Original Research Article

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

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A R T I C L E   I N F O

Article history:
Received 7 August 2018
Received in revised form 6 November 2018
Accepted 17 January 2019
Available online 25 February 2019

Keywords:
Amino acids
Ileal digestibility
Kafirin
Pig
Sorghum

A B S T R A C T

The effects of kafrins on protein and amino acid ileal digestibility have not been evaluated in vivo. 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 kafrin profile. Sorghum with greater content of total kafrins had less content of γ- and 1-kafrins and higher content of β- and 2-kafrins than that with lower content of total kafrins. Two sorghum-soybean meal (SBM) diets were formulated: 1) low kafrin (LK) content (32.2 g/kg) and 2) high kafrin (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 BW0.75). 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 kafrin 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. 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 HK and LK 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-kafrins in HK sorghum negatively affected threonine and serine digestibility, implying that the AID of amino acids is affected more by the profile than the content of kafrins.

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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...
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 Involving Animals (CIOMS, 1985) and the Official Mexican Standard for Production, Care and Use of Laboratory Animals (Diario Oficial de la Federación, 2001).

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 of low tannins sorghums.

| Item                        | Soybean meal | Maize | LK sorghum | HK sorghum | Difference | Percentage of variation |
|-----------------------------|--------------|-------|------------|------------|------------|------------------------|
| Energy, kcal/kg             | 4,283        | 4,045 | 3,981      | 3,940      | 41         | –1.0                   |
| Protein                     | 44.0         | 40.7  | 39.5       | 39.1       | –0.1       | –0.1                   |
| NDF                         | 9.5          | 6.6   | 10.6       | 10.5       | 1.1        | 13.4                   |
| Ether extract               | 0.5          | 3.7   | 2.2        | 2.6        | 0.4        | 19.6                   |
| Ash                         | 1.1          | 1.5   | 1.5        | 2.5        | 1.0        | 66.7                   |
| Na/Kafrins or zeins as protein | 5.7       |       | 74.0       | 73.6       | 2.0        | 42.6                   |
| γ-kafrins                   | 25.1         | 21.0  | 23.2       | 21.7       | –3.7       | –14.7                  |
| α1-kafrins                  | 26.7         | 22.3  | 23.6       | 22.8       | –4.4       | –16.5                  |
| α2-kafrins                  | 17.2         | 18.6  | 18.1       | 18.4       | 0.4        | 8.1                    |
| β-kafrins                   | 31.0         | 37.7  | 34.9       | 35.3       | 6.7        | 21.6                   |
| Total kafrins               | 100          |       | 100        | 100        |            |                        |
| Alanine                     | 1.83         | 0.51  | 0.63       | 0.79       | 0.16       | 25.4                   |
| Arginine                    | 3.33         | 0.36  | 0.32       | 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       | 16.5                   |
| 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 kafrins; HK – high kafrins; ND – non detectable.

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 BW0.75) (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 kafirins profile (Table 1) were used. The 2 CB-107 sorghums were low-kafirins (LK sorghum) and high-kafirins (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 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 are not consistent (Mariscal-Landin 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.

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 are not consistent (Mariscal-Landin 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.
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 diet 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 for collection). Ileal digesta were collected in plastic bags (11 cm x 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 using an adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL). Titanium dioxide content was determined 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 van Soest et al. (1991). Gross energy was estimated using an adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL).

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):

\[
AID_D = 100 - 100 \times \frac{[ID \times AF]}{[AD \times IF]} \tag{1}
\]

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.

\[
AID_{AN} = \frac{[AID_{AD} - (AID_{RF} \times L_{RN})]}{L_{AN}} \tag{2}
\]

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 (HOMVTEST) 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):

\[
Y_{ij} = \mu + T_i + B_j + e_{ij} \tag{3}
\]

where, \(Y_{ij}\) is the response of interest variable, \(\mu\) 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-kafrin sorghum had higher protein content and lower NDF content than LK sorghum, and the relative difference in kafrin content was 42.6% (Table 1). Changes were also observed in the ratio of different types of kafrins in HK sorghum: γ- and α-1-kafrins decreased by 14.7% and 16.5%, respectively, and α2- and β-kafrins 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 kafrins 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 kafrins 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 kafrins 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 kafrin profile. In the present study, the increase in $\alpha$- and $\beta$-kafrins in HK sorghum might account for the decrease in the content of proline and cystine as $\alpha$- and $\beta$-kafrins are less abundant in cystine and proline than $\gamma$-kafrins (Belton et al., 2006; Shewry and Halford, 2002, 2003). Furthermore, lysine presented the smallest increase, possibly because it is at low content in kafrins; the low content of lysine is a factor that contributes to the low nutritive value of sorghum proteins (Shewry and Halford, 2003). The kafrin content observed in sorghum was within the range reported, as kafrins 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 kafrins is a factor affecting the digestibility of proteins, AA, and starch in sorghum. Recently, Selle et al. (2018) classified kafrins as the most important factor that negatively influences the performance of broiler chickens offered sorghum-based diets. In particular, $\gamma$-kafrins 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, $\gamma$- and $\beta$-kafrins surround the proteins in sorghum grain (Wong et al., 2010). Therefore, if these kafrins have low digestibility, they can reduce the digestibility of proteins, mainly $\gamma$-kafrins that is located within the protein bodies (Wong et al., 2010). In the present study, there was no evidence that kafrins modulate protein and AA ileal digestibility. This discrepancy with the findings of previous reports (Elkonn 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, kafrins 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 $\gamma$-kafrins than that in HK sorghum. The $\gamma$-kafrins have a more effect on the digestibility of protein bodies than other kafrins as they have spatial stability due to their disulphide bonds (Crevieux-Gabriel, 1999). Therefore, the higher content of $\gamma$-kafrins in LK sorghum could cause a more loss of the endogenous proteins in response to its lower digestibility.
mainly because endogenous proteins are rich in threonine, proline, serine, and glycine (Mariscal-Landvn and Reis de Souza, 2006; Ravindran, 2016; Reis de Souza et al., 2013). This method explains 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 were fed diets with low-tannin sorghums and those that were fed other diets with high-tannin sorghums, and the difference observed in feed efficiency among pigs were not affected by the ka-

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5. Conclusions

The higher protein content in HK sorghum changed its protein profile, decreasing γ- and α1-kafrin content and increasing β- and α2-kafrin content. The higher content of γ-kafrins in HK sorghum negatively affected threonine and serine digestibility. These AA are abundant in endogenous protein, and this could explain their lower digestibility.
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