Influence of caeca micro flora and tannin on true amino acid availability in grain sorghum cultivars

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Abstract: This study was conducted to determine caeca micro flora and tannin effects on amino acid availability (AAA) values for different grain sorghum cultivars (GSC). Three GSCs including low tannin (0.09%, LTS), medium tannin (0.19%, MTS) and high tannin (0.37%, HTS) were selected to determine AA availability compare to the corn by using the true metabolizable energy (TME) assay with both intact (INT) and caecectomized (CEC) roosters. The results showed that true AA availability (TAAA) of LTS was high (95%) whereas, corresponding value for MTS (82.7%) and HTS (41.6%) was significantly lower ($P < 0.05$). The correlation between tannin and availability of all of the AAs was negative and significant (r >-0.78, $P < 0.001$). However, the availability of proline was the most affected by tannin, which was 93.4% for LTS, 77.6% for MTS and 18.4% for HTS. The influence of caeca micro flora on AAs availability was found to vary between corn and GSCs and among different AAs. For the corn, CEC rooster exhibited lower AAs digestibility coefficient than INT rooster. In contrast, corresponding value for the GSCs in CEC rooster was higher than INT rooster.

Key words: Sorghum; Tannin; Amino acid; Caeca micro flora; Poultry.

Introduction

Amino acid contents in hybrid sorghum varieties are considerably different (Hullan & Proudfoot, 1982). Workers have reported reduced nitrogen and protein retention due to consumption of tannic acid. They have reported that activities of the intestinal proteolysis 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 differences existed in amino acid digestibility values in one hybrid and also among different hybrids. The use of excreta as a means of determining digestibility coefficients has been questioned (Farrell, 1989) because of the possibly spurious influence of bacteria in the hind gut. It has been suggested that they may be able to synthesize amino acids or utilize undigested amino acids without benefit to the bird. Because the caeca are the main sites of bacterial activity in the hind gut, caecectomy has been proposed a method of reducing bacterial influence (Parsons, 1984). In this trial the influence of caecectomy and tannin content on the measurement of digestibility of amino acids in three sorghum grain varieties compare to the corn was investigated.

Materials and Methods

Twelve varieties of grain sorghum and one corn sample were grown in the same location. After harvesting of the plants at maturity, 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 compare to the corn. Amino acids availability of grains was determined by using precision fed caecectomized and intact 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).After recovery period, twenty four roosters were selected for availability trails. Following a 24-h period of feed deprivation, six roosters were randomly given 30 g 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 et al.,1988). The excreta were collected over a 48-hour period. 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 & 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 & 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 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. This observation indicates that variations in the percentage and ratio of amino acids does not match the variations in crude protein (NRC, 1994) and that, as shown in Table 1, the quantities of hystidine, isoleucine, and methionine in the HTS are higher than the same in the other two varieties.

Increasing tannin content corresponded to a decreasing availability value for all amino acids in sorghum grains, such that the average amino acid availability for caecectomized rooster was 96.07% ± 3.4 in the LTS, 87% ± 7.4 in the MTS, and 46.87% ± 13.5 in the HTS variety. Elkin et al (1990) reported a negative and significant correlation between tannin content and amino acid availability ($p < 0.001$; r = -0.97). Availability of all amino acids was 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 proteolysis 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. 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 & Butler, 1981). 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 the brush border membrane. It has been established that changes in membrane fluidity

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
Aspratic 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	20.0	2.71	22.9	2.32	23.1	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	10.8	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	12.7	1.33	13.3	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	

[1] Each value designates the average of two replications; N/A, no data were available

Table 2. Effect of tannin and caeca micro flora on amino acids availability (%)

	Sorghum Grain Varieties and Corn								
	LTS		MTS		HTS		CORN		
	Bird								
Amino acid[1]	Cec	Int	Cec	Int	Cec	Int	Cec	Int	SEM [1]
Asparatic acid	97.5^{a}	93.8^{a}	93.3^{a}	80.2^{b}	53.5^{c}	41.6^{d}	92.7^{a}	93.8^{a}	3.5
Threonine	96.1^{a}	89.9^{a}	85.8^{ab}	76.3^{b}	41.5^{c}	22.8^{d}	91.6^{a}	92.2^{a}	4.7
Serine	96. 7^{a}	94.6^{a}	89. 7^{a}	76.6^{b}	45.2^{c}	29.9^{d}	97.8^{a}	97.^{a}	4.0
Glutamic acid	98.3^{a}	97.2^{a}	95. 5^{a}	78.5^{b}	49.1^{c}	43.7^{c}	96.6^{a}	95.9^{a}.	2.5
Proline	91.5^{ab}	95.4^{a}	84.8^{b}	71.8^{c}	22.8^{d}	15.0^{d}	86.6^{b}	97.4^{a}	3.2
Alanine	98.1^{a}	97.7^{a}	93.4^{a}	79.8^{b}	45.2^{c}	45.3^{c}	91.0^{a}	97.3^{a}	2.6
Cystine	96.5^{a}	92.7^{a}	82.8^{b}	72.9^{c}	54.3^{d}	31.7^{e}	95.6^{a}	97.7^{a}	2.8
Valine	94.3^{a}	94.6^{a}	86.1^{a}	75.9^{b}	52.7^{c}	47.3^{c}	91.7^{a}	94.1^{a}	3.0
Methionine	107.7^{a}	100.2^{a}	92.6^{ab}	87.1^{bc}	75.9^{c}	54.1^{d}	100.4^{a}	101.3^{a}	5.0
Isoleucine	96.6^{a}	97.3^{a}	82.7^{b}	82.7^{b}	55.9^{c}	49.1^{c}	91.0^{ab}	96.8^{a}	3.3
Leucine	98.3^{a}	98.1^{a}	93.4^{a}	79.8^{b}	38.8^{c}	34.4^{c}	94.9^{a}	98.2^{a}	3.0
Tyrosine	98.4^{a}	97.2^{a}	89.3^{ab}	82.7^{b}	24.7^{c}	31.3^{c}	84.4^{b}	97.9^{a}	4.6
Phenylalanine	96.7^{a}	96.3^{a}	92.7^{a}	81.0^{c}	33.7^{d}	27.0^{d}	86.0^{bc}	96.6^{a}	3.6
Histidine	85.7^{a}	69.0^{b}	66.9^{b}	66.5^{b}	45.2^{c}	46.8^{c}	87.0^{a}	98.4^{a}	4.6
Lysine	96.0^{a}	94.9^{a}	78.6^{ab}	90.5^{a}	65.1^{b}	42.0^{c}	90.0^{a}	90.3^{a}	6.2
Arginine	96.3^{a}	83.9^{b}	79.5^{b}	86.0^{b}	44.5^{c}	38.7^{c}	92.9^{ab}	102.7^{a}	4.7
Average	96. 7^{a}	93.3^{a}	87.1^{bc}	79.3^{c}	46.8^{d}	37.6^{e}	91.9^{ab}	96.7^{a}	

[1] Standard Error of Mean; Cec=Caecectomized rooster, Int= Intact rooster
a-e Means within rows with different superscripts are significantly different ($p < 0.05$).

are related to intestinal transport functions (Hayashi & 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). The influence of caeca microflora on AAs availability was found to vary among different AAs. Methionine, threonine, aspartic acid, cystine, glutamic acid, and serine were the most affected by caeca micro flora (table 2). Average of amino acids availability for three sorghum grain varieties in intact roosters was lower than caecectomized roosters, while for corn caecectomized roosters showed lower coefficients of amino acids availability. Since amount of non starch polysaccharides in sorghum grains were more than corn, seems fermentation of these sugars by hind gut micro flora led to microbial protein synthesis, which its excretion in manure make lower coefficients of amino acids digestibility (Misiri & Sauer, 1982). Results of the present study indicated that profile and amino acids availability of sorghum grain varieties are different and are not in agreement with standard tables. In addition, the influence of caeca micro flora on amino acids digestibility is depending on feedstuff and amino acid assayed.

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