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Technical note

Standardized ileal digestibility of protein and amino acids of sesame meal in growing pigs

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Abstract:

To determine the apparent (AID) and standardized ileal digestibility (SID) of the amino acids of sesame meal (SM), 10 pigs of 78.6 ± 5.2 kg, housed in metabolic cages, were used; located in a room with controlled temperature (19 to 22 °C). The pigs were implanted with a “T” cannula in the ileum and fed twice a day, at 2.5 times their digestible energy requirement for maintenance (110 kcal per kg of $LW^{0.75}$). Two diets were prepared with 160 g of CP/kg of feed: one with SM and one with soybean meal (SBM). The results show that the AID of the following amino acids was higher in SM than in SBM: arginine (P<0.0001) 7.3 units; alanine, glutamic acid, glycine, methionine and valine, it was on average 6.8 units higher (P<0.01); cysteine, it was higher by 11.5 units (P<0.05). On the contrary, the AID of proline (P<0.0001), leucine (P<0.01) and lysine (P<0.05) was lower by 21.9, 2.8 and 2.5 units, respectively, in SM than in SBM. The SID of arginine (P<0.0001) was 6.7 % higher; valine (P<0.001) 10.6 % higher, alanine, glutamic acid, glycine and threonine (P<0.01), it was higher on average by 6.4 %; and cysteine, histidine, isoleucine, and tyrosine (P<0.05), it was 7.45 % higher in SM than in SBM. The SID of proline (P<0.01) and leucine (P<0.05) was lower by 4.7 and 2.1 SM than in SBM. It is concluded that SM is a good source of digestible amino acids for pig feeding.

Key words: Sesame meal, Ileal digestibility, Amino acids, Pigs.

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The high cost of soybean meal has led to the search for alternative sources of protein to be used in pig feeding(1). Within these alternative sources, sesame is characterized by its high content of oil, 40 to 50 %, and 27 % protein(2). As a by-product of the extraction of sesame oil, sesame meal is obtained, which is characterized by containing between 45 and 50 % protein, 10 to 12 % ether extract in the meal obtained by pressure and between 1 and 2 % in that obtained with solvents(3). This meal is characterized by being a good source of amino acids, mainly sulfur amino acids, arginine and leucine; however, it is poor in lysine(3). The use of a protein source depends on its contribution of amino acids, since feeds are formulated using the concepts of ideal protein(4) and standardized ileal digestibility of amino acids(5), the way in which the nutritional requirements of the pig are expressed(6,7). The use of these concepts and the availability of crystalline amino acids (Lysine-HCL, L-Threonine, L-Tryptophan and DL-Methionine) have allowed the inclusion of different protein sources in the pig's diet, although these sources are deficient in some of those amino acids, as would be the case of sesame meal with respect to lysine. However, the available information on the digestibility of protein and amino acids of sesame meal in pigs is scarce and contradictory, as low digestibility of sesame amino acids has been reported in growing pigs(8). Therefore, the objective of this work was to determine the ileal digestibility in growing pigs of the amino acids of sesame meal obtained by pressure, since the existing information has been obtained in meals extracted with solvents.

The work was carried out in the experimental farm of CENID Physiology of INIFAP. The protocol was reviewed and approved by the Bioethics Committee of the Faculty of Natural Sciences of the Autonomous University of Querétaro. The handling used in the animals respected the guidelines of the Official Mexican Standard for the production, care and use of laboratory animals(9), as well as those of the International Guiding Principles for Biomedical Research Involving Animals(10).

Ten pigs (castrated males) from a cross (Landrace × Large-White) were used, with an average weight of 78.6 ± 5.2 kg, divided into two groups of 5 pigs. The pigs were housed in individual metabolic cages, provided with a feeder and drinker; located in a temperature controlled room, which fluctuated between 19 and 22 °C. The four days after their entry to the experimental unit served as a period of adaptation to the metabolic cages; for the first three days, they were offered the diet they previously consumed; on the fourth day, they fasted and on the fifth day, the pigs were implanted with a "T" cannula at the level of the terminal ileum(11). The post-surgical period lasted 21 days, in which the pigs had free access to water and the feed offered during that period was gradually increased until reaching pre-surgery consumption. During the experimental period, the pigs were fed twice a day at the rate of 2.5

times their digestible energy requirement for maintenance, which was estimated at 110 kcal per kilo of $LW^{0.75}$[12].

Sesame meal was compared with soybean meal (Table 1). Two experimental diets were formulated with these raw materials (Table 2), a diet with sesame meal (SM) and another with soybean meal (SBM), both diets provided 160 g of CP/ kg of feed; the level of inclusion of both meals depended on their protein content. To both diets, sucrose was added at the rate of 65 g/kg to increase their palatability, a source of fiber (Arbocel™) 40 g/kg and corn oil at a rate of 30 g/kg. Vitamins and minerals were added to provide or exceed the requirements recommended by the NRC[6], chromium oxide was included at the rate of 3 g/kg as a digestibility marker.

Table 1: Composition of the raw materials

	Sesame meal	**Soybean meal**
Crude protein	53.50	50.10
Ether extract	11.10	1.30
NDF	17.50	13.80
TIA, mg TIA/g[1]	1.00	6.00
PA, g sodium phytate/100 g[2]	4.00	2.80
Alanine	1.90	2.00
Arginine	4.90	3.40
Aspartic acid	3.60	5.40
Glutamic acid	7.50	9.30
Glycine	2.10	2.20
Histidine	1.00	1.20
Isoleucine	1.50	2.20
Leucine	2.80	3.40
Lysine	1.00	2.80
Methionine + Cysteine	2.10	1.40
Phenylalanine	1.80	2.50
Proline	1.60	3.70
Serine	1.90	2.30
Threonine	1.50	1.90
Tyrosine	1.60	1.80
Valine	1.90	2.30

[1]TIA= trypsin inhibitor activity.
[2]PA= phytic acid.

Table 2: Composition of experimental diets

	Sesame meal	**Soybean meal**
Corn starch	53.60	46.37
Soybean meal		36.40
Sesame meal	30.00	
Sugar	6.50	6.50
Cellulose[1]	4.00	4.00
Corn oil	3.00	3.00
Salt	0.40	0.40
Calcium carbonate	0.07	1.10
Dicalcium phosphate	1.90	1.70
Mineral premix[2]	0.07	0.07
Vitamin premix[3]	0.16	0.16
Chromium oxide	0.30	0.30
Chemical analysis:		
Crude protein	16.30	16.10
NDF	8.80	11.90

[1]Arbocel= fiber concentrate.

[2]Mineral premix= it provides the following amounts per kilo of feed: Co, 0.60 mg; Cu, 14 mg; Fe, 100 mg; I, 0.80 mg; Mn, 40 mg; Se, 0.25 mg; Zn, 120 mg.

[3]Vitamin premix= it provides the following amounts per kilo of feed: vitamin A, 4,250 IU/g; vitamin D3, 800 IU/g; vitamin E, 32 IU/g; vitamin K_3 menadione, 1.5 mg/kg; biotin, 120 mg/kg; cyanocobalamin, 16 µg/kg; choline, 250 mg/kg; folic acid, 800 mg/kg; niacin, 15 mg/kg; pantothenic acid 13 mg/kg; pyridoxine 2.5 mg/kg; riboflavin 5 mg/kg; thiamine, 1.25 mg/kg.

Water was provided freely through a nipple drinker located on a wall of the metabolic cage. The experimental period lasted 7 d (five days of adaptation to the diet and two for the collection of the ileal digesta). The ileal digesta was collected in plastic bags (11 cm long × 5 cm wide), 10 ml of a solution of HCl 0.2 M was added to the bags in order to block all bacterial activity. The bags were fixed to the cannula with a band at 0800 h on day one and the ileal digesta was collected from 0800 to 1800 h. As the bags were filled with the ileal digesta, this was transferred to a container to proceed immediately to freeze it at -20 °C until lyophilization.

Digesta samples from the experiment were lyophilized and subsequently ground through a 0.5 mm mesh with a laboratory mill (Arthur H. Thomas Co. Philadelphia, PA). The following analyses were performed on the experimental diets and ileal digesta samples: dry matter (DM) and crude protein (CP) according to methods 934.01 and 976.05 of the AOAC(13), chromium oxide according to Fenton and Fenton(14). The preparation of the samples for the determination of AA was carried out following method 994.12 of the AOAC(13), which consists of hydrolyzing the samples at 110 °C for 24 h in HCl 6M; in the case of methionine and cysteine, a previous oxidation with performic acid was carried out. AA analyses were performed by reversed-phase chromatography according to the method described by Henderson *et al*[15] on a Hewlett Packard HPLC, model 1100. The trypsin inhibitor activity (TIA) of the raw materials and experimental diets was determined according to the method described by Kakade *et al*[16]; phytic acid was analyzed according to Vaintraub and Lapteva[17]; and neutral detergent fiber (NDF) was analyzed according to van Soest *et al*[18].

Calculations to estimate the apparent ileal digestibility (AID) of protein, amino acids, and energy of the experimental diets were performed using the equation used by Fan and Sauer[19].

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

Where AID is the apparent ileal digestibility of a nutrient in the diet in percentage, ID is the concentration of the indicator in the diet (mg/kg of DM), AF is the concentration of the nutrient in the ileal digesta (mg/kg of DM), AD is the concentration of the nutrient in the diet (mg/kg of DM), IF is the concentration of the indicator in the ileal digesta (mg/kg of DM).
Calculations to estimate the standardized ileal digestibility (SID) of protein and amino acids were performed using the formula proposed by Furuya and Kaji[20].

$$SID = AID +[(EndoN/ConsN) \times 100]$$

Where SID is the standardized ileal digestibility of a nutrient in percentage. AID is the apparent ileal digestibility of a nutrient. EndoN is the endogenous amount excreted of the nutrient in mg/kg of dry matter consumed. ConsN is the amount of nutrient consumed in mg/kg of dry matter consumed.

For the calculations, the endogenous reported by Mariscal-Landín and Reis de Souza[21] was used.

Data on the AID and SID of protein and amino acids in growing pigs were analyzed using the GLM procedure of the SAS statistical package[22], according to a completely randomized design[23]. Treatment means were compared using Tukey's method[23]. The differences were considered significant when (P<0.05), and a trend was recognized when (0.05<P<0.10).

The results show that the pigs consumed completely their ration. Sesame meal had 6.8 % more CP; 42.9 % more phytic acid and 26.8 % more NDF than soybean meal. The ether extract content of sesame meal was 8.5 times higher (111 *vs* 13 g/kg) than that of soybean meal (Table 1). On the contrary, the trypsin inhibitor content was 6 times higher in soybean meal (6 mg/g) than in sesame meal (1 mg/g). Regarding the total amino acid content, sesame meal had 50 % more sulfur amino acid content (methionine + cysteine) and 44 % more arginine than soybean meal. In contrast, soybean meal had a lysine content 280 % higher than sesame meal, as in the case of proline 231 %; as well as 50 % more aspartic acid, 46 % more isoleucine, 38 % more phenylalanine and 26 % more threonine.

The AID of the crude protein of sesame meal was higher (*P*<0.01) by 5.6 percentage units than that of soybean meal (Table 3). The AID of arginine was higher (*P*<0.0001) by 7.3 percentage units in sesame meal than in soybean meal; the digestibilities of alanine, glutamic acid, glycine, methionine and valine were on average 6.8 percentage units higher (*P*<0.01) in sesame meal than in soybean meal. The AID of cysteine was higher by 11.5 percentage units (*P*<0.05) in sesame meal than in soybean meal. On the contrary, the AID of proline (*P*<0.0001), as well as that of leucine (*P*<0.01) and lysine (*P*<0.05) was lower by 21.9, 2.8 and 2.5 percentage units, respectively, in sesame meal than in soybean meal (Table 3).

Table 3: Apparent ileal digestibility

	Sesame meal	**Soybean meal**	**Probability**	**SEM**
Crude protein	83.8 [a]	78.2 b	0.01	0.8
Alanine	76.1 [a]	67.8 [b]	0.01	0.8
Arginine	92.9 [a]	85.6 [b]	0.0001	0.4
Aspartic acid	83.6	85.3	NS	0.4
Cysteine	72.9 [a]	61.6 [b]	0.05	2.3
Glutamic acid	90.5 [a]	87.1 [b]	0.01	0.4
Glycine	82.1 [a]	77.0 [b]	0.01	0.6
Histidine	90.8 [a]	87.1 [b]	0.05	0.7
Isoleucine	82.8	81.4	NS	0.5
Leucine	84.4 [b]	87.2 [a]	0.01	0.4
Lysine	91.6 [b]	94.1[a]	0.05	0.4
Methionine	83.4 [a]	72.4 [b]	0.01	1.3
Phenylalanine	82.2	81.2	NS	0.5
Proline	59.6 [b]	81.5 [a]	0.0001	0.5
Serine	84.1	83.6	NS	1
Threonine	76.9	76.0	NS	0.4
Tyrosine	76.2	72.9	NS	0.7
Valine	78.5 [a]	72.0 [b]	0.01	0.7

SEM= Standard error of the mean.

The SID of the protein of sesame meal was higher (*P*<0.01) by 6.4 %. The SID of arginine (*P*<0.0001) was 6.7 % higher; of valine (*P*<0.001) 10.6 % higher, of the amino acids alanine, glutamic acid, glycine and threonine (*P*<0.01), it was higher on average by 6.4 %; and the SID of cysteine, histidine, isoleucine and tyrosine (*P*<0.05) was 7.45 % higher in sesame meal than in soybean meal. However, the SID of proline (*P*<0.01) and leucine (*P*<0.05) was 4.7 % and 2.1 % lower in sesame meal compared to those in soybean meal (Table 4).

Table 4: Standardized ileal digestibility

	Sesame meal	**Soybean meal**	**Probability**	**SEM**
Crude protein	91.6[a]	86.1[b]	0.01	0.8
Alanine	83.4[a]	75.2[b]	0.01	0.8
Arginine	95.8[a]	89.8[b]	0.0001	0.4
Aspartic acid	88.3	88.4	NS	0.4
Cysteine	75.9[a]	65.0[b]	0.05	2.3
Glutamic acid	94.2[a]	90.5[b]	0.01	0.4
Glycine	88.8[a]	83.5[b]	0.01	0.6
Histidine	93.8[a]	90.2[b]	0.05	0.7
Isoleucine	90.5[a]	87.3[b]	0.05	0.5
Leucine	89.4[b]	91.3[a]	0.05	0.4
Lysine	95.5	96.2	NS	0.4
Methionine	86.3	76.2	NS	1.3
Phenylalanine	86.00	84.2	NS	0.5
Proline	86.3[b]	90.6[a]	0.01	0.5
Serine	94.0	91.5	NS	1
Threonine	88.2[a]	84.5[b]	0.01	0.4
Tyrosine	81.6[a]	77.6[b]	0.05	0.7
Valine	86.7[a]	78.4[b]	0.001	0.7

SEM= Standard error of the mean.

According to the FAO, the world production of sesame was 6’448,961 metric tons in 2018, with Mexico ranking 15th with a production of 57,256 t, FAO STAT[(24)]. Sesame is mainly grown as an oil source, as it contains on average 44 to 58 % oil, but it is also rich in protein 18 to 25 % CP[(25)]. Sesame oil is characterized by being very stable, due to the presence of natural antioxidants (sesamolina, sesamin and sesamol)[(26)]. Sesame proteins consist predominantly of four protein fractions, which are designated according to their molecular weight as 2S, 7S, 11S (low, medium and high molecular weight) and 15-18S (polymeric proteins resulting from a possible aggregation of 2S, 7S or 11S)[(27)]. High-molecular-weight proteins are the main ones in sesame, and they are characterized by being rich in glutamic acid and aromatic amino acids, and low in lysine; in addition to having a low proportion of the α-helix conformation and a high proportion of βeta sheet(27); the sesame protein is characterized by being rich in arginine(28). Globulin 11S (insoluble in water) and albumin 2S (soluble) are called α-globulin and β-globulin respectively; and they are the two main storage proteins of sesame, constituting between 80 and 90 % of the total sesame proteins(29).

It has been reported that phytic acid can interfere with protein digestibility due to its chelating ability(30). Phytate is formed during the maturation period of the plant and its function is to be storage of P and minerals, playing an important role in the metabolism of seeds during germination. In addition, myo-inositol (the chemical component of the phytate molecule) is used for the formation of cell walls(31). Sesame is characterized by containing high levels of phytate, (14.6 g of phytate-P/kg of seed, up to 51.8 g of phytate/kg of meal)(28). The phytate molecule contains twelve reactive protons, six can dissociate at acidic pH, three at neutral pH and the remaining three at basic pH, allowing it to bind with charged molecules from the diet and with endogenous secretions such as digestive enzymes and mucin in all pH conditions found in the intestine(32). Among the amino acids that are most easily chelated by phytate are the basic amino acids and richness in arginine of the sesame protein was already previously mentioned(28,30). However, the pepsin digestibility of sesame protein isolate is high 89.57 %; so, it can be assumed that what would negatively modulate the digestibility of sesame protein is the amount of phytate.

The AID of protein was 83.8 and the average AID of sesame amino acids was 81.7, very similar to the average AID of soybean meal amino acids, which was 79.6; however, differences were observed in some amino acids, with the AID of leucine, lysine and proline being higher in the case of soybean meal. In the case of lysine, its higher AID may be due to the higher lysine content in soybean meal: 2.8 times higher than that of sesame meal. A similar situation is observed in the case of arginine (since the AID of arginine in the sesame meal was higher by 7.3 percentage units), methionine (the AID in the sesame meal was higher by 11.0 percentage units) and cysteine (the AID in the sesame meal was higher by 11.3 percentage units). In the three cases, the sesame was richer: arginine 30.6 %; sulfur amino acids 50.0 %. The AID of the protein and amino acids reported in the present work are similar to those previously reported(33,34,35).

Regarding the SID of the protein and amino acids of sesame, this was higher by 5.5 and 3.8 percentage units respectively, maintaining the difference in the SID of arginine, which was higher by 6 percentage units in sesame than in soybean meal and in the case of the SID of lysine, this was similar in sesame and soybean meal; similarly, the SID of the sesame amino acids reported in the present work are similar to those previously reported[33,34,35]. The difference found in the SID of the protein and amino acids of the sesame meal reported by Son *et al*[8] could be due to the quality of the sesame meal that they used in their study, since the same authors mention that the low digestibility could be due to a different process of extraction of the oil (without particularizing in it); and to the content of NDF, since the sesame meal used by Son *et al*[8] was much richer in NDF, which contained 28 % NDF and the one used in this work contained 10 percentage units less NDF (17.5 %); although it is known that fiber increases endogenous amino acid losses[36,37], affecting, in some cases, their ileal digestibility[36]. However, despite its high content of phytates, the good digestibility of the protein and amino acids of sesame meal allows it to be used in the feeding of pigs[38] and poultry[39] at any productive stage, without impairing the productive aspect.

The results allow concluding that sesame meal is an alternative source of protein and digestible amino acids for pig feeding, since the average SID of its amino acids is 88.5 %. In addition to being a rich source of arginine and sulfur amino acids.

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