Gastrointestinal morphophysiology and presence of kafirins in ileal digesta in growing pigs fed sorghum-based diets

José Guadalupe Gómez Soto, Tércia Cesária Reis de Souza, Gerardo Mariscal Landín, Araceli Aguilera Barreyro, María Guadalupe Bernal Santos, and Konisgmar Escobar García

We evaluated the effects of three sorghum-based diets with different levels of tannins and kafirins on gastrointestinal morphophysiological characteristics of growing pigs. We also evaluated the pigs’ performance and the presence of kafirin fractions in sorghum samples and in ileal digesta. We used 24 pigs that weighed 22.1 ± 0.65 kg randomly assigned to four different cereal–soybean meal diets: a corn-based (control diet; C), a low tannin and low kafirin sorghum-based (LTLK), a low tannin and high kafirin sorghum-based (LTHK), and a high tannin and high kafirin sorghum-based (HTHK). We evaluated the pH of the gastrointestinal digesta, the liver and pancreas weight, and the total and specific trypsin and chymotrypsin enzymatic activities in the pancreas, and the intestinal villi morphology. The results indicated that the different sorghum diets did not affect the performance of the pigs (P > .05) or the majority of the evaluated morphophysiological parameters. The total trypsin activity was higher in pigs fed the HTHK diet (P < .001). The highest intensity/area of the kafirin fractions was noted in ileal digesta from pigs fed LTHK and HTHK diets. The simultaneous presence of high levels of tannins and kafirins could affect the digestion of sorghum proteins.

1. Introduction

During the last few years, sorghum has been the fifth most economically important grain in the world, after wheat, corn, rice, and barley (Monk et al. 2014). It is considered a primary feed crop in the semiarid tropical regions of Asia, Africa, and South America, because it is cultivated in dry areas with low soil moisture (Rakshit et al. 2014; Mateus et al. 2016). In Mexico, sorghum is the second most produced cereal after corn and is almost exclusively used in animal feeds (Mariscal-Landín et al. 2004) mainly in pig nutrition. Sorghum and corn have similar crude protein content, but the nutritional value of sorghum may be lower due to the presence of tannins (Fialho et al. 2002) and kafirins (Aboubacar et al. 2001; Chiquito-Almanza et al. 2011). Tannin content in sorghum grains can vary considerably among different genetic backgrounds (Wu et al. 2012), and their hybrid can be divided into high and low tannin (Mariscal-Landín et al. 2004). Sorghums with low tannin content are the most commonly cultivated sorghums in Mexico and in the world (Oria et al. 2000; Ramírez et al. 2005), despite the fact that the sorghum hybrids with high tannins have added protection from birds and fungal infestations (Wu et al. 2012). Tannins are polyphenolic compounds of high molecular weight that are present in the pericarp of the grain (Mueller-Harvey 2006). Tannins are classified as either hydrolysable or condensed (Lee et al. 2010; Van Parys et al. 2010), and there are the condensed tannins that are present in sorghum (Fayyaz and Naser 2015). Tannins form complexes with polysaccharides and proteins (dietary and endogenous), and bind to digestive enzymes, amino acids, fatty acids and nucleic acids (Serrano et al. 2009; Jezierny et al. 2011; Piluzza et al. 2014), which may affect digestive secretions and nutrients use.

Kafirins are the most abundant proteins in sorghum (De Mesa-Stonestreet et al. 2010). Based on differences in solubility, molecular weight, structure, and amino acid composition (Bansal et al. 2008), the kafirins are classified into α, β, γ and δ fractions. To separate these fractions many researchers have used electrophoresis test (Hamaker et al. 1995; El Nour et al. 1998; Duodu et al. 2002; Nunes et al. 2005). However, western blot test can also be employed using specific anti-kafirin antibodies (Mazhar and Chandrashekar 1995; Wong et al. 2010).

Kafirins can affect sorghum nutrient utilization (Kumar et al. 2012) because they are non-soluble in water (Xiao et al. 2015), and contain high levels of proline, an amino acid that has a high affinity to form complexes and bind to tannins (Taylor et al. 2007; Piluzza et al. 2014), which further limits the action of the digestive enzymes on dietary proteins. Kafirins are also the last proteins to be digested in sorghum flour (Hamaker et al. 1986), which is probably because they are encapsulated in protein bodies in the endosperm of the grain (Chandrashekar and Mazhar 1999; Bansal et al. 2008) and they form polymers, trimers and dimers (Nunes et al. 2005; Belton et al. 2006) affecting the digestive process.
Because tannins and kafirins in sorghum may affect enzyme activity, and consequently the digestion and absorption of nutrients that would be used for the development of the pig, our hypothesis was that high tannin and high kafirin sorghum-based diets fed to pigs could decrease pigs’ performance. The objective of this study was to investigate the effect of different sorghum-based diets, with different levels of tannins and kafirins, on some morphophysiological characteristics of the digestive tract in growing pigs (pH of gastrointestinal content, weight of liver and pancreas, enzymatic activity of trypsin and chymotrypsin, and morphology of the intestinal mucosa). We also evaluated kafirin profiles in samples of sorghums and ileal digesta samples using two different tests (electrophoresis and western blot).

2. Materials and methods

The experiment was performed at the National Center of Disciplinary-Research Physiology of the National Institute of Forestry, Crops and Livestock (INIFAP) in Mexico. The protocol was reviewed and approved by the Autonomous University of Queretaro’s Natural Science Faculty’s Bioethics Committee (authorization: 90-FCN-2014). The management of the experimental animals was performed according to the Mexican Official Norm ‘NOM-062-ZOO-1999’ guidelines (Diario Oficial de la Federación 2001) and the International Guiding Principles for Biomedical Research Involving Animals (CIOMS/ICLAS 2012). The laboratory analyses were performed at the Natural Science Faculty’s Animal Nutrition Laboratory.

2.1. Experimental treatments

The chemical composition of the three sorghum hybrids and the corn used in the experiment were determined before formulating the diets. The content of prolamins (true kafirins in sorghum and true zeins in corn, which are homologous proteins, according to Oom et al. 2008) were determined (Hamaker et al. 1995), as well as the condensed tannin content of the sorghum hybrids (Price et al. 1978). Then we classified the three sorghum hybrids into low in tannins and low in kafirins (LTLK), low in tannins and high in kafirins (LTHK), and high in tannins and high in kafirins (HTHK) (Table 1). The gross energy of the sorghum and corn were quantified using calorimetry (Bateman 1970). Crude protein, ashes, and ether extract were also determined, based on the AOAC (2002) methods 976.05, 923.03, and 920.35, respectively. Neutral detergent fibre was also quantified (van Soest et al. 1991). Amino acid determination was performed according to the 994.12 method of AOAC (2002), which involved samples being hydrolysed at 110°C for 24 h in HCl 6M. Amino acid quantification was performed using ion exchange, applying the post-column derivatization test in a HPLC (Model 1100 HPLC, Hewlett Packard®), attached to a Pickering Pinnacle PCX. To determine methionine and cysteine content, a previous oxidation with performic acid was performed (Csapó et al. 2005).

Based on the results of chemical analysis of the major ingredients, four different cereal–soybean meal diets were formulated: a corn-based diet (control diet; C), an LTLK sorghum-based diet, an LTHK sorghum-based diet, and an HTHK sorghum-based diet (Table 2). All diets were formulated using the ideal protein concept, according to the digestible amino acid requirements recommended by the NRC (2012). To formulate the HTHK diet, a lower amino acid digestibility of the high tannin sorghum was considered (Mariscal-Landin et al. 2004).

Table 1. Chemical composition of the sorghum hybrids and corn as fed.

| Ingredients (% of total) | LTLK | LTHK | HTHK | C |
|-------------------------|------|------|------|---|
| Dry Matter (DM)         | 89.16| 89.06| 88.76| 88.31|
| Crude protein,%         | 8.02 | 9.10 | 7.45 | 7.74 |
| True kafirins           | 58.69| 73.64| 69.53| –   |
| True zeins              | –    | –    | –    | 56.80|
| Tannins,%               | ND   | ND   | 5.40 | ND  |
| Gross energy, Mcal/kg   | 3981 | 3940 | 3999 | 4045|
| Ashes,%                 | 1.50 | 2.50 | 1.90 | 1.10|
| Neutral detergent fibre,%| 10.24| 8.49 | 8.71 | 6.55|
| Ether extract,%         | 2.19 | 2.62 | 2.45 | 3.65|
| Amino acids (g/kg of DM)|     |      |      |     |
| Alanine                 | 0.63 | 0.79 | 0.56 | 0.51|
| Arginine                | 0.30 | 0.34 | 0.28 | 0.36|
| Aspartic acid           | 0.22 | 0.60 | 0.45 | 0.44|
| Cysteine                | 0.17 | 0.14 | 0.14 | 0.17|
| Glutamic acid           | 1.44 | 1.79 | 1.27 | 1.23|
| Glycine                 | 0.21 | 0.24 | 0.20 | 0.26|
| Histidine               | 0.19 | 0.21 | 0.17 | 0.24|
| Isoleucine              | 0.33 | 0.38 | 0.29 | 0.27|
| Leucine                 | 1.04 | 1.25 | 0.87 | 0.86|
| Lysine                  | 0.26 | 0.28 | 0.25 | 0.32|
| Methionine              | 0.17 | 0.16 | 0.14 | 0.20|
| Proline                 | 0.72 | 0.44 | 0.41 | 0.49|
| Phenylyalanine          | 0.43 | 0.50 | 0.37 | 0.37|
| Serine                  | 0.22 | 0.34 | 0.27 | 0.31|
| Threonine               | 0.16 | 0.29 | 0.23 | 0.26|
| Tyrosine                | 0.29 | 0.34 | 0.26 | 0.26|
| Valine                  | 0.40 | 0.47 | 0.35 | 0.37|

*In the case of corn are true prolamins or true zeins. Expressed as the percentage content of prolamins regarding the content of crude protein.
ND: None detected.

Table 2. Composition of experimental diets as fed.

| Ingredients (% of total) | LTLK | LTHK | HTHK | C |
|-------------------------|------|------|------|---|
| Sorghum                 | 68.69| 72.13| 69.09| – |
| Corn                    | –    | –    | –    | 68.51|
| Soybean meal            | 25.75| 22.45| 25.45| 25.86|
| Soybean oil             | 2.14 | 1.86 | 1.92 | 2.28|
| L-Lysine                | 0.41 | 0.49 | 0.45 | 0.40|
| L-Threonine             | 0.10 | 0.11 | 0.13 | 0.09|
| DL-Methionine           | 0.14 | 0.14 | 0.17 | 0.10|
| L-Tryptophan            | –    | 0.01 | 0.01 | 0.01|
| Common salt             | 0.50 | 0.50 | 0.50 | 0.50|
| Calcium carbonate       | 0.74 | 0.75 | 0.74 | 0.73|
| Dicalcium phosphate     | 1.19 | 1.22 | 1.20 | 1.18|
| Vitamins                | 0.24 | 0.24 | 0.24 | 0.24|
| Minerals                | 0.10 | 0.10 | 0.10 | 0.10|
| Chemical composition    |     |      |      |     |
| Dry Matter,%            | 91.94| 91.99| 92.35| 92.64|
| Crude protein,%         | 17.62| 15.88| 16.69| 16.80|
| Ashes,%                 | 5.87 | 7.24 | 5.11 | 4.57|
| Metabolizable energy, Mcal/kg | 3.30 | 3.30 | 3.30 | 3.30|
| Gross energy, Mcal/kg   | 4.23 | 4.34 | 4.33 | 4.39|

*Each kg of product contains vitamin A 10.20 international units (IU), vitamin D 1.99 IU, vitamin E 0.06 IU, vitamin K 1.20 mg, riboflavin (B2) 7.20 mg, vitamin B12 (cyanocobalamin) 0.04 mg, choline 968.58 mg, niacin 36 mg, pantothenic acid 16.55 mg, thiamin (B1) 0.30 mg, pyridoxine (B6) 0.31 mg, biotin 0.08 mg, folic acid 0.75 mg.

*Each kg of product contains sulphur 200 mg, cobalt 0.72 mg, copper 14.4 mg, iron 120 mg, manganese 36 mg, selenium 0.30 mg, iodine 0.96 mg, zinc 144 mg, and chloride 300 mg.
which favoured the inclusion of more digestible amino acids from soybean meal and the addition of crystalline amino acids. Dry matter, crude protein, ashes (AOAC 2002), and gross energy (Bateman 1970) of the diets were analysed (Table 2).

2.2. Animals

Twenty-four growing pigs (Fertilis 20x G Performance, Genetipurc®) of both sexes, with an average body weight (BW) of 22.1 ± 0.65 kg were used. The experiment was performed in two blocks (different months) with 12 pigs in each one, and a total of 6 animals being assigned to one of the four diets. The pigs were housed for 12 d in individual pens (elevated 50 cm), equipped with an individual feeder and a nipple drinker. Pigs had ad libitum access to their diet and water during the entire experiment. Average daily feed intake (ADFI) and average daily gain (ADG) were recorded, and the gain to feed ratio (G:F) was calculated.

2.3. Sampling

At the day 12 of the experiment, the pigs were weighed and tranquilized with azaperone (2 mg/kg BW). They were then anaesthetized with isoflurane (inhaled) and slaughtered using a cardiac overdose of sodium pentobarbital (50 mg/kg BW). An incision was done in the abdomen midline, and the pancreas and liver were collected and weighed. The pancreas was maintained at −80°C until the total and specific activities of trypsins (Reboud et al. 1962) and chymotrypsins (Bergmeyer et al. 1974) were measured. Protein concentration in the pancreatic tissue was quantified as described by Lowry et al. (1951). The stomach, jejunum, ileum, caecum, and colon were dissected and emptied, and the pH of their content was measured using a potentiometer (Model PH211, Hanna Instruments®). The ileal digesta from the pigs on the sorghum-based diets were collected to perform the electrophoresis and western blot tests. They were kept at −80°C, until they were lyophilized and grounded using a mill with 1 mm sieve (Thomas-Wiley®), and the crude protein (AOAC 2002) was determined.

In order to measure the villi height and crypts depth, 10 cm sections of the duodenum, jejunum, ileum, and caecum were collected, washed with a saline solution, and preserved in a 10% neutral formalin solution. Afterwards, the sections were fixed in paraffin, sectioned into 5 μm slices, stained with haematoxylin and eosin, and inspected using an optical microscope (Vert. A1, Zeiss®). Ten villi and crypts of each intestinal section were measured (Nabuurs et al. 1993) using AxioVision 4.9 software (Zeiss®).

2.4. Electrophoresis and western blot tests

A total of six 12% polyacrylamide gels (SDS-PAGE) were used for the identification and analyses the profiles of kafirins in the three sorghums and in the ileal digesta from four pigs of each diet (Laemmli 1970). Four of these gels were used for the electrophoresis tests and two for western blot tests. The sorghums and ileal digesta samples (50 μg of crude protein), under reducing conditions (Duodu et al. 2002), were placed in the wells of each gel. Gels for the electrophoresis tests were stained with Coomassie Brilliant Blue R-250 (Xiao et al. 2015). In three wells of each electrophoresis gel, 3, 6, and 12 μg of bovine serum albumin were placed as a known concentration of internal standard protein to make the standard curve (Sato et al. 1986) to quantify the amount of monomeric kafirin fractions in the ileal digesta.

To perform the western blot tests, all bands were transferred to nitrocellulose membranes (Cat. 162-0115, Bio-Rad), and blocked with a 5% concentration of skimmed milk (Le Gall et al. 2005). After being blocked, the membranes were incubated with polyclonal antibodies (produced by rabbits) that acted against the sorghum kafirins. In order to produce the anti-kafirin antibodies, three rabbits were each inoculated three times in the back (intradermally), with an interval of 15 d between inoculations. For the first inoculation, the rabbits received 0.5 mg of emulsified kafirins in complete Freund's adjuvant (Cat. F5881, Sigma). For the other inoculations, the rabbits received 0.25 mg of emulsified kafirins in an incomplete Freund's adjuvant (Cat. F5506, Sigma). Seven days after the last inoculation, the rabbits were slaughtered, and plasma samples were obtained from each one (Stills 2012). The anti-kafirin antibodies were used in a 1:600 dilution. A commercial anti-rabbit IgG produced in donkeys was used as a second antibody (A120-208P, Bethyl Lab.) in a 1:2000 dilution. The dilutions of antibodies were established using a dot blot test (Hawkes et al. 1982). Finally, the western blot was revealed with a peroxidase substrate kit (SK-4800, Vector Lab).

The gels of electrophoresis and the nitrocellulose membranes were digitized using a Molecular Imager Gel Doc XR System (Universal Hood II, Bio-Rad) and analysed using the Quantity One V 4.6.7 software (Bio-Rad). The amount of protein of each monomeric kafirin band was expressed as ng/μg of ileal digesta protein in electrophoresis, and as intensity/area in western blot (Wong et al. 2009; Elkonin et al. 2013).

2.5. Statistical analysis

Data were analysed as a randomized complete block design, and measures were compared using Tukey’s test (Steel and Torrie 1980) with the GLM procedure of SAS (2008). Statistical differences were considered significant at P < .05.

3. Results

3.1. Chemical composition

The chemical composition of the sorghum hybrids and corn are shown in Table 1. The three sorghum hybrids were classified as low (undetectable) in tannins and low (58.6%) in kafirins (LTLK), low (undetectable) in tannins and high (73.6%) in kafirins (LTHK), and high (5.4%) in tannins and high (69.5%) in kafirins (HTHK). The true zein content of the corn was similar to the true kafirin content of the LTLK sorghum hybrid. We observed some differences between the sorghum hybrids for crude protein, neutral detergent fibre, and ether extract content. However, gross energy content was similar. There were some differences in composition between the corn and sorghum...
hybrids, mainly for the neutral detergent fibre and ether extract content, as well as for the amino acids profile.

### 3.2. Performance and morphophysiological characteristics

There was no effect of diet on pig performance (P > .05). The average values observed in all pigs were an ADFI of 1.568 kg and an ADG of 0.685 kg, with a G:F ratio of 0.448. Table 3 shows that the experimental diets also did not affect (P > .05) the pH of the digesta in the stomach and intestinal portions, the liver and pancreas relative weights, the specific trypsin activity, the total and specific activities of chymotrypsin, the villi height, and crypt depth in all intestinal portions. However, pigs fed the HTHK diet had a higher total trypsin activity (TTA; P = .003).

### 3.3. Identification of kafirins

Figure 1 shows an electrophoresis gel that is representative of the four analysed gels. We detected the kafirin fractions \( \alpha_1, \alpha_2, \text{ and } \beta \) in the sorghum samples. In the ileal digesta samples, the kafirin fractions \( \gamma_1, \alpha_1, \text{ and } \alpha_2 \) were still present, whereas the \( \beta \) fraction was no longer present.

Table 4 shows the analysis of the kafirin bands present in the electrophoresis gels. The \( \alpha_1 \) kafirins had more (P ≤ .01) concentration in the ileal digesta from the pigs fed HTHK diet than in the pigs fed the low tannin diets (LTLK and LTHK). The concentration of the \( \alpha_2 \) and \( \gamma \) kafirin fractions was not affected (P > .05) by the diets.

Figure 2 shows a western blot representative of the two analysed in the study. We detected dimers and trimers with a molecular weight of 61–81 kDa, as well as the kafirin fractions \( \gamma \) (27.5 kDa), \( \alpha_1 \) (25.5 kDa), \( \alpha_2 \) (23.1 kDa), and \( \beta \) (18.9 kDa), in the sorghum samples and in the ileal digesta. The intensity/area of kafirin polymers was considerably lower in the ileal digesta than in the sorghum samples. Also we detected in the ileal digesta some residues of kafirin digestion with a very low molecular weight, regardless of the diet.

Table 4 also shows the analysis of the kafirin bands present in the western blot tests. In addition, for the \( \gamma, \alpha_1, \alpha_2 \) and \( \beta \) kafirin fractions, a higher intensity/area was noted for the high kafirin diets (LTHK and HTHK) than for the LTLK diet (P < .05).

### 4. Discussion

The variation in the chemical composition of the sorghum hybrids (Mariscal-Landin et al. 2004; Ramirez et al. 2005) indicates an essential need for the feed industry to perform more frequent analyses of their ingredients. Regarding the crude protein level, there was a lower variation (7.4%–9.1%) in values for the sorghums used in this study than for the sorghums mentioned in the literature (6.6–15%; Ramirez et al. 2005). The crude protein level of the LTHK sorghum and the corn used in the present study were in accordance with the NRC (2012), while the crude protein levels of the LTLK and HTHK sorghums were in accordance with Rostagno et al. (2011).

There was also considerable variation in the levels of tannins for the three sorghum hybrids used in this study. In two of the hybrids, no tannins were detected, whereas the third hybrid had a high (5.4%) level of tannins, similar to the values for typical high tannin varieties (Ramírez et al. 2005; Gómez-Soto et al. 2015).

The values of kafirins observed in this study varied between 58.6% and 73.6%. These data were within the range (37.5% and 72.9%) reported by Selle et al. (2010). A high proportion of sorghum proteins are kafirins (Wong et al. 2009), and therefore an increase in total crude protein is likely related to an increase of kafirins (Mossé et al. 1988), as we observed for the low tannin sorghums.

Our results suggest that low tannin sorghums can replace corn in the diets of pigs between 10 and 30 kg without negatively affecting ADFI, ADG, and the feed conversion ratio (Fialho et al. 2004). We also observed that, when diets are formulated based on the digestible amino acid content, it is possible to use high tannin sorghums without affecting the growth of animals.

One of the hypotheses of this study was that high tannin and kafirin levels in sorghum would have a negative effect on the growth of animals and the morphophysiology of the intestinal tract. This is because many authors have noted that tannins form complexes with digestive enzymes (Jansman et al. 1993; Emmambux and Taylor 2003), polysaccharides, and proteins (Duodu et al. 2002; Serrano et al. 2009), which may affect...
digestion and absorption of the nutrients. Furthermore, the complexes formed between the proline of kafirins and tannins reduce the possibility of enzymatic action over the proteins (Emmambux and Taylor 2003) and decrease the digestibility of most of the essential amino acids (Jansman et al. 1993). This occurs mainly in the high tannin sorghums (>4%) (Mariscal-Landín et al. 2004, 2010). Thus, with a lower digestive utilization of sorghum proteins, we expected to observe a negative effect of high kafirin diets (LTHK and HTHK) on the parameters evaluated for the pigs. However, our results showed no negative effects on performance nor on the morphophysiological characteristics of the digestive tract, corroborating with the results of Mariscal-Landín et al. (2004) who reported that the

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Table 4. Concentrations of monomeric kafirin bands detected by electrophoresis and western blot tests.

| Kafirin fraction | Sorghum-based diets | P     | SEM  |
|------------------|---------------------|-------|------|
|                  | LTLK    | LTHK  | HTHK |       |
| Electrophoresis  |          |       |      |       |
| (ng/μg of ileal digesta protein) |        |       |      |       |
| Gamma (γ)        | 28.1    | 28.0  | 33.8 | 3.40  |
| Alfa 1 (α1)      | 147.8b  | 128.4b| 181.5a| 4.3   |
| Alfa 2 (α2)      | 38.2    | 39.8  | 38.9 | 3.3   |
| Western blot     |          |       |      |       |
| (intensity/area) |        |       |      |       |
| Gamma (γ)        | 5056b   | 6121a | 6167a| 102.3 |
| Alfa 1 (α1)      | 5367b   | 6402a | 6098a| 105.9 |
| Alfa 2 (α2)      | 3454b   | 5331a | 5746a| 199.9 |
| Beta (β)         | 6262b   | 10807a| 13412a| 633.3 |

Note: Values in the same row without a common superscript letter are significantly different (P < .05).

SEM, standard error of the mean.
tannin content did not affect pancreas weight and specific activity of trypsin.

Our findings showed that TTA in pancreatic tissue increased in response to the HTHK diet, whereas Jansman et al. (1994) reported that animals fed high tannin sorghums have a reduction in trypsin activity in their ileal content. The increase in pancreatic trypsin activity is probably due to the interaction between tannins and trypsin in the intestine, decreasing the amount of trypsin available for hydrolysis and increasing the amount of cholecystokinin secreted which stimulate the pancreatic secretion (Fushiki and Iwai 1989; Ahmed et al. 1991; Thaela et al. 1995) and, in turn, the trypsin secretion.

The weight of the pigs is an important factor that needs to be considered, because we used growing pigs that had a functional and mature gastrointestinal tract (Mariscal-Landín et al. 2010), which allows them to take advantage of the nutrients of the food in a better way, and when the pigs have up to 25 kg BW, the presence of tannins showed little effect on growth performance (Lizardo et al. 1995). In fact, the pigs weighed 22.1 ± 0.65 kg at the start of the experiment and they weighed 30.5 ± 1.36 kg at the end of the experiment. So two factors could explain the absence of an adverse effect of tannins on pigs’ performance. The first was that diets were formulated to furnish the same amount of digestible amino acids, diminishing the deleterious tannin effect on amino acids digestibility (Selle et al. 2010). The second factor was the pig weight (22–30 kg) because they were able to maintain the same feed intake avoiding the main adverse effect of tannins (reduction of feed intake) on performance (Lizardo et al. 1995).

The fact that we found the kafirin fractions in the ileal digesta confirmed the effect of tannins and kafirins on the digestibility of sorghum-based diets observed by Mariscal-Landín et al. (2004). Regarding the electrophoresis test, the HTHK diet resulted in a higher concentration of $\alpha_1$ kafirins, which indicates a lower digestibility (Aboubacar et al. 2001) of this fraction compared to the $\alpha_1$ kafirins in the other diets. Several authors reported that $\alpha$ kafirins are located in the central and internal part of the protein body of sorghum, while $\gamma$ kafirins (Nunes et al. 2005; De Mesa-Stonestreet et al. 2010) and $\beta$ kafirins are located in the peripheral area of the grain (Taylor et al. 2007). Because of the location of the $\alpha$ kafirins, the digestive enzymes probably have difficulty to hydrolyse these kafirins, whereas the $\beta$ and $\gamma$ kafirins are the first fractions to be degraded, owing to their peripheral location (Oria et al. 1995, 2000). This may be the reason for the larger quantity of $\alpha_1$ kafirins in the ileal digesta, especially for the pigs fed the HTHK diets.

In the ileal digesta, $\beta$ kafirins were not detected by electrophoresis, and this suggests that they were totally digested. However, two factors need to be considered. First, $\beta$ kafirins represent only 8–13% of the kafirins present in sorghum grain, while $\alpha$ kafirins represent 66–84% (Chamba et al. 2005; Wong et al. 2009; De Mesa-Stonestreet et al. 2010). Second, Coomassie blue staining has a limited capacity to detect proteins (Westermeier 2011).

In the western blot test, all kafirin fractions were detected in the ileal digesta, even $\beta$ and the low-molecular-weight kafirins, due to the high specificity of this test (Sambrook and Russell 2001) because specific antibodies against sorghum kafirins were used. The presence of $\beta$ kafirins in the western blot test and the higher concentration observed in the ileal digesta for pigs fed high kafirin diets (LTHK and HTHK) indicate that $\beta$ kafirins were not totally digested, and their digestibility is probably related to the kafirin level in the sorghum.

The $\gamma$ kafirin fractions were present in the ileal digesta in the electrophoresis and western blot tests (Figures 1 and 2). The $\gamma$ kafirin fractions are rich in cysteine and proline (Oria et al. 2000; Duodu et al. 2003; Belton et al. 2006) and have an inherent capacity to form disulphide bonds and protease-resistant complexes (Chandrashekar and Mazhar 1999; Wong et al. 2010). Pigs fed high kafirin diets (LTHK and HTHK) had a large concentration of the $\gamma$ kafirin fraction, indicating that this fraction is poorly digested, because a higher intensity/area in ileal digesta indicates a lower digestibility of the proteins (Aboubacar et al. 2001; Wong et al. 2009).

The presence of kafirins in trimers and dimers or in a monomeric state in the ileal digesta suggests that kafirins are also resistant to enzymatic digestion, regardless of their position in the protein body in the grain, and this is probably due to their hydrophobic nature (Hamaker et al. 1986, 1987; Xiao et al. 2015). The low-molecular-weight kafirin bands detected in the ileal digesta were not found in the sorghum samples, which suggests that they are not the $\delta$ kafirins described by Belton et al. (2006) and De Mesa-Stonestreet et al. (2010), and are probably peptides of kafirins partially digested.

The effect of the intake of an elevated kafirin level in the ileal digesta is demonstrated in the western blot test, by a higher quantity in the $\gamma$, $\alpha_1$, $\alpha_2$, and $\beta$ kafirin fraction bands in pigs fed the high kafirin diets independently of the tannin levels (LTHK and HTHK), corroborating that not only tannin levels are a factor to consider when pigs fed diets with sorghum but also that kafirin levels must be considered (Elkin et al. 1996; Mariscal-Landín et al. 2010), as they could negatively affect the digestibility of the sorghum protein.

5. Conclusion

There was no adverse effect of the sorghum tannins and kafirins on the performance of the growing pigs and in most of the morphophysiological characteristics of their gastrointestinal tract. Western blot was more sensible and specific than electrophoresis to detect kafirin fractions. The raised concentration of kafirins detected by western blot test in the ileal digesta of pigs fed LTHK and HTHK diets suggests that kafirins negatively affect the digestibility of dietary protein. Thus, not only tannin but also kafirin content in sorghums fed by pigs affected the digestion of dietary proteins.

Disclosure statement

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