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Amino Acid Content and Availability in Low, Medium and High Tannin Sorghum Grain for Poultry

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Abstract: In order to assess and determine amino acids profile and availability in different sorghum grain (SG) varieties, three SG varieties including low tannin (0.09%, LTS), medium tannin (0.19%, MTS), and high tannin (0.37%, HTS) were grown in the same location. The grains were analyzed for their proximate analyses, tannin, and amino acids. True amino acid availability (TAAA) was obtained by caecectomized single comb leghorn cockerels and Sibbald's method. The results showed that the first and the second limiting amino acids in LTS and MTS were methionine and cystine, respectively. Whereas in HTS, lysine (0.166%) was the first and methionine (0.176%) was the second limiting amino acid. Methionine contents in LTS, MTS, and HTS were 0.114%, 0.182%, and 0.176%, respectively. Although crude protein was lower in HTS (10%) than the other SG, histidine, methionine, and isoleucine were higher for HTS as compared to LTS and MTS. TAAA decreased when tannin content increased and all amino acids had a lower availability in HTS than in LTS and MTS ($p<0.05$). Methionine in HTS and LTS had the highest availability as had glutamic acid in MTS. The availability of proline was most affected by tannin, which was 91.55% for LTS, 84.82% for MTS, and 22.82% for HTS ($p<0.05$).

Key words: Tannin, sorghum grain, amino acid, poultry diet

Introduction

The nutritional importance of sorghum grain in human food and animal and poultry feed is well established worldwide. This is reflected in the fact that in terms of cultivated area on a global scale, sorghum grain ranks the fifth among cereals and in Asia it ranks the third only after rice and corn (Ravidran and Blair, 1991). Recent droughts and increasing world prices of corn and barley have caused both farmers and poultry and livestock breeders in Iran to pay more attention to substitute cereals, especially to sorghum. Numerous studies have been carried out over the years on the possibility of replacing sorghum for corn in poultry diets and different results have been reported in the literature (Gualtieri and Rapaccini, 1990). Variety, climate conditions, soil and fertilizers types are listed among the factors involved in the chemical composition and nutritional value of sorghum. In one study carried out on 36 varieties of sorghum cultivated in Iran, the protein content was reported to vary from 9.5 to 14, ADF from 3 to 18, fat from 1.5 to 4.5, and tannin from 0.02 to 1 percent. Also the range of metabolizable energy (AMEn) varied from 2750 to 3540 Kcal/Kg for LTS and HTS varieties, respectively (Ebadi *et al*., 1998). Chang and Fuller (1964) reported that HTS varieties and dark brown sorghums with high tannin contents had lower nutritional values. Later studies, however, showed that grain color was not a relevant index of nutritional value in sorghum varieties (Damron *et al*., 1968; Stephenson *et al*., 1970). Even though sorghum enjoys a higher protein content than corn, its protein quality is lower (Gualtieri and Rapaccini, 1990; Diygkas *et al*., 1991). Amino acid contents in hybrid sorghum varieties are considerably different (Deyoe and Shellenberger, 1965; Hullan and Proudfoot, 1982). Only after cystine, tryptophan, whose content is higher in sorghum than in corn but lower than in wheat (Hullan and Proudfoot, 1982), is considered to be the first limiting amino acid in sorghum. Despite identical sulfur amino acid contents in sorghum and corn, these amino acids seem to have a lower availability in sorghum as compared to those in corn (Diygkas *et al*., 1991). Workers have reported reduced nitrogen and protein retention due to consumption of tannic acid. They have reported that activities of the intestinal proteolytic enzymes in the intestinal contents of the rats with 5% tannic acid in their diets were three times higher than those in the control group. They also found that a major portion of the excreta protein in these rats had an endogenous origin (Elkin *et al*., 1990). Stephenson *et al*. (1970), Using total excreta collection method in chicks, determined the digestibility values of amino acids in 24 varieties of sorghum. They concluded that vast

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differences existed in amino acid digestibility values in one hybrid and also among different hybrids. When they supplemented one or two limiting amino acids to the diets containing HTS the negative effects of these varieties on poultry performance were lifted and they were able to obtain similar results with those obtained from low tannin varieties or even from experiments with corn (Rostango *et al*., 1973; Elkin *et al*., 1990). The results showed that there are differences in both the profile and availability of amino acids among various varieties. It is essential, therefore, to have adequate information in order to identify, select, and apply different varieties of sorghum in livestock and poultry diets. The present study was carried out to determine the amino acid profile and also the effects of tannin quantities in Iranian sorghum varieties on amino acids availability.

Materials and Methods

Twelve varieties of grain sorghum were grown at Shahid Fozveh Research Station affiliated to Isfahan Research Center for Agricultural & Natural Resources. Growing conditions were kept identical to avoid any environmental differences such that the same irrigation and fertilizing practices were employed for all samples. After harvesting of the plants at maturity, the grains were removed from their clusters and two random samples from each variety were transferred to lab for proximate analyses. Dry matter, crude fibers, crude fat, and crude protein of all varieties were determined and the tannin contents were measured according to Folin Denis method (AOAC, 1990). From the twelve varieties, three low, medium, and high-tannin varieties with tannin contents of 0.09, 0.19 and 0.37%, respectively, were selected to determine the amino acid profile and availability. The ingredients were analyzed for amino acid availability using precision fed caecectomized roosters assay of Sibbald's method (Sibbald, 1986). At 30 wk of age, fifty Single Comb Leghorn roosters were subjected to caecectomy according to Parson's method (Parsons, 1985), and were not used in availability trials for at least 10 wk following surgery. After recovery period, the birds were weighed and twenty four roosters weighing around 2100±100g were selected for availability trials. Following a 24-h period of feed deprivation, six roosters were randomly given 30g of feedstuff via crop intubation. Six additional roosters were fed with 30 g of glucose to measure endogenous amino acids excreted (Green *et al*., 1987; McNab and Blair, 1988). After feeding, the birds were returned to their cages and an excreta collection tray was placed under each cage. The excreta were collected over a 48-hour period. After removing external materials and feathers from the trays, the excreta from each bird was placed into special bags and stored in a freezer. The moisture content of the excreta was removed by freeze-drying and, after weighing, the excreta were ground and sieved through a 1mm sieve. Amino acid concentrations in sorghum grains and excreta samples were analyzed by ion exchange chromatography following hydrolysis in 6 N HCL for 24-h at 110°C in sealed tubes, with at least two replicates per samples. Derivation with ninhydrin was accomplished (Andrews *et al.*, 1985) and the quantity of each amino acid was determined using the Bechman Biocrom 20 Amino Acid Analyzer at the University of Manitoba, Canada. Methionine and cystine were determined on samples that had been oxidized in performic acid prior to acid hydrolysis (Moore, 1963). Tryptophan was determined by alkaline hydrolysis (Hugli and Moore, 1972). True amino acids availability were calculated by the method of Sibbald (1986), with digestibility referring the amount of dietary amino acid not appearing in the feces plus urine. Analysis of variance using the General Linear Model (GLM) procedure of SAS (SAS Institute, 1999) was performed on data where appropriate. Treatment mean differences were tested ($P < 0.05$) by Duncan multiple-range test (Snedecore and Cochran, 1980).

Results and Discussion

The quantities of the amino acids in the three sorghum varieties determined in this study are compared with the values reported by NRC (1994) in Table 1. The results show that these three varieties are considerably different in terms of their amino acid contents, especially in the case of those generally regarded as limiting amino acids in poultry. Methionine content varied from 0.114% in low-tannin to 0.182% in medium-tannin, and 0.176% in high-tannin SG varieties, with a difference of 0.068% between the low and high-tannin varieties. The values obtained in the three SG varieties studied ware around 0.04% lower and higher than those reported by NRC (1994). The other sulfur amino acid, i.e. cystine, recorded lower differences among the three SG varieties (0.015%) but all three varieties had considerably higher cystine contents as compared to those reported by NRC such that the cystine content in the varieties in the present study showed to be about two times the cystine content in the variety containing more than high protein sorghum reported by NRC (0.205% vs. 0.11%). Methionine and cystine were the first and the second limiting amino acids in the LTS and MTS, respectively. This is while in the HTS, lysine was the first and methionine is considered to be the second limiting amino acid. Stephenson *et al.* (1970) also reported methionine and cystine to be the first and the second limiting amino acids in the 24 SG varieties studied. This observation corresponds to the low- and medium-tannin varieties in the present study. Douglas and Sullivan (1991) reported the percentages for cystine in low- and high-tannin SG proteins to be 2.2 and 1.9%, respectively, which corresponds to the values obtained in this study. However, methionine content in these varieties is found

Table 1: Amino acid compositions for different SG varieties[1] (air dried percentage)

	Sorghum Grain Varieties						NRC, 1994			
	LTS		MTS		HTS		CP < 10 %		CP > 10 %	
Amino acid	% of total	% of CP	% of total	% of CP	% of total	% of CP	% of total	% of CP	% of total	% of CP
Aspartic acid	0.82	7.21	0.89	7.58	0.75	7.49	0.29	3.18	0.33	3
Threonine	0.34	2.99	0.39	3.23	0.34	3.45	0.4	4.39	0.45	4.09
Serine	0.51	4.44	0.56	4.73	0.47	4.69	N/A	N/A	N/A	N/A
Glutamic acid	2.28	20.03	2.71	22.98	2.32	23.16	N/A	N/A	N/A	N/A
Proline	0.83	7.29	1.08	9.19	0.78	7.80	N/A	N/A	N/A	N/A
Glycine	0.20	2.64	0.30	2.58	0.29	2.92	0.31	3.4	0.32	2.9
Alanine	1.02	8.91	1.24	10.5	1.08	10.82	N/A	N/A	N/A	N/A
Cystine	0.19	1.73	0.21	1.80	0.21	2.06	0.17	1.86	0.11	1
Valine	0.53	4.64	0.63	5.30	0.59	5.9	0.44	4.83	0.54	4.90
Methionine	0.11	1	0.18	1.54	0.18	1.76	0.16	1.75	0.15	1.36
Isoleucine	0.41	3.64	0.44	3.71	0.47	4.68	0.35	3.84	0.43	3.9
Leucine	1.31	11.5	1.50	12.73	1.33	13.37	1.14	12.5	1.37	12.45
Tyrosine	0.40	3.53	0.42	3.59	0.38	3.76	0.34	3.73	0.17	1.54
Phenylalanine	0.52	4.54	0.57	4.81	0.47	4.68	0.47	5.16	0.52	4.72
Histidine	0.22	1.89	0.23	1.95	0.30	2.96	0.22	2.41	0.23	2.09
Lysine	0.21	1.88	0.21	1.82	0.17	1.66	0.21	2.31	0.22	2
Arginine	0.32	2.85	0.33	2.83	0.27	2.68	0.35	3.84	0.35	3.18
Tryptophan	0.23	2.05	0.27	2.27	0.24	2.39	0.8	8.79	0.09	0.81
CP	11.4		11.8		10		9.1		11	

Each value designates the average of two replications. N/A, no data were available

in the present study to be far lower than those reported by Douglas and Sullivan (1.76 vs. 2.1% in protein). Stephenson *et al.* (1970) also reported the average methionine content in the varieties under study to be 0.2% but it ranged from 0.15 to 0.24% in the 24 SG varieties they studied. The lysine content found in the present study for LTS and MTS was identical to that reported by NRC but its content in the HTS was lower than the NRC value. Also, on the basis of proportion of amino acids to protein, lysine in all three SG varieties in this experiment had lower proportion of lysine to protein compare to NRC values. Higher proportion of lysine to protein in SG have been reported by other investigators. For instance, Douglas and Sullivan (1991) reported 2.3 and 2.1% of lysine to protein for low- and high-tannin varieties, respectively. Okoh *et al.* (1989) found the lysine ratio in sorghum protein to be 2.74%. There were some differences for threonine and phenylalanine among different varieties and with other reports (Okoh *et al.,* 1989; Douglas and Sullivan, 1991). Unlike the two former amino acids, arginine contents in the LTS and MTS were almost similar to those reported by NRC but their percentages in the protein section were lower than the NRC values and those reported in other studies (Okoh *et al* .,1989; Douglas and Sullivan, 1991; NRC, 1994). Glutamic acid formed the highest protein part in these varieties (22%), which agrees well with values reported by Okoh *et al*. (1989) but was higher by about 5% than those reported by Douglas and Sullivan (1991). Despite the fact that the protein content and most amino acids were lower in HTS, most amino acids in the HTS made a higher proportion of the protein as compared to the LTS and MTS. This observation indicates that variations in the percentage and ratio of amino acids don't match the variations in crude protein (NRC, 1994) and that, as shown in Table 1, the quantities of histidine, isoleucine, and methionine in the HTS are higher than the same in the other two varieties.

The differences in the availability of amino acids (Table 2) were even greater than was the variability of the total content. Increasing tannin content corresponded to a decreasing availability value for all amino acids such that the average amino acid availability was 96.07% ± 3.4 in the LTS, 87% ± 7.4 in the MTS, and 46.87% ± 13.5 in the HTS variety. Average amino acid availability values for low and medium-tannin varieties in this experiment were found to be higher than those in previous studies (Rostango *et al*., 1973; Elkin *et al*., 1990). Elkin *et al.* (1990) obtained the amino acid availability values of 79% for low-tannin varieties and 68.5% for high-tannin varieties. They also reported a negative and significant correlation between tannin content and amino acid availability ($p < 0.001$; $r = -0.97$). These differences may be due to differences in quantities or to tannin measurement methods (Hullan and Proudfoot, 1982; Gualtieri and Rapaccini, 1990). Other sources of differences may be whether intact or caecectomized birds were used or ileum digestibility was measured

Table 2: Actual amino acid availability in different SG varieties (%)

Amino acid[1]	Sorghum Grain varieties			
	LTS	MTS	HTS	SEM[2]
Aspartic acid	97.55[a]	93.27[a]	53.53[b]	0.041
Threonine	96.10[a]	85.85[a]	41.52[b]	0.058
Serine	96.67[a]	89.67[a]	45.20[b]	0.058
Glutamic acid	98.30[a]	95.45[a]	49.15[b]	0.054
Proline	91.55[a]	84.82[a]	22.82[b]	0.075
Alanine	98.12[a]	93.37[b]	45.20[c]	0.056
Cystine	96.45[a]	82.85[b]	54.27[c]	0.046
Valine	94.32[a]	86.15[b]	52.70[c]	0.045
Methionine	98.34[a]	92.60[a]	75.97[b]	0.039
Isoleucine	96.60[a]	87.47[b]	55.90[c]	0.043
Leucine	98.30[a]	87.42[b]	38.80[c]	0.064
Tyrosine	98.37[a]	89.30[b]	24.72[c]	0.077
Phenylalanine	96.75[a]	92.72[a]	33.75[b]	0.066
Histidine	85.75[a]	66.97[b]	45.25[c]	0.048
Lysine	96.00[a]	78.60[b]	65.16[c]	0.040
Arginine	96.32[a]	79.55[b]	44.52[c]	0.057
Average ± SD	96.07 ± 3.4	87 ± 7.4	46.78 ± 13.5	

[1]Glycine and Tryptophan present in excreta were not determined. [2]Standard Error of Mean

[a-c]Means within rows with different superscripts are significantly different ($p < 0.05$).

(Parsons, 1985). Barnabas *et al.* (1985) reported a value of 43-73% for amino acids availability in high-tannin variety and 84-93% for low-tannin varieties, which is in good agreement with the results from this investigation. Availability of all amino acids were significantly ($P < 0.05$) lower in HTS.

Glick and Joslyn (1970) observed a 3 to 4-fold increase in the level of activity of intestinal proteolytic enzymes of rats fed tannic acid. So, the lower amino acid availability for HTS can be accounted for by the increased excretion of endogenous amino acids. The highest availability values were recorded for methionine in the low- and high-tannin varieties and for glutamic acid in the medium-tannin variety. Except for methionine, which showed no significant differences in terms of availability for the low- and medium-tannin varieties, the two varieties showed significant differences for other essential amino acids ($P < 0.05$). Histidine had the lowest availability in the LTS and MTS but in the HTS variety, proline was most affected by tannin content and had the lowest availability such that it showed the greatest range of variation in availability, showing a reducing trend of 91.6% for the low-tannin to 22.8% in the high-tannin variety ($P < 0.05$). Investigations have shown that tannin preferentially binds to peptide bonds adjacent to proline (Damron *et al*., 1968; Hagerman and Butler, 1981). Stephenson *et al*. (1970) also found the greatest effect of tannin on proline. They reported its availability to be 19.4% for high-tannin to 93.3% for low-tannin varieties. At least two mechanisms have been proposed for the preventive effect of tannin on the amino acids transport across brush border membranes. One account is that tannin may bind to non-transport proteins and change the membrane fluidity of the brush border membrane. It has been established that changes in membrane fluidity are related to intestinal transport functions (Hayashi and Kawasaki, 1982). Tannin may also bind directly to transport proteins and change the structure of these proteins. Conceivably, these structural changes could change both the affinities of transport proteins for amino acids and their capacities to transport amino acids from the intestinal lumen (King *et al*., 2000).

As already mentioned, the varieties under study were grown under identical conditions and they were subjected to similar levels of irrigation and fertilizers. It seems, therefore, that the differences in amino acids content and their digestibility are more genetically motivated than otherwise. Since the differences in the quantities and availabilities of amino acids both within one variety and among varieties were considerable, it is not logical to use overall means or the results obtained from other studies on poultry diets formulation, as this may bias nutritionists. The results from the present study reveal that both the plant geneticists and the nutritionists will need to consider the genetics of the SG varieties when evaluating their nutritional value in order to identify the best and most appropriate varieties in each case.

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