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Original Research Article

Is the kafirin profile capable of modulating the ileal digestibility of amino acids in a soybean meal-sorghum diet fed to pigs?

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ABSTRACT

The effects of kafirins on protein and amino acid ileal digestibility have not been evaluated *in vivo* in pigs. The aim of this study was to determine the effects of protein profile on apparent ileal digestibility (AID) of amino acids. We used a sorghum hybrid with low tannin content (<0.5%). The same hybrid was harvested from 2 different plots with different kafirin profile. Sorghum with greater content of total kafirins had less content of γ- and α1-kafirins and higher content of β- and α2-kafirins than that with lower content of total kafirins. Two sorghum-soybean meal (SBM) diets were formulated: 1) low kafirin (LK) content (32.2 g/kg) and 2) high kafirin (HK) content (48.1 g/kg). A control diet (maize-SBM) and a reference SBM-diet were also prepared. The reference diet was fed to all pigs following the experimental period and was used to estimate the AID of cereals by the difference method. "T" cannulas were fixed in the distal ileum of 18 barrows (6 by treatment), divided into 2 groups of 9 pigs. The pigs were fed 2.5 times their maintenance requirement of digestible energy (110 kcal/kg $BW^{0.75}$). The AID of dry matter, protein, amino acids, and energy of the experimental diets was measured; the AID of cereals (maize, LK sorghum and HK sorghum) was estimated by the difference method. The maize-SBM diet was more digestible than the sorghum-SBM diets, only with respect to valine ($P < 0.05$). The AID of valine in the maize-SBM diet was higher than that in sorghum-SBM diets. The changes in kafirin profile between the diets only affected the AID of threonine ($P < 0.01$), which decreased by 9.5 percentage units in LK diet compared with HK diet. Regarding the AID of cereals, maize exhibited greater AID than sorghum, with respect to valine ($P < 0.01$) and serine ($P < 0.10$). A comparison of sorghum with LK and HK content showed that the AID of threonine and serine increased by 50.5 ($P < 0.001$) and 19.2 percentage units ($P < 0.05$) in the latter, respectively. The higher content of γ-kafirins in LK sorghum negatively affected threonine and serine digestibility, implying that the AID of amino acids is affected more by the profile than the content of kafirins.

1. Introduction

Sorghum (*Sorghum bicolor* L. Moench) is widely used to feed animals as it is a good source of amino acids (AA) and energy (Selle et al., 2010). However, sorghum exhibits considerable variation in its chemical composition and nutritive value (Mossé et al., 1988). Tannins are the primary cause of these differences as they have adverse effects on proteins (Mariscal-Landín et al., 2004, 2010). It might also be attributed to energy digestion (Pan et al., 2016) owing to the inhibition of trypsin, lipase, and amylase activities (Horigome

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et al., 1988; Jansman et al., 1994). However, feeding low-tannin sorghum results in lower feed efficiency in pigs than if maize is fed (Marques et al., 2007). This lower feed efficiency can be explained by lower digestibility of energy (Xie et al., 2017) and protein, due to the high number of sulphuric bonds in the reserve proteins of sorghum (Cremer et al., 2014). Results of *in vitro* studies indicated that kafirins (sorghum reserve proteins), particularly α-kafirins, are resistant to digestion (Oria et al., 1995a; Wong et al., 2009). This detrimental property is more evident in sorghums with low tannin content (<1.0%) than in those with high tannin content, because the adverse effects of tannins in low-tannin sorghum is not consistent (Mariscal-Landín et al., 2004). However, the effects of kafirins on protein and AA ileal digestibility have not been evaluated *in vivo* in pigs. Therefore, the objective of this study was to measure the apparent ileal digestibility (AID) of sorghum-soybean meal diets with different kafirin content in growing pigs, to verify if kafirins (amount or profile) affect the AID of protein and AA.

2. Materials and methods

The study was approved by the Scientific Associate Technical Group Committee of the National Center for Disciplinary Research in Physiology (CENID Physiology, INIFAP, México). The experiment was conducted at the experimental farm of CENID-Physiology. The experimental animals were treated according to the guidelines of the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1985) and the Official Mexican Standard for Production, Care and Use of Laboratory Animals (Diario Oficial de la Federación, 2001).

2.1. Animals

Eighteen barrows from the cross Fertilis 21 × G Performance (Genetiporc) were used; the mean body weight of pigs was 23.9 ± 2.16 kg. Pigs were divided into 2 groups of 9 pigs and placed in individual metabolic cages equipped with a self-feeder and a low-pressure drinking nipple connected to a watering system that controlled water supply. The first 3 d served as an adaptation period to the cages. On the 4th day, pigs were fasted and on the 5th day, a cannula was implanted at the terminal ileum (Reis de Souza et al., 2000). The post-surgery period lasted for 21 d. The pigs had free access to water during this period, and the amount of feed was gradually increased until it reached the pre-surgery level. Pigs were fed twice a day, at 08:00 and 18:00, with diet 2.5 times their digestible energy requirement for maintenance (110 kcal/kg $BW^{0.75}$) (INRA, 1984).

2.2. Treatments

A sorghum hybrid was used in this study: CB-107, which is a low-tannin sorghum hybrid (<0.5%). Two CB-107 sorghums with different kafirin profile (Table 1) were used. The 2 CB-107 sorghums were low-kafirin (LK) sorghum and high-kafirin (HK) sorghum (Table 1). These sorghums were used to formulate 2 sorghum-soybean meal diets (Table 2). A yellow maize (DAS-3359 hybrid)-soybean meal diet was also formulated and served as a control diet. The 3 diets were formulated to furnish the requirements of digestible AA using the standardized ileal digestibility (SID) coefficient reported by Mariscal et al. (1997). A fourth diet with soybean meal as the sole source of

Table 1
Chemical composition (%, as-fed basis) of raw materials used to formulate the experimental diets and effect of fertilization on chemical composition and kafirins profile[1] of low tannins sorghums.

Item	Raw materials				Difference	Percentage of variation
	Soybean meal	Maize	LK sorghum	HK sorghum		
Energy, kcal/kg	4,283	4,045	3,981	3,940	41	−1.0
Dry matter	89.7	88.3	89.2	89.1	−0.1	−0.1
Protein	44.0	7.7	8.0	9.1	1.1	13.4
NDF	9.5	6.6	10.2	8.5	−1.7	−17.1
Ether extract	0.5	3.7	2.2	2.6	0.4	19.6
Ash		1.1	1.5	2.5	1.0	66.7
Tannins	ND	ND	ND	ND		
Kafirins			4.7	6.7	2.0	42.6
Zeins		5.7				
Kafirins or zeins as protein		74.0	58.8	73.6	14.8	25.2
γ-kafirins			25.1	21.4	−3.7	−14.7
α1-kafirins			26.7	22.3	−4.4	−16.5
α2-kafirins			17.2	18.6	1.4	8.1
β-kafirins			31.0	37.7	6.7	21.6
Total kafirins			100	100		
Alanine	1.83	0.51	0.63	0.79	0.16	25.4
Arginine	3.33	0.36	0.30	0.34	0.04	13.3
Aspartic acid	4.50	0.44	0.22	0.60	0.38	172.7
Cysteine	0.64	0.17	0.17	0.14	−0.03	−17.7
Glutamic acid	7.59	1.23	1.44	1.79	0.35	24.3
Glycine	1.68	0.26	0.21	0.24	0.03	14.3
Histidine	1.31	0.24	0.19	0.21	0.02	10.5
Isoleucine	2.18	0.27	0.33	0.38	0.05	15.2
Leucine	3.36	0.86	1.04	1.25	0.21	20.2
Lysine	3.24	0.32	0.26	0.28	0.02	7.7
Methionine	0.67	0.20	0.17	0.16	−0.01	−5.9
Phenylalanine	2.29	0.37	0.43	0.50	0.07	16.3
Proline	1.93	0.49	0.72	0.44	−0.28	−38.9
Serine	1.93	0.31	0.22	0.34	0.12	54.6
Threonine	1.74	0.26	0.16	0.29	0.13	81.3
Tyrosine	1.83	0.26	0.29	0.34	0.05	17.2
Valine	2.23	0.37	0.40	0.47	0.07	17.5

LK = low kafirins; HK = high kafirins; ND = non detectable.
[1] Kafirins analysis reported in Gómez-Soto et al. (2018).

Table 2
Experimental diets composition (%, as-fed basis).

Item	Diets			
	LK sorghum	HK sorghum	Maize	Soybean meal
Ingredients				
Soybean meal (46%)	25.75	22.45	25.86	38.70
LK sorghum	68.39			
HK sorghum		71.83		
Maize			68.21	
Maize starch				60.16
Soybean oil	2.14	1.86	2.28	
L-lysine	0.41	0.49	0.40	
L-threonine	0.10	0.11	0.09	
DL-methionine	0.14	0.14	0.10	
L-tryptophan		0.01	0.01	
Salt	0.50	0.50	0.50	0.50
Calcium carbonate	0.74	0.75	0.73	
Dicalcium phosphate	1.19	1.22	1.18	
Vitamins[1]	0.24	0.24	0.24	0.24
Minerals[2]	0.10	0.10	0.10	0.10
Titanium dioxide	0.30	0.30	0.30	0.30
Analysis				
Dry matter	90.0	90.0	89.5	90.4
Crude protein	17.6	15.9	16.8	16.1

LK = low kafirins; HK = high kafirins.
[1] Vitamins supplied per kilogram of diets: vitamin A, 4,250 IU; vitamin D_3, 800 IU; vitamin E, 32 IU; menadione, 1.5 g; biotin, 120 g; cyanocobalamin, 16 μg; choline, 250 g; folic acid, 800 g; niacin, 15 g; pantothenic acid 13 g; pyridoxine 2.5 g; riboflavin 5 g; thiamine, 1.25 g.
[2] Minerals supplied per kilogram of diets: $CoCO_3$, 1.43 mg; $CuSO_4 \cdot 5H_2O$, 55.5 mg; $FeSO_4 \cdot H_2O$, 333.3 mg; $C_2H_8N_22HI$, 1.01 mg; $MnSO_4 \cdot H_2O$, 135 mg; Na_2SeO_3, 0.5 mg; $ZnSO_4 \cdot H_2O$, 338 mg.

protein (reference diet) was used to calculate the AID coefficient of proteins and AA of sorghum and maize by the difference method (Fan and Sauer, 1995). All diets were fortified with vitamins and minerals to meet or exceed the requirements of the NRC (2012). Additionally, titanium dioxide (3 g/kg) was added to the diets as an indigestible index for the AID of nutrients.

2.3. Sampling ileal digesta

The experimental period lasted for 7 d (5 d for adaptation and 2 d for collection). Ileal digesta were collected in plastic bags (11 cm × 5 cm), containing 10 mL of 0.2 mol/L HCl solution to block further bacterial activity. Ileal digesta were collected from 08:00 to 18:00 after the attachment of bags to the cannula with a rubber band. When the bags were full, the ileal digesta were transferred to a container and frozen at −20 °C until lyophilization.

2.4. Preparation of samples and chemical analysis

The ileal digesta were lyophilized, ground, and passed through a 0.5-mm mesh of a laboratory mill (Arthur H. Thomas Co., Philadelphia, PA, USA). Experimental diets and ileal digesta were analyzed for dry matter (DM) and protein content using the 934.01 and 976.05 methods of the Association of Official Analytical Chemists (AOAC, 2000), respectively. Fiber fractions were analyzed as described by van Soest et al. (1991). Gross energy was estimated using an adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL). Titanium dioxide content was determined as described by Myers et al. (2004). The samples of raw materials, diets, and digesta were hydrolyzed at 110 °C for 24 h in 6 mol/L HCl for use by the AA analysis method 994.12 (AOAC, 2000). To determine the content of methionine and cysteine, oxidation with performic acid was carried out before acid hydrolysis. The content of AA was determined by ion exchange column chromatography with post-column derivatization as recommended by Csapó et al. (2005).

2.5. Data analysis

The AID of DM, protein, and AA in the experimental diets was calculated using the following equation (Fan and Sauer, 1995):

$$AID_D = 100 - 100 \times [(ID \times AF)/(AD \times IF)] \quad (1)$$

where, AID_D is the AID of a nutrient in the diet (%), ID is the concentration of the index in the diet (mg/kg of DM), AF is the concentration of nutrient in the ileal digesta (mg/kg of DM), AD is the concentration of nutrient in the diet (mg/kg of DM), and IF is the concentration of the index in the ileal digesta (mg/kg of DM). The calculation excluded crystalline AA (L-Lys-HCl, DL-Met, L-Thr, and L-Trp) as they are considered to be completely digested by the end of the ileum (INRA, 2002; INRA et al., 2008).

To estimate the AID of sorghum by the difference method (Fan and Sauer, 1995), we used soybean meal as the basal feed ingredient.

$$AID_{AN} = [AID_{AD} - (AID_{RF} \times L_{RN})]/L_{AN} \quad (2)$$

where, AID_{AN} is the AID of a nutrient in the assay ingredient under the assumption of additivity of digestible or indigestible components (%), AID_{AD} is the AID of a nutrient in the assay diet, AID_{RF} is the AID of a nutrient in the reference feed ingredient, L_{RN} is the contribution of a nutrient in the reference feed ingredient to the assay diet, and L_{AN} is the contribution of a nutrient in the assay ingredient to the assay diet (in a decimal proportion).

2.6. Statistical analyses

Homogeneity of variance for all the data was tested by Levene's test using the test for homogeneity of variances (HOVTEST) of the SAS (SAS Inst. Inc, Cary, NC USA) software. The protein and AA AID data were analyzed as a Randomized Complete Block Design in the general model (Steel and Torrie, 1980):

$$Y_{ij} = \mu + T_i + B_j + e_{ij} \quad (3)$$

where, Y_{ij} is the response of interest variable, μ is the general mean of the population, T_i is the variation attributed to the effect of the treatments, B_j is the variation that is attributed to the blocks, and e_{ij} is the variation of the uncontrolled factors (the experimental error). The comparisons were: maize vs. sorghum and LK sorghum vs. HK sorghum (Steel and Torrie, 1980). The experimental unit was each cannulated pig; and the statistical differences were considered significant at $P < 0.05$.

3. Results

3.1. Chemical composition

High-kafirin sorghum had higher protein content and lower NDF content than LK sorghum, and the relative difference in kafirin content was 42.6% (Table 1). Changes were also observed in the ratio of different types of kafirins in HK sorghum: γ- and α1-kafirins decreased by 14.7% and 16.5%, respectively, and α2- and β-kafirins increased by 8.1% and 21.6%, respectively. Thus, the amino acid profile changed, with increases in amino acid content: from 7.7% for lysine (the amino acid with the lowest increase) to 172.7% for aspartic acid. However, the content of the following 3 AA decreased: proline, cysteine, and methionine.

3.2. Apparent ileal digestibility of experimental diets

The maize-soybean meal diet was more digestible than the sorghum-soybean meal diets, with respect to valine ($P < 0.05$). The AID of valine varied between maize and sorghum diets (Table 3).

Changes in kafirin content between the diets only affected threonine digestibility ($P < 0.01$), which decreased by 9.5 percentage units in LK diet compared with HK diets (Table 3).

3.3. Apparent ileal digestibility of cereals

Maize had higher AID than sorghums, with differences with respect to the AID of valine ($P < 0.01$) and serine ($P < 0.10$) (Table 4). A comparison of sorghums with low and high content of kafirins showed that threonine AID increased ($P < 0.001$) in the latter (50.5 percentage units), as did the AID of serine ($P < 0.05$, 19.2 percentage units).

4. Discussion

An increase in protein content in sorghum due to over-fertilization has been reported previously (Kaufman et al., 2013). This increase indicates the augmentation of all proteins, although the effect is proportionally higher for reserve proteins (Mossé, 1990). This explains the higher proportion of kafirins in over-fertilized sorghum as they are the main reserve proteins (Belton et al., 2006; Nunes et al., 2005). As one protein content changes, the synthesis of other proteins is modified in grains (Kumar et al., 2012), which might explain the observed change in kafirin profile. In the present study, the increase in α2-and β-kafirins in HK sorghum might account for the decrease in the content of proline and cystine as α- and β-kafirins are less abundant in cystine and proline than γ-kafirins (Belton et al., 2006; Shewry and Halford, 2002, 2003). Furthermore, lysine presented the smallest increase, possibly because it is at low content in kafirins; the low content of lysine is a factor that contributes to the low nutritive value of sorghum proteins (Shewry and Halford, 2003). The kafirin content observed in sorghum was within the range reported, as kafirins represent 48% to 84% of the total protein present in sorghum grain (Oria et al., 1995a).

Similar AID of DM (70.2%) and protein (71.5%) were observed in the 3 diets as these diets were formulated taking into account the estimated SID of protein and AA of each feed ingredient (Mariscal et al., 1997). Consequently, the inclusion of crystalline AA differed among the diets. This SID predictive capacity has been previously recognized (Columbus and de Lange, 2012; Mariscal-Landín et al., 2009), and it is known to decrease with dietary fiber content or the presence of anti-nutritional factors (Columbus and de Lange, 2012; Dégen et al., 2007).

The content of kafirins is a factor affecting the digestibility of proteins, AA, and starch in sorghum. Recently, Selle et al. (2018) classified kafirins as the most important factor that negatively influences the performance of broiler chickens offered sorghum-based diets. In particular, γ-kafirins with high cysteine content is resistant to the action of pepsin, affecting the digestibility of AA and energy (Oria et al., 1995b) and have a high affinity of binding to tannins (Taylor et al., 2007). Additionally, γ- and β-kafirins surround the proteins in sorghum grain (Wong et al., 2010). Therefore, if these kafirins have low digestibility, they can reduce the digestibility of proteins, mainly α-kafirins that is located within the protein bodies (Wong et al., 2010). In the present study, there was no evidence that kafirins modulate protein and AA ileal digestibility. This discrepancy with the findings of previous reports (Elkonin et al., 2013; Wong et al., 2009) might be because those studies were performed *in vitro*. Moreover, as previously described (Qiao et al., 2004; Tavano et al., 2016), studies with *in vitro* techniques do not take into account the capacity of the animal to compensate the difficulty to digest a protein by increasing enzyme secretion and the role of the intestinal membrane with brush border enzymes. In the present study, kafirins adversely affected the AID of threonine in diets, and threonine and serine in sorghum, as the lowest digestibility of threonine and serine was observed in LK sorghum, which was richer in γ-kafirins than that in HK sorghum. The γ-kafirins have a more effect on the digestibility of protein bodies than other kafirins as they have spatial stability due to their disulphide bonds (Crevieu-Gabriel, 1999). Therefore, the higher content of γ-kafirins in LK sorghum could cause a more loss of the endogenous proteins in response to its lower digestibility,

Table 3
Apparent ileal digestibility (%) of experimental diets.

Item	Diets			SEM
	LK Sorghum	HK Sorghum	Maize	
Dry matter	71.2	71.4	70.2	1.39
Energy	72.2	72.3	70.6	1.51
Protein	72.4	72.6	73.6	1.97
Alanine	69.9	74.5	72.8	1.95
Arginine	86.7	87.5	88.6	0.80
Aspartic acid	77.9	79.6	79.5	1.99
Cystein	76.8	63.7	69.1	8.80
Glutamic acid	80.0	83.7	83.7	1.81
Glycine	55.0	63.5	62.3	3.34
Histidine	80.9	81.3	82.5	1.27
Isoleucine	78.1	79.8	80.1	1.34
Leucine	79.0	81.0	81.2	1.37
Lysine	79.5	80.2	81.9	1.40
Methionine	61.2	65.3	78.0	6.86
Phenylalanine	78.4	79.8	80.4	1.36
Proline	71.9	73.0	71.4	4.70
Serine	73.2	76.3	77.2	1.70
Threonine[1]	64.5	74.0	69.6	1.83
Tyrosine	78.5	80.0	79.6	1.22
Valine[2]	74.2	76.7	80.4	1.49

LK = low kafirins; HK = high kafirins; SEM = standard error of the mean.
[1] Contrast: LK sorghum diet vs. HK sorghum diet ($P < 0.01$).
[2] Contrast: maize diet vs. sorghums diets ($P < 0.05$).

Table 4
Apparent ileal digestibility (%) of cereals.

Item	LK sorghum	HK sorghum	Maize	SEM
Protein	55.7	57.6	61.1	5.03
Alanine	62.2	70.4	67.7	3.79
Arginine	67.4	74.0	79.2	3.72
Aspartic acid	25.8	64.1	55.9	12.80
Cystein	31.3	29.9	47.9	19.19
Glutamic acid	65.4	76.6	71.9	4.84
Glycine	14.7	43.9	43.9	10.94
Histidine	64.6	68.0	73.2	3.83
Isoleucine	59.8	68.0	66.2	4.54
Leucine	71.6	76.6	75.8	2.98
Lysine	41.6	48.9	60.8	7.00
Methionine	23.8	36.3	68.8	14.80
Phenylalanine	63.7	69.6	68.0	4.29
Proline	73.2	73.7	66.2	11.01
Serine[1, 2]	44.3	63.5	67.8	5.92
Threonine[3]	13.7	64.2	52.1	7.27
Tyrosine	65.1	70.3	68.6	3.93
Valine[4]	56.6	66.0	77.0	4.25

LK = low kafirins; HK = high kafirins; SEM = standard error of the mean.
[1] Contrast: Maize cereal vs. sorghums cereals ($P < 0.10$).
[2] Contrast: LK sorghum cereal vs. HK sorghum cereal ($P < 0.05$).
[3] Contrast: LK sorghum cereal vs. HK sorghum cereal ($P < 0.001$).
[4] Contrast: Maize cereal vs. sorghums cereals ($P < 0.01$).

mainly because endogenous proteins are rich in threonine, proline, serine, and glycine (Mariscal-Landνn and Reis de Souza, 2006; Ravindran, 2016; Reis de Souza et al., 2013). This might explain the comparatively low digestibility of threonine and serine in LK sorghum, and the difference observed in feed efficiency among pigs that were fed diets with low-tannin sorghums and those that were fed a corn diet (Marques et al., 2007). Myrie et al. (2008) reported that the antinutritive factors are mucin secretagogues that increase endogenous amino acid loss. The γ-kafirins are not an antinutritive factor, but its less digestibility can increase endogenous protein loss and consequently endogenous threonine loss as suggested by Mariscal-Landín et al. (2004).

5. Conclusions

The higher protein content in HK sorghum changed its protein profile, decreasing γ- and α1-kafirin content and increasing β- and α2-kafirins content. The higher content of γ-kafirins in LK sorghum negatively affected threonine and serine digestibility. These AA are abundant in endogenous protein, and this could explain their lower AID. Our results show that amino acid ileal digestibility is more affected by the kafirin profile than the kafirin amount. These effects of protein content and kafirin profile on ileal digestibility of protein and AA in sorghum diets have not been previously described *in vivo* in pigs.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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