn particle size as small as 400 µm may improve feed efficiency and nutrient digestibility (Healy et al., 1994; Rojas and Stein, 2015), especially for piglets. Extrusion might also provide further benefits: the heat, humidity, and pressure of the extrusion induce changes on the vegetable cell matrix, facilitating digestion and absorption (Sun et al., 2006). Extrusion also improves the flavor and palatability of diets for weanling pigs (Solà-Oriol et al., 2009).

Thus, extruded corn has been used in starter diets due to its potential to improve the performance of weaned piglets. However, after extrusion, corn is milled in smaller sieves, which makes it difficult to determine if the quality of the ingredient is due to the extrusion or fine-milling process. Performance or digestibility improvements in piglets due to corn extrusion have been previously reported (Medel et al., 1999; Rojas et al., 2016), whereas other reports found no benefits (Oliveira et al., 2011; Liu et al., 2014). However, none of these authors specifically investigated particle size of milled vs. extruded corn, nor investigated the possible effects of processing on intestinal development.

Our hypothesis was that ground corn and extruded corn with similar particle sizes would have similar effects on performance and gut parameters of newly weaned piglets. Therefore, the objective of the present study was to compare the effects of corn processing on performance and intestinal parameters of weanling piglets.

2. Material and Methods

All experimental procedures abided by the ethical principles in animal research (CONCEA, 2017) and were reviewed and approved by the institutional Ethical Committee on Animal Use, case no. 085/2018. The experiment was conducted on an experimental farm located in Viçosa, MG, Brazil (20°45'14" S and 42°52'55" W).

2.1. Animals and housing

A total of 42 castrated male piglets (AGPIC 415 × Camborough, Agroceres PIC, Patos de Minas, MG, Brazil), weaned at 21 days old and with 7.18±1.0 kg body weight (BW), were used in a 14-day feeding trial. Pigs were allotted to suspended pens (0.35 m^2/pig) in an environmentally controlled nursery building (kept at 26 °C and 60% humidity) with 24-h day lighting. Each pen was equipped with a one-sided, stainless steel self-feeder and a nipple drinker that allowed access to mash diet and water *ad libitum*.

2.2. Experimental design and diets

Pigs were randomly allotted to pens according to their initial BW (two BW blocks), and pens were randomly assigned to one of the two corn processing methods in a completely randomized block design. There were seven replicated pens per treatment with three pigs per pen. Dietary treatments consisted of two types of corn: ground corn – corn ground through a hammer mill (1.0-mm screen; Ferraz M-1000, Ribeirão Preto, SP, Brazil) after being ground by a hammer mill with a 3.0 mm screen; and extruded corn – corn was ground by a hammer mill (2.0-mm screen) and wet extruded using a single screen extruder (Ferraz E-240 R, Ribeirão Preto, SP, Brazil), and then ground again through a hammer mill (1.0-mm screen) after extrusion. In both methods, corn from the same batch was used.

Particle size distribution and mean particle size were determined using ABNT sieves with numbers 5, 10, 16, 30, 50, and 100, and a pan. Briefly, sample was placed on top of the test sieves, which were stacked from the greater to the smallest aperture size. The test sieves were then located in a vibratory sieve shaker for 10 min. The feedstuff material in each of the test sieves was recorded and weighed for calculations of particle size distribution and mean particle size (Zanotto and Bellaver, 1996), and the water absorption index (WAI) was determined as described by Anderson et al. (1969) (Table 1).

Diets were formulated following the ideal amino acid profile recommended by the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017; Table 2), with the respective corn processing method.

Table 1 - Physical composition of diets and corn used in ground and extruded corn treatments

Item	Ground corn	Extruded corn
Corn		
Water absorption index (%)	104.6	231.1
Particle size (µm)	428	414
SD of particle size	1.36	1.48
Diet		
Particle size (µm)	529	508
SD of particle size	1.81	1.85

SD - standard deviation.

Table 2 - Ingredients and calculated nutritional composition of the diet offered from 21 to 35 days old

Ingredient	g/kg
Corn (ground or extruded)[1]	401.6
Soybean meal	220.1
Dried whey	150.0
Micronized soybean	130.0
Sugar	30.0
Soybean oil	17.0
Dicalcium phosphate	15.2
Limestone	9.9
Fumaric acid	5.0
Salt	3.4
Zinc oxide	2.5
Mineral-vitamin premix[2]	2.0
Copper sulfate	0.6
L-lysine, 78%	5.0
L-threonine	3.0
DL-methionine	2.6
L-valine	1.5
L-tryptophan	0.6
Calculated nutritional composition (g/kg)	
Metabolized energy (kcal/kg)	3400
Crude protein	210.0
SID lysine	14.5
SID threonine	9.7
SID Met + Cys	8.1
SID tryptophan	2.7
SID valine	10.0
Calcium	8.5
Available phosphorus	5.0
Lactose	112.5
Sodium	2.8

SID - standardized ileal digestible.
[1] Experimental diets were obtained by using ground corn or extruded corn.
[2] Vitamin-mineral premix provided the following per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,250 IU; vitamin E, 65 IU; vitamin K, 3 mg; thiamine, 2.25 mg; riboflavin, 6 mg; pyridoxine, 2.25 mg; vitamin B12, 27 mcg; folic acid, 400 mcg; biotin, 150 mcg; pantothenic acid, 22.5 mg; niacin, 45 mg; copper, 10 mg; iodine, 1.5 mg; iron, 100 mg; manganese, 40 mg; selenium, 0.3 mg; zinc, 100 mg.

2.3. Performance and sample collection

Throughout the trial, feed intake and feed wastage were collected and weighed daily to determine average daily feed intake (ADFI). At 21 and 35 days old, pigs were individually weighed to estimate final BW, average daily gain (ADG), and feed conversion. Incidence of diarrhea was visually assessed at 26, 29, 32, and 35 days and ranked as 0 = absence or 1 = presence for each pen.

At 35 days, one pig (no fasting) per pen with BW closest to the pen average was euthanized. The pig was rendered unconscious using head-only electrical stunning (240V, 1.3A), immediately exsanguinated, dehaired, and eviscerated. Ten grams of colon digesta were immediately removed and stored at −20 °C for bacterial genomic DNA extraction. From the duodenum (10 cm from pylorus) and jejunum (mid-section), fragments of 2.0 cm were removed (Yang et al., 2014) for morphological evaluation. Another 2 cm of duodenum and jejunum were collected and immediately frozen in liquid nitrogen and stored at −80 °C for RNA extraction and gene expression analysis.

2.4. Histology

The intestinal sections (duodenum and jejunum) were washed in physiological solution and then placed in 4.0% paraformaldehyde fixative solution for 4 h at room temperature. Next, tissues were washed with a PBS solution, then dehydrated in a graded ethanol series (70-95%), and embedded in historesin (Leica Biosystems Inc, Nussloch, Germany). The slices were semi-serial, using one in 10 slices. After sectioning, the resulting 3-µm-thick slices were stained with toluidine blue and examined using a light microscope (Upright microscope BX53, Olympus Corporation). Quantitative histomorphometry measurements of intestinal villus and crypt length measurements were performed using ImageJ software (ImageJ 1.50i; java1.6.0_20*;* National Institutes of Health, USA). Ten fields were randomly photographed with a 40-objective lens to measure fifteen villus heights and their respective crypt depths. Villus to crypt ratios using the length data were then calculated. All measurements were made by a single individual.

2.5. RNA extraction, cDNA synthesis, and RT-qPCR

Total RNA extraction was performed from 50 mg of duodenum and jejunum using TRIzol® (Invitrogen™) according to the manufacturer's instructions. The final precipitate was rehydrated with 30 µL of UltraPure® DNase/RNase-Free water. The RNA concentration was estimated using a NanoVue Plus TM spectrophotometer (GE Healthcare, Munich, Germany), with A260:A280 ratios between 1.8 and 2 serving as the purity control. The quality and integrity of the extracted RNA were verified using a 1% agarose gel. Subsequently, the samples (100 ng) were reverse-transcribed into cDNA using the GoScript Reverse Transcription (RT) Kit (Promega, Madison, WI, USA), following the manufacturer's recommendations. The cDNA samples were stored at −20 °C until use in a real-time quantitative polymerase chain reaction (RT-qPCR).

Primers for amplification of the target and endogenous gene fragments were designed using the PrimerQuest software provided by Integrated DNA Technologies, Inc. (Coralville, IA) using the nucleotide sequences obtained from the GeneBank database (https://www. ncbi.nlm.nih.gov) (Table 3).

The β-actin, 18S-ribosomal RNA (18S), and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) genes were tested as reference genes to be used in standardization analyses to minimize possible variations in the amount of initial mRNA and the efficiency of reverse transcription. The selection of the reference gene was based on the amplification efficiency of candidate genes from the efficiency calculation for each primer pair using the formula $E = 10^{(-1/\text{slope of the line})} - 1$, in which E is the reaction efficiency (Pfaffl, 2001). Due to its lack of difference in mRNA expression between treatments ($P<0.10$), 18S was chosen as the reference gene.

The RT-qPCR analyses were performed in duplicate in an ABI Prism 7300 Sequence Detection Systems thermocycler (Applied Biosystems, Foster City, CA, USA) using the relative quantification method

Table 3 - List of primers sequences for gene expression analysis by RT-qPCR

Target gene[1]	GenBank number	Primer sequence[2]	Size (bp)
Ocludin	NM_001163647.1	F: 5'TCCTGGGTGTGATGGTGTTC3' R: 5'CGTAGAGTCCAGTCACCGCA3'	145
ZO-1	XM_003353439.2	F: 5'AAGCCCTAAGTTCAATCACAATCT3' R: 5'ATCAAACTCAGGAGGCGGC3'	130
TGF-β1	NM_214015.1	F: 5'GGACCTTATCCTGAATGCCTT3' R: 5'TAGGTTACCACTGAGCCACAAT3'	133

[1] ZO-1 - zonula occludens 1; TGF-β1 - transforming growth factor beta 1.
[2] F and R indicate forward and reverse primers, respectively.

and applying the SYBR® Green system (Applied Biosystems, Foster City, CA, USA) and the GoTaq® qPCR Master Mix kit (Promega Corporation, Madison, USA). The PCR reactions were subjected to the cycles protocol according to the following program: 95 °C for 3 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min, and 72 °C for 30 s. The target gene values of the threshold cycle (Ct) obtained were later normalized (ΔCt) based on the Ct values obtained for the endogenous control gene (18S). The calculation of the relative gene expression levels was performed according to the $2^{-\Delta\Delta Ct}$ method of Livak and Schmittgen (2001).

2.6. Genomic DNA extraction, qPCR, and bacterial quantification

Total genomic DNA was extracted from colon digesta following methods described by Stevenson and Weimer (2007) with adaptations. Briefly, samples were thawed at room temperature and centrifuged (10,000 × *g* for 10 min at 4 °C), and the supernatant was discharged. The cells were resuspended in 3 mL of cold extraction buffer, and 1 mL of cell suspension was lysed by heating and mechanical disruption (Beadbeater, Bartlesviille, Oklahoma, USA). The DNA was purified by phenol and phenol:chloroform:isoamyl alcohol extraction and resuspended in TE buffer. The amount of DNA was measured using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE), and the quality was measured in 0.8% agarose gel.

Real-time PCR analysis was performed to investigate the copy numbers of the 16S rDNA genes of *Lactobacillus spp.* and the *Enterobacteriaceae* family, following the protocol of Li et al. (2009) with some modifications. The quantification of DNA for each bacterial group was performed by using the ABI Prism 7300 Sequence Detection Systems thermocycler (Applied Biosystems, Foster City, CA, USA) with the SYBR Green chemistry (Fast SYBR® Green Master Mix; Applied Biosystems, Foster City, CA, USA).

The standard curve was constructed based on a tenfold serial dilution of PCR products from *L. delbrueckii and E. coli* cultures using universal bacterial primer set 27F-1492R (27F, 5'-AGAGTTTGATYMTGGCTCAG-3' and 1492R, 5'-TACGGYTACCTTGTTACGACT-3'), ranging from 6.23×10^9 to 6.23×10^4 copies/µL. The mass concentration of the PCR product was measured using Qubit 4® Fluorometer (ThermoFisher, MA, USA) spectrophotometer and converted to molecular concentration using the following equation:

$$= \frac{[(6.02 \times 10^{23})(molecules/mol) \times DNA\ amount\ (g)]}{(DNA\ length\ (pb) \times 660\ ((g/mol)/pb))}$$

Each standard dilution and samples were assayed in duplicate in a 10-µL reaction mixture containing 5 µL Fast SYBR Green Master Mix, 1 µL of each primer (Table 4) (10 µM), 2.2 µL ultrapure water, and 10 ng DNA template. Amplification involved one cycle at 95 °C for 10 min for initial denaturation and then 40 cycles of 95 °C for 15 s followed by annealing/extension for 30 s. After the amplification, a melt curve analysis with a temperature gradient of 0.1 °C/s from 60 to 95 °C was performed to confirm that only specific products were amplified. The copy numbers of total bacteria and each species in 10 ng

Table 4 - List of primers used for bacterial quantitation by qPCR analysis (16S rDNA sequences)

Bacteria group target	Primer sequence	Reference
Total bacteria	F: GCAGGCCTAACACATGCAAGTC R: CTGCTGCCTCCCGTAGGAGT	Castillo et al., 2006
Enterobacteria spp.	F: ATGTTACAACCAAAGCGTACA R: CTCTACGAGACTCAAGCTTGC	Takahashi et al., 2017
Lactobacillus spp.	F: TGGAAACAGRTGCTAATACCG R: GTCCATTGTGGAAGATTCCC	Byun et al., 2004

F and R indicate forward and reverse primers, respectively.

DNA were determined by relating the Ct values to standard curves. The efficiency was calculated for each primer pair using the formula $E = 10^{(-1/\text{slope of the line})} - 1$. The corresponding qPCR efficiency ranged between 85 and 105%.

2.7. Statistical Analysis

Performance and bacterial abundance data were analyzed using the following fixed effect model:

$$Y_{ijk} = \mu + T_i + B_j + e_{ijk},$$

in which Y_{ijk} is the response variable, μ is the overall mean, T_i is the fixed effect of the i-th treatment ($i = 1, 2$), B_j is the fixed effect of the j-th block ($j = 1, 2$), and e_{ijk} is the residual error. A normality test based on the Shapiro-Wilk statistics was applied for the residuals. When the normality assumption was not met ($P<0.01$; and visual assessment of the residual variance), a log-transformation was applied. In addition, initial BW was tested as a covariate in the model, but it was not significant ($P<0.01$), and thus removed from subsequent analyses. Because of the multiple sub-sampling for small intestine morphology traits (villus/crypt), the model included an additional random effect of a sample within residual. For the analyses of gene expression (Occludin-1, zonula occludens-1 (ZO-1) and transforming growth factor beta (TGF-β1)), data were analyzed according to methods proposed by Steibel et al. (2009). The results between extruded and ground corn treatments were calculated based on the relative expression of each gene ($2^{-\Delta Ct}$) and are shown as fold change (FC). The results of bacterial abundance are shown as $\log_2$FC. Results were considered statistically significant when $P<0.05$ and a tendency when $P<0.10$. Analyses were performed using the GLIMMIX procedure of SAS (Statistical Analysis System, version 9.4).

3. Results

Pigs fed ground or extruded corn diet had similar ($P>0.05$) final BW, ADG, ADFI, and feed conversion (Table 5). Corn processing did not affect fecal score (data not shown).

Duodenum and jejunum of pigs fed extruded corn had greater ($P<0.02$ and $P<0.01$, respectively) villus height compared with those of pigs fed ground corn (Table 6). Crypt depth was not influenced by corn processing ($P>0.05$). Duodenum and jejunum of pigs fed extruded corn had greater ($P = 0.04$ and $P = 0.02$, respectively) villus:crypt ratio compared with those of pigs fed ground corn.

Table 5 - Performance of piglets fed ground or extruded corn diets[1]

Item	Ground corn	Extruded corn	SEM	P-value
Initial BW (kg)	7.25	7.30	-	-
Final BW (kg)	9.53	9.49	0.27	0.91
ADG (g)	163	155	12.03	0.62
ADFI (g)	203	192	11.95	0.54
Feed conversion	1.25	1.25	0.04	0.98

BW - body weight; ADG - average daily weight gain; ADFI - average daily feed intake; SEM - standard error of the mean.
[1] Ground corn – corn ground through a hammer mill with a 1.0-mm screen after being ground by a hammer mill with a 3.0-mm screen; extruded corn – wet extruded after being ground by a hammer mill with a 2.0-mm screen, and after extrusion, it was ground by a hammer mill with a 1.0-mm screen.

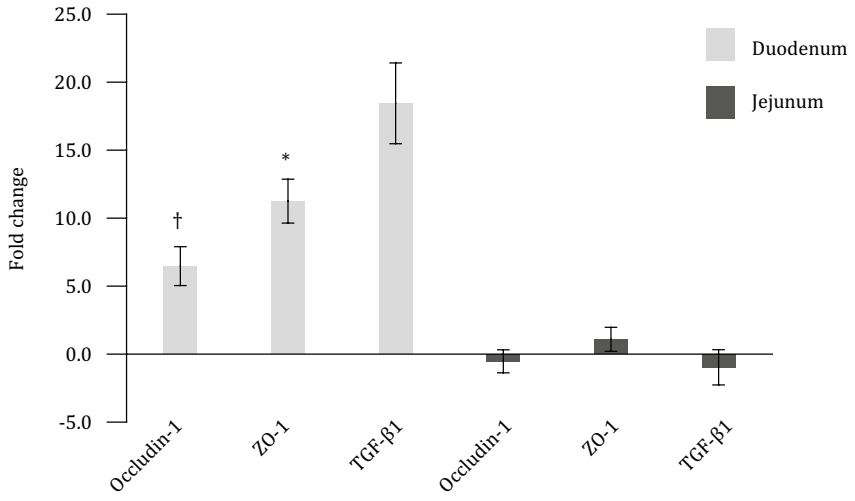
In the duodenum, expression of ZO-1 was greater (P<0.05), and the expression of Occludin-1 tended to be greater (P = 0.08) in pigs fed the extruded corn diets (Figure 1). There was no effect (P>0.05) of corn processing on ZO-1 and Occludin-1 expression in the jejunum, nor TGF-β1 expression in duodenum and jejunum.

There was no effect (P>0.05) of the type of corn processing on colon total bacteria or *Enterobacteriaceae* and *Lactobacillus* abundance (Table 7).

Table 6 - Small intestine morphology of piglets fed ground or extruded corn diets[1]

Item	Ground corn	Extruded corn	SEM	P-value
Duodenum				
Villus height (µm)	183	240	15.6	0.02
Crypt depth (µm)	151	153	8.6	0.83
Villus:crypt ratio	1.20	1.62	0.17	0.04
Jejunum				
Villus height (µm)	183	254	13.6	0.01
Crypt depth (µm)	162	155	10.1	0.64
Villus:crypt ratio	1.10	1.64	0.15	0.02

SEM - standard error of the mean.
[1] Ground corn – corn ground through a hammer mill with a 1.0-mm screen after being ground by a hammer mill with a 3.0-mm screen; extruded corn – wet extruded after being ground by a hammer mill with a 2.0-mm screen, and after extrusion, it was ground by a hammer mill with a 1.0-mm screen.



* P<0.05; † P<0.10.

Figure 1 - Fold change between extruded and ground corn treatments on the relative expression (positive values mean higher mRNA expression for extruded corn-fed pigs) of Occludin-1, zonula occludens-1 (ZO-1) and transforming growth factor beta (TGF-β1) in duodenum and jejunum of pigs with 35 days of age.

Table 7 - Intestinal microbiota (log 16S rDNA copy number/g) in the colon of piglets fed ground or extruded corn diets[1]

Item	Ground corn	Extruded corn	SEM	P-value
Total bacteria	9.57	9.43	0.12	0.43
Enterobacteriaceae	9.73	8.62	0.49	0.37
Lactobacillus spp.	8.48	7.59	0.35	0.10

SEM - standard error of the mean.
[1] Ground corn – corn ground through a hammer mill with a 1.0-mm screen after being ground by a hammer mill with a 3.0-mm screen; extruded corn – wet extruded after being ground by a hammer mill with a 2.0-mm screen, and after extrusion, it was ground by a hammer mill with a 1.0-mm screen.

4. Discussion

We hypothesized that diets with ground or extruded corn would have similar effects on weaned piglets. Thus, once grinding is a cheaper process than extrusion, it would meet the demand of the pig production industry for better performance and intestinal parameters of weaned piglets in the starter phases. There are many approaches to reduce post-weaning gut disorders. One involves the manipulation of grain processing. In this experiment, milling and extrusion were used to determine their effects on weaned piglet performance and gut parameters. The WAI data indicate that extrusion processing techniques were effective. The expected similarity of particle size among the two sources of corn was obtained.

In the present work, corn grinding and extrusion resulted in a similar performance, consistent with other studies that also did not find the extrusion process to result in better pig growth performance than grinding (Oliveira et al., 2011; Liu et al., 2014; Veum et al., 2017). Extrusion is generally believed to provide superior performance due to its improvement in nutrient digestibility (Medel et al., 1999; Rojas et al., 2016). However, improved nutrient digestibility does not always reflect the performance of piglets, probably due to interference from other factors, such as feed intake, length of the experiment, and type of extruder (Hongtrakul et al., 1998; Rodrigues et al., 2016).

Although the performance of piglets fed the ground corn and the extruded corn diets were similar, pigs fed extruded corn diets had significantly improved duodenum and jejunum villus height and villus:crypt ratio. Greater villus height and villus:crypt ratio are indicative of higher surface area and cell ability to produce digestive enzymes and increased absorptive capabilities (Pluske et al., 1996; Wang et al., 2017). Thus, our findings reinforce that extrusion improves digestion and absorption of the nutrients (Medel et al., 1999; Liu et al., 2014), even when compared with ground ingredients with similar particle sizes.

Tight junctions play a crucial role in the maintenance of intestinal barrier integrity. They seal the paracellular space between epithelial cells, thus preventing paracellular diffusion of microorganisms and other antigens across the epithelium (Ulluwishewa et al., 2011). The extruded corn diet improved ZO-1 and Occludin-1 gene expression in the duodenum by 11- and 6-fold, respectively, compared with the ground corn diet. This improvement is likely due to the higher starch digestibility provided by the extrusion process, increasing intestinal nutrient uptake as well as reducing the fraction of carbohydrates potentially harmful to the epithelium (Fadel et al., 1988; Rojas et al., 2016). The extrusion may also increase the digestion of non-starch polysaccharides and, although the fiber content of corn is low, approximately 50% of the total dietary fiber in corn-based diets may be attributed to corn (Liu et al., 2014). In addition, thermal processing acts to reduce pathogen concentration in the diet, a condition that favors the digestive tract epithelium (Kelley and Walker, 1999; Okelo et al., 2008). Together with the morphology results, these findings support the notion that extruded corn beneficially affected the intestinal structure.

It has been demonstrated that TGF-β1 upregulates barrier function (Roselli et al., 2003) and may be associated with the maturation of intestinal tissue (Nguyen et al., 2007). In the present study, an 18-fold increase was observed for TGF-β1 expression in the duodenum of piglets fed the extruded corn. Although not statistically significant, this result corroborates the morphological and tight junction results obtained and reinforces that extruded corn may improve intestinal digestion and barrier function.

The reduction of undigested nutrients in the large intestine, especially resistant starch, leads to a change in bacterial concentration (Pluske et al., 2002). Thus, based on intestinal morphology and tight junction protein results of the present study, changes in the concentration of bacteria were expected. However, there was no effect of the experimental treatment on total bacteria, *Enterobacteriaceae*, and *Lactobacillus* group in the colon of pigs.

As we hypothesized, piglets fed extruded or ground corn showed similar performance. However, in the present study, pigs fed diets containing extruded corn had improved gut variables. Therefore, from a

physiological point of view, extruded corn would be more suitable than ground corn. This impact on gut parameters could serve as a basis for further works on the assessment of the possible effects of extruded corn on subsequent nursery phases.

5. Conclusions

Piglets fed diets with ground or extruded corn have similar growth performance. However, piglets fed diets with extruded corn present improved intestinal morphology and tight junction protein expression compared with those fed ground corn.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: T. Scalco and G.C. Rocha. Data curation: G.A.C. Valini, G.A. Rodrigues, L.M.P. Sanglard and G.C. Rocha. Formal analysis: L.M.P. Sanglard and G.C. Rocha. Funding acquisition: A.A. Calderano and G.C. Rocha. Investigation: T. Scalco, G.A.C. Valini, C.B. Silva, G.A. Rodrigues, H.C. Mantovani and G.C. Rocha. Methodology: T. Scalco, G.A.C. Valini and G.C. Rocha. Project administration: T. Scalco, G.A.C. Valini and G.C. Rocha. Resources: G.C. Rocha. Supervision: G.C. Rocha. Validation: G.C. Rocha. Visualization: A.A. Calderano, P.H.R.F. Campos, H.C. Mantovani and G.C. Rocha. Writing-original draft: T. Scalco, A.A. Calderano, G.A. Rodrigues, P.H.R.F. Campos and G.C. Rocha. Writing-review & editing: A.A. Calderano, P.H.R.F. Campos and G.C. Rocha.

Acknowledgments

We thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, grant #001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Instituto Nacional de Ciência Animal (INCT-CA) for the financial support. Appreciation is extended to Cooperalfa (Santa Catarina, Brazil) for the donation of corn for the experimental diets.

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