Condensed Tannins Are Only Partially Responsible for Variations in Nutrient Digestibilities of Sorghum Grain Cultivars†

Robert G. Elkin,*,‡ Marisue B. Freed,‡ Bruce R. Hamaker,§ Ye Zhang,⊥ and Carl M. Parsons⊥

Departments of Animal Sciences and Food Science, Purdue University, West Lafayette, Indiana 47907, and Department of Animal Sciences, University of Illinois, Urbana, Illinois 61801

Nitrogen-corrected true metabolizable energy (TME_n) and true amino acid digestibility values were determined, using cecectomized cockerels, for 20 sorghum [*Sorghum bicolor* (L.) Moench] cultivars that ranged in catechin equivalent (CE) percentages from 0 to 3.88. There were significant ($P \leq 0.01$) overall inverse relationships between CE content and (1) the individual digestibilities of lysine, methionine, threonine, isoleucine, leucine, valine, phenylalanine, histidine, and arginine; (2) the mean digestibility of essential amino acids; (3) the mean digestibility of nonessential amino acids; and (4) TME_n values. In contrast to these overall trends, several cultivars of similar condensed tannin content had markedly different nutrient digestibilities. The dissimilarity of protein digestibilities in sorghum genotypes of similar tannin content was confirmed by subjecting samples of the ground grains to *in vitro* pepsin digestion with subsequent visualization of the undigested proteins by SDS−PAGE. The present work suggests that other components besides tannins are responsible for variations in the availability of nutrients in sorghum.

Keywords: *Sorghum; tannin; amino acid digestibilities; true metabolizable energy; TME_n; pepsin digestibility; chicken*

INTRODUCTION

Sorghum bicolor (L.) Moench is the world's fifth most abundant cereal (Doggett, 1988). It is indigenous to arid and semiarid regions of the globe and is one of the most drought-resistant cereal crops. Sorghum is unique among the cereals in that certain cultivars are able to produce relatively large amounts of polymeric, polyphenolic antinutritional compounds known as tannins. Tannins have come to be regarded as nonspecific protein-binding agents, and their wide variety of biological effects have generally been interpreted as manifestations of their ability to bind, coagulate, and precipitate proteins (Butler *et al*., 1984).

The major adverse effects that commonly result from feeding high-tannin sorghum cultivars to poultry include reduced growth rates, poorer efficiency of feed utilization, increased incidence of leg abnormalities, and lower egg production [for reviews see Butler (1989), Elkin *et al*. (1990), Gualtieri and Rapaccini (1990), and Jansman (1993)]. In addition, amino acid digestibilities and metabolizable energy content both have been observed to vary inversely with sorghum tannin content, a relationship that has been investigated over the past two decades by several laboratories (Rostagno *et al*., 1973; Nelson *et al*., 1975; Kirby *et al*., 1983; Mitaru *et al*., 1983, 1985; Banda-Nyirenda *et al*., 1987; Douglas *et al*., 1990; Schang *et al*., 1991). However, in each of the above studies a limited number of samples (12 sorghums or fewer) were examined, with several low-tannin cultivars being compared with high-tannin varieties over a narrow range of tannin contents within each group. Thus, the objective of the present work was to more completely define the relationship between sorghum tannin content and both true amino acid digestibilities and nitrogen-corrected true metabolizable energy (TME_n) values over a broad range of tannin contents.

MATERIALS AND METHODS

Sorghum Cultivars. Twenty-five sorghums from the World Collection, grown at the Purdue University Agronomy Farm in 1992, were provided by Dr. Gebisa Ejeta. The grains were hand cleaned to remove glumes, and ~100 g samples of each cultivar were ground and assayed for condensed tannin content according to the vanillin procedure (Price *et al*., 1978). Because catechin, a monomeric flavan-3-ol unit of condensed tannins, is used to standardize the assay rather than purified condensed tannin, results are expressed as catechin equivalents. On the basis of their catechin equivalent content, 20 cultivars were selected for further study. Dry matter, crude protein, and gross energy analyses were performed on the selected sorghums according to methods described by the AOAC (1984). Cultivar amino acid contents were determined by cation exchange chromatography following hydrolysis in 6 N HCl for 22 h at 110 °C (Zhang *et al*., 1993). Because of their lability during acid hydrolysis (Spindler *et al*., 1984), cyst(e)ine and methionine were converted to cysteic acid and methionine sulfone, respectively, by oxidizing additional samples of each sorghum with performic acid prior to hydrolysis with 6 N HCl (Moore, 1963). Excess performic acid was removed by lyophilization after dilution with water (Zhang *et al*., 1993).

True Metabolizable Energy and Amino Acid Digestibility Assays. Adult Single Comb White Leghorn roosters (35 weeks of age), which had been cecectomized at 25 weeks of age according to the procedure of Parsons (1985), were housed in individual cages in an environmentally regulated room with 16 h of light provided daily. Feed and water were provided for *ad libitum* consumption prior to the start of the digestibility trials. With minor modifications, the digestibility assays were conducted as previously described by Sibbald (1979). All birds were fasted for 24 h, and then four cockerels

* Author to whom correspondence should be addressed [telephone (317) 494-4820; fax (317) 494-9346; e-mail belkin@www.ansc.purdue.edu].

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‡ Department of Animal Sciences, Purdue University.

§ Department of Food Science, Purdue University.

⊥ Department of Animal Sciences, University of Illinois.

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Table 1. Catechin Equivalent, Dry Matter, Crude Protein, and Amino Acid Contents of Selected Sorghum Cultivars from the World Collection[a]

sample	ID[b]	catechin equiv (%)	dry matter (%)	crude protein (%)	amino acid (g/100 g, on "as is" basis)															
					Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg
1	IS 10246	0.00	90	10.4	0.73	0.34	0.48	2.16	0.33	0.91	0.22	0.49	0.23	0.37	1.29	0.22	0.54	0.22	0.23	0.36
2	IS 2217	0.00	93	12.3	0.89	0.35	0.64	3.33	0.31	1.36	0.21	0.63	0.21	0.49	1.93	0.26	0.77	0.24	0.28	0.38
3	IS 10644	0.11	92	12.6	0.85	0.39	0.56	2.64	0.35	1.15	0.24	0.60	0.22	0.45	1.67	0.27	0.69	0.25	0.25	0.38
4	IS 10484	0.20	93	10.4	0.74	0.35	0.49	2.14	0.34	0.95	0.19	0.52	0.15	0.41	1.39	0.28	0.56	0.22	0.21	0.34
5	IS 6451	0.39	92	10.9	0.82	0.36	0.54	2.49	0.33	1.07	0.23	0.54	0.15	0.45	1.58	0.29	0.63	0.24	0.25	0.38
6	IS 8583	0.70	92	9.9	0.71	0.34	0.48	2.18	0.32	0.95	0.21	0.51	0.22	0.39	1.41	0.34	0.54	0.21	0.21	0.36
7	IS 9180	0.98	92	10.5	0.72	0.33	0.48	2.11	0.33	0.94	0.19	0.49	0.24	0.39	1.36	0.18	0.56	0.23	0.22	0.31
8	IS 16046	1.08	93	11.7	0.81	0.35	0.51	2.46	0.32	1.06	0.19	0.53	0.18	0.43	1.59	0.25	0.51	0.25	0.23	0.39
9	IS 2670	1.19	91	10.4	0.71	0.29	0.47	2.40	0.27	0.98	0.16	0.47	0.17	0.37	1.39	0.20	0.55	0.21	0.21	0.32
10	IS 16327	1.35	92	12.0	0.82	0.36	0.48	2.14	0.36	0.93	0.23	0.50	0.20	0.40	1.35	0.25	0.51	0.28	0.24	0.46
11	IS 15526	1.50	92	9.0	0.66	0.30	0.40	1.90	0.27	0.87	0.16	0.41	0.12	0.36	1.26	0.11	0.46	0.18	0.19	0.26
12	IS 15106	1.63	92	8.1	0.58	0.26	0.35	1.71	0.25	0.74	0.18	0.38	0.12	0.32	1.13	0.14	0.41	0.16	0.19	0.25
13	IS 15070	1.72	92	11.5	0.86	0.37	0.54	2.56	0.32	1.11	0.22	0.60	0.20	0.45	1.62	0.25	0.48	0.25	0.24	0.37
14	IS 4904	1.80	92	11.2	0.85	0.38	0.56	2.67	0.33	1.17	0.24	0.71	0.17	0.46	1.67	0.35	0.51	0.24	0.25	0.42
15	IS 8671	1.90	92	9.7	0.72	0.32	0.45	2.04	0.32	0.89	0.20	0.49	0.15	0.38	1.29	0.21	0.51	0.22	0.23	0.34
16	IS 9282	2.06	93	11.2	0.77	0.33	0.52	2.48	0.31	1.03	0.18	0.51	0.17	0.41	1.47	0.20	0.61	0.23	0.22	0.36
17	IS 8070	2.50	93	9.5	0.66	0.28	0.43	2.04	0.29	0.83	0.15	0.43	0.19	0.35	1.19	0.18	0.52	0.20	0.20	0.33
18	IS 15346	2.83	92	10.8	0.70	0.32	0.45	2.11	0.30	0.90	0.21	0.45	0.16	0.39	1.38	0.26	0.51	0.21	0.22	0.35
19	IS 15612	3.05	92	9.8	0.72	0.30	0.43	1.83	0.30	0.80	0.18	0.45	0.14	0.34	1.15	0.13	0.38	0.21	0.20	0.33
20	IS 1291	3.88	93	11.7	0.86	0.39	0.59	2.84	0.36	1.22	0.24	0.62	0.25	0.48	1.81	0.34	0.66	0.21	0.29	0.39

[a] Means of duplicate measurements. [b] India Sorghum (IS) number.

each were given, via crop intubation, 40 g of a sorghum administered in two 20-g portions 8 h apart. Four additional cockerels were fasted throughout the trial to measure endogenous energy, nitrogen, and amino acid excretion. Excreta samples, quantitatively collected on a plastic tray placed under each cage for 56 h following the initial feeding, were lyophilized, weighed, and ground to pass through a 60-mesh screen. Analytical methods for energy, nitrogen, and amino acids in ground excreta were the same as those described for the sorghum samples. True digestibilities of amino acids were calculated according to the method of Sibbald (1979) and TME_n according to the method of Parsons *et al.* (1982).

***In Vitro* Pepsin Digestion Procedure and Visualization of the Indigestible Proteins by SDS−PAGE.** Sorghum samples were ground in a Cyclotec 1093 sample mill (Tecator, Höganäs, Sweden) and passed through a 0.5-mm-mesh screen. Approximately 200 mg of each ground sample, which contained exactly 3.33 mg of nitrogen, was then subjected to *in vitro* pepsin digestion as described by Mertz *et al.* (1984). The digestion was stopped after 2 h by the addition of 2 M NaOH and the reaction mixture was then centrifuged at 10000*g* for 20 min at 4 °C. After the supernates were discarded, the pellets were suspended in water, shaken for 10 min, and centrifuged as described above. The supernates were discarded, and 2.5 mL of a buffer (pH 10) containing 0.0125 M sodium borate, 1% SDS, 10 mM phenylmethanesulfonyl fluoride, and 2% β-mercaptoethanol was added to each pellet, which was then resuspended and extracted for 2 h on a shaker at room temperature. The suspensions were then centrifuged at 10000*g* for 20 min and the supernates saved. Two hundred microliters of each supernate was mixed with 100 μL of sample buffer [2% SDS, 1% β-mercaptoethanol, 0.066 M Tris (pH 6.8), 10% glycerol, and bromophenol blue] and heated for 3 min at 95 °C. Fifty microliters of each sample mixture was loaded onto a 10−18% polyacrylamide gel. The gel was calibrated with SDS−PAGE broad range standards (No. 161-0317, Bio-Rad, Hercules, CA), and electrophoresis was carried out at 70 V for 18 h. Proteins were stained with 0.25% Coomassie Brilliant Blue R-250 in 46% methanol and 8% acetic acid and destained in 20% ethanol and 10% acetic acid. A black and white photograph of the gel was made and the negative scanned on a Model JX-610 Extra-Hi Resolution scanner (Sharp Electronics Corp., Mahwah, NJ) using a gamma value (contrast) of 1 and a G setting (exposure) of 20. In each lane, the area of the band corresponding to α-kafirin, the principal storage protein of sorghum (Hamaker *et al.*, 1995a), was quantitated from a one-dimensional intensity profile (resolution of 300 dpi) using Adobe Photoshop 3.0 (Adobe Systems, Inc., Mountain View, CA) and IP Gel 1.1 (Signal Analytics Corp., Vienna, VA) software.

Statistical Analyses. Analysis of variance was used to statistically analyze all data (Steel and Torrie, 1980). In addition, linear regression was performed to quantify the relationship between (1) catechin equivalent percentages and the digestibilities of lysine, methionine, essential amino acids (averaged), and TME_n; and (2) α-kafirin band area from an SDS−polyacrylamide gel of pepsin-digested sorghum extracts and the mean essential amino acid digestibilities of the sorghum cultivars determined *in vivo*.

RESULTS

The catechin equivalent, dry matter, crude protein, and amino acid contents of 20 sorghums from the World Collection are presented in Table 1. The cultivars were selected for evaluation in this study solely on the basis of their condensed tannin content, expressed as catechin equivalents, which ranged from 0 to 3.88%. While dry matter contents of the sorghums were very similar (range 90−93%), crude protein and amino acid compositions varied markedly and were unrelated to a cultivar's catechin equivalent content.

The influence of sorghum catechin equivalent content on true amino acid digestibilities and TME_n is shown in Table 2. Overall, there was an inverse relationship between the catechin equivalent content of a cultivar and (1) the digestibilities of threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, and arginine; (2) the mean digestibility of all essential amino acids (except for tryptophan, which was not determined); (3) the mean digestibility of all nonessential amino acids (except for proline, which was not determined); and (4) TME_n values. Correlation coefficients between catechin equivalent percentage and both amino acid digestibilities and TME_n values were highly significant [all $P \leq 0.001$, except for phenylalanine ($P \leq 0.01$)] and ranged from 0.30 (phenylalanine) to 0.61 (valine). Not unexpectedly, mean essential amino acid and nonessential amino acid digestibility values within a cultivar agreed closely.

In contrast to these overall trends, there were several instances in which cultivars of similar catechin equivalent contents had markedly different nutrient di-

Table 2. Influence of Catechin Equivalent Content on True Amino Acid Digestibilities (Percent) and Nitrogen-Corrected True Metabolizable Energy (TME_n) Values (Kilocalories per Gram of Dry Matter) of Selected Sorghum Cultivars from the World Collection[a]

	catechin	amino acid										
sample	equiv (%)	Thr	Val	Met	Ile	Leu	Phe	Lys	His	Arg	$\bar{x}$EAA[b]	$\bar{x}$NEAA[c]	TME_n
1	0.00	72.9 ± 4.1	81.2 ± 5.2	93.0 ± 3.9	72.7 ± 4.6	87.0 ± 3.2	77.1 ± 11.5	62.2 ± 10.4	76.3 ± 7.0	72.2 ± 8.1	77.3 ± 4.2	75.8 ± 5.7	3.780 ± 0.044
2	0.00	76.2 ± 8.6	88.6 ± 5.2	96.1 ± 6.0	83.5 ± 4.6	94.5 ± 1.8	57.0 ± 2.5	72.7 ± 7.9	86.3 ± 4.7	95.4 ± 13.5	83.4 ± 4.8	86.5 ± 4.6	3.613 ± 0.079
3	0.11	72.6 ± 10.5	82.9 ± 7.6	91.7 ± 7.7	72.3 ± 8.1	82.4 ± 7.1	85.1 ± 7.6	78.2 ± 13.3	75.1 ± 15.0	74.5 ± 10.8	79.4 ± 9.4	77.7 ± 9.5	3.667 ± 0.110
4	0.20	59.4 ± 11.3	74.1 ± 8.6	74.9 ± 8.9	63.6 ± 10.5	72.1 ± 11.7	75.7 ± 10.6	71.1 ± 7.9	58.9 ± 13.2	65.1 ± 8.9	68.3 ± 9.9	65.3 ± 11.1	3.371 ± 0.094
5	0.39	89.3 ± 1.3	97.5 ± 1.9	104.3 ± 5.4	91.5 ± 1.4	96.5 ± 1.0	99.7 ± 1.8	88.2 ± 4.1	88.8 ± 2.5	93.6 ± 2.8	94.4 ± 2.1	94.1 ± 2.0	3.739 ± 0.062
6	0.70	60.9 ± 6.3	73.1 ± 8.1	83.1 ± 4.1	62.6 ± 7.3	69.6 ± 9.7	72.2 ± 10.1	68.0 ± 4.3	47.7 ± 7.5	69.9 ± 8.9	67.5 ± 6.9	69.1 ± 6.3	3.483 ± 0.046
7	0.98	74.7 ± 6.3	88.9 ± 4.1	98.6 ± 1.9	78.6 ± 4.3	90.0 ± 2.0	92.4 ± 1.7	73.7 ± 5.0	71.4 ± 5.9	74.6 ± 6.1	81.7 ± 3.8	79.0 ± 3.9	3.559 ± 0.039
8	1.08	29.6 ± 3.3	41.7 ± 1.5	56.6 ± 1.6	31.3 ± 3.7	33.5 ± 0.9	31.1 ± 1.1	66.8 ± 3.5	34.1 ± 3.5	48.0 ± 1.2	41.4 ± 1.0	29.4 ± 0.9	3.427 ± 0.067
9	1.19	58.2 ± 10.2	77.3 ± 10.2	84.5 ± 10.0	66.4 ± 9.6	76.6 ± 9.0	87.5 ± 5.0	77.4 ± 10.0	66.2 ± 7.4	71.5 ± 10.3	73.2 ± 6.8	71.9 ± 10.2	3.612 ± 0.107
10	1.35	57.4 ± 5.0	59.7 ± 2.6	77.8 ± 3.4	54.5 ± 4.5	52.0 ± 2.1	58.8 ± 1.7	71.8 ± 3.5	52.2 ± 4.7	69.4 ± 2.3	61.2 ± 2.2	52.2 ± 2.7	3.316 ± 0.049
11	1.50	64.4 ± 4.4	68.2 ± 1.9	75.1 ± 7.3	63.1 ± 2.8	65.2 ± 1.3	69.8 ± 2.0	73.5 ± 6.6	55.4 ± 2.6	66.4 ± 4.0	65.3 ± 2.2	58.9 ± 3.0	3.630 ± 0.111
12	1.63	47.6 ± 4.2	52.4 ± 2.4	62.1 ± 3.7	49.6 ± 2.3	52.8 ± 1.9	55.0 ± 3.7	51.2 ± 6.3	40.0 ± 2.1	47.0 ± 5.5	50.9 ± 2.7	44.0 ± 3.7	3.404 ± 0.056
13	1.72	29.5 ± 2.4	45.7 ± 1.6	62.4 ± 2.3	32.9 ± 3.6	35.3 ± 0.7	25.1 ± 1.0	51.2 ± 2.5	34.4 ± 2.5	42.2 ± 0.6	40.7 ± 0.5	34.1 ± 1.1	3.234 ± 0.102
14	1.80	61.5 ± 10.6	71.1 ± 6.0	69.7 ± 9.4	61.8 ± 6.7	62.0 ± 6.6	55.1 ± 9.6	65.5 ± 3.9	52.0 ± 7.9	63.0 ± 8.7	61.1 ± 6.3	59.6 ± 9.1	3.612 ± 0.082
15	1.90	44.1 ± 4.9	52.3 ± 2.9	57.8 ± 5.8	46.5 ± 3.5	50.6 ± 1.0	55.2 ± 1.7	46.6 ± 11.4	40.1 ± 3.8	48.3 ± 4.7	52.0 ± 4.1	44.5 ± 2.6	3.342 ± 0.096
16	2.06	54.6 ± 11.3	72.0 ± 14.3	84.1 ± 12.2	60.0 ± 15.7	72.2 ± 12.7	94.5 ± 2.5	59.0 ± 13.2	64.4 ± 13.7	65.7 ± 15.8	69.4 ± 12.5	65.7 ± 15.4	3.570 ± 0.043
17	2.50	36.1 ± 5.2	50.7 ± 1.9	75.1 ± 3.9	49.4 ± 15.8	49.3 ± 2.5	82.0 ± 8.4	44.7 ± 4.2	39.1 ± 3.4	48.4 ± 2.7	52.8 ± 2.9	41.7 ± 2.0	3.375 ± 0.062
18	2.83	51.5 ± 6.0	55.0 ± 3.9	82.6 ± 14.1	52.8 ± 4.0	55.8 ± 3.5	59.5 ± 4.8	58.1 ± 5.2	43.6 ± 1.2	55.8 ± 4.7	57.2 ± 4.7	48.8 ± 5.4	3.385 ± 0.088
19	3.05	30.6 ± 7.8	33.9 ± 5.2	57.8 ± 3.4	31.0 ± 11.7	27.4 ± 13.4	43.8 ± 17.1	56.6 ± 9.2	47.1 ± 13.9	46.2 ± 6.7	41.6 ± 6.2	28.4 ± 5.1	3.446 ± 0.026
20	3.88	43.1 ± 5.4	52.4 ± 2.5	62.7 ± 1.9	47.0 ± 2.9	50.2 ± 2.4	52.4 ± 3.0	49.1 ± 7.6	40.7 ± 2.1	48.4 ± 4.8	48.7 ± 3.0	45.7 ± 3.5	3.299 ± 0.038
	r[d]	0.54***	0.61***	0.46***	0.51***	0.60***	0.30**	0.46***	0.52***	0.52***	0.58***	0.59***	0.44***

[a] Values are mean ± SE of four cockerels per sorghum. [b] $\bar{x}$EAA = mean value ± SE of Thr, Val, Met, Ile, Leu, Phe, Lys, His, and Arg. [c] $\bar{x}$NEAA = mean value ± SE of Asp, Ser, Glu, Gly, Ala, Cys, and Tyr. [d] Correlation coefficients between the catechin equivalent content and amino acid digestibility or TME_n values. **$P \leq 0.01$; *** $P \leq 0.001$.

Figure 1. Relationship between catechin equivalent content and true lysine digestibility of sorghum grain. Each point represents the mean value of four roosters. ***$P \leq 0.001$.

Figure 2. Relationship between catechin equivalent content and true methionine digestibility of sorghum grain. Each point represents the mean value of four roosters. ***$P \leq 0.001$.

Figure 3. Relationship between catechin equivalent content and true essential amino acid (EAA) digestibility of sorghum grain. Mean percent EAA digestibility for each cultivar was calculated from the individual digestibility percentages for Thr, Val, Met, Ile, Leu, Phe, Lys, His, and Arg. Each point represents the mean value of four roosters. ***$P \leq 0.001$.

gestibilities (note in particular essential amino acid and nonessential amino acid values for sample 5 *vs* samples 4 and 6; sample 8 *vs* samples 7 and 9; sample 14 *vs* samples 13 and 15; and sample 19 *vs* samples 18 and 20 in Table 2). The existence of these outliers is further depicted in plots of sorghum catechin equivalent content *vs* the digestibilities of lysine, methionine, essential amino acids, and TME_n (Figures 1−4, respectively). Attention was focused on lysine and methionine digestibilities because of the importance of these two amino acids in the nutrition of domestic fowl. Lysine is the first-limiting amino acid in sorghum (Rooney and Clark,

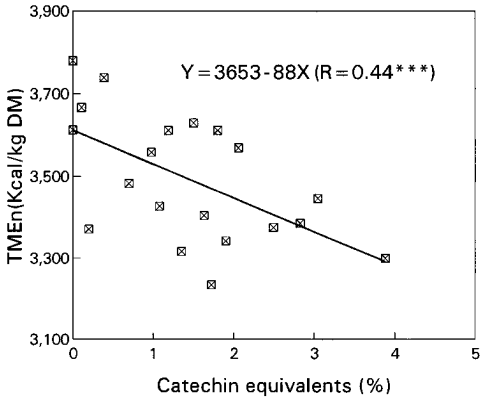

Figure 4. Relationship between catechin equivalent content and nitrogen-corrected true metabolizable energy (TME_n) value of sorghum grain. Each point represents the mean value of four roosters. ***$P \leq 0.001$.

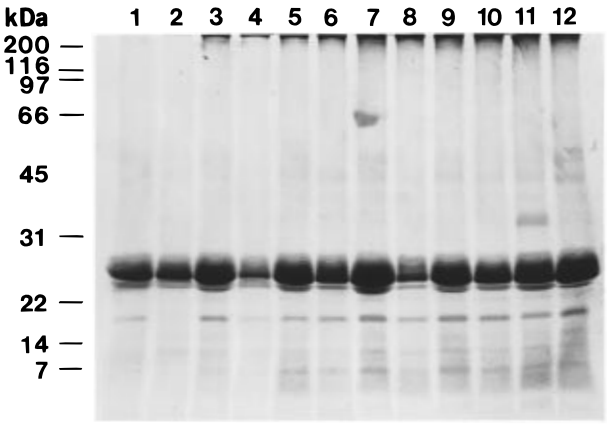

Figure 5. Visualization of indigestible sorghum proteins by SDS−PAGE following *in vitro* digestion with pepsin. Ground sorghum samples, containing 3.3 mg of nitrogen each, were subjected to a 2-h *in vitro* pepsin digestion (Mertz *et al.*, 1984). Indigestible proteins were then extracted, mixed with sample buffer, and reduced. Fifty microliters of each sample mixture was loaded onto a 10−18% polyacrylamide gel. Lanes 1−12 correspond to sorghum numbers 4−6, 7−9, 13−15, and 18−20, respectively, in Table 1. Proteins were stained with Coomassie Brilliant Blue R-250. M_r standards (kDa) are indicated on the left. The band with an M_r of approximately 26 000 is α-kafirin, the major storage protein of sorghum.

1968), while methionine is typically the first-limiting amino acid in practical broiler and layer diets (Scott *et al.*, 1982; Fernandez *et al.*, 1994).

Visualization of indigestible proteins by SDS−PAGE following a 2-h *in vitro* pepsin digestion of sorghum samples 4−6, 7−9, 13−15, and 18−20 (Figure 5) supported the *in vivo* findings (Table 2) in that selected cultivars of similar tannin contents had markedly different protein digestibilities. The areas of the α-kafirin bands (Table 3) were inversely related ($P = 0.018$) to the mean essential amino acid values, and their relationship was described by the following model:

$$\alpha\text{-kafirin band area} = -1479.6\,(\text{mean EAA digestibility \%}) + 172735$$

The coefficient of determination (r^2) was 0.45.

DISCUSSION

Although the metabolizable energy content of sorghum grain is slightly lower than that of corn, the presence of tannins in sorghum is of much greater concern to nutritionists, production managers, purchasing agents, and feed mill managers with regard to its employment as a feedstuff for poultry (Boren and Waniska, 1992). In domestic fowl, the antinutritional effects of sorghum tannins have been primarily attributed to inhibition of dietary protein digestion (Gualtieri and Rapaccini, 1990; Jansman, 1993). However, recent evidence in rats suggests that the protein affected is largely endogenous, not dietary, and that this inhibition accounts for only a portion of the antinutritional effects (Butler and Rogler, 1992). This concept is supported by chick and rat studies in which supplementation of a low-protein, sorghum-based diet with isolated soy protein overcame essentially all of the adverse effects of high-tannin sorghum on growth rate, but supplementation with an equivalent amount of free amino acids (not requiring digestion) did not overcome the diminished weight gains due to tannin (Rogler *et al.*, 1985).

An alternative explanation for the toxicity of high-tannin sorghum may lie in the inhibition of postdigestive metabolism, a systemic effect. In a study with rats, Mole *et al.* (1990) found that the toxic effects of dietary condensed tannins were due to the impaired efficiency with which digested and absorbed nutrients were converted to new body substance and did not involve inhibition of food consumption or digestion. Butler and Rogler (1992) suggested possible systemic effects to include direct inhibition of a key metabolic pathway and/or the diversion of metabolism into detoxification of polyphenols or their degradation products. Although condensed tannins from sorghum are not absorbed from the digestive tract of chickens, it is possible that lower molecular weight polyphenols associated with tannin in the sorghum seed, which *can* be absorbed from the gastrointestinal tract and distributed in various tissues (Jimenez-Ramsey *et al.*, 1994), may be partially responsible for the toxic effects seen in chickens fed high-tannin sorghum diets. Alternately, Clausen *et al.* (1990) suggested that the toxicity of condensed tannins may result from their depolymerization and subsequent absorption from the digestive tract; however, the data of Jimenez-Ramsey *et al.* (1994) do not support this hypothesis. Thus, the adverse biological effects result-

Table 3. Comparison of α-Kafirin Band Areas from an SDS−Polyacrylamide Gel of Pepsin-Digested Sorghum Samples with Their Mean Essential Amino Acid (EAA) Digestibilities[a]

sample[b]	catechin equiv (%)	α-kafirin peak area[c]	mean EAA digestibility[d] (%)
4	0.20	79 811	68.3
5	0.39	54 183	94.4
6	0.70	103 106	67.5
7	0.98	28 392	81.7
8	1.08	94 126	41.4
9	1.19	54 293	73.2
13	1.72	138 055	40.7
14	1.80	32 924	61.1
15	1.90	105 240	52.0
18	2.83	68 197	57.2
19	3.05	89 060	41.6
20	3.88	148 554	48.7

[a] SDS−polyacrylamide gel is depicted in Figure 5. [b] See Table 1 for sample identities. [c] Quantitated from a negative of a black and white photo of the gel in Figure 5 as a one-dimensional intensity profile. [d] Data from Table 2.

ing from the consumption of high-tannin sorghum by poultry are most likely due to a combination of condensed tannin-associated impairment of protein utilization (both dietary and endogenous) *and* systemic effects due to absorbable, lower molecular weight polyphenols associated with condensed tannins.

In agreement with previous work (Rostagno *et al.*, 1973; Mitaru *et al.*, 1983, 1985; Douglas *et al.*, 1990; Schang *et al.*, 1991), results of the present study show that, in general, protein and energy digestibilities are inversely related to the tannin content of a sorghum cultivar. However, several exceptions to this trend were noted in which cultivars with similar catechin equivalent contents had markedly different nutrient digestibilities (Tables 2 and 3 and Figures 1−5). Although fewer varieties were employed and the cultivars contained a narrower range of tannin content than in the present study, other investigators have also observed an inconsistency in the relationship between tannin content and nutrient utilization in sorghum grain. Maxson *et al.* (1973) reported that sorghum catechin equivalent content was not correlated to protein efficiency ratio (PER; grams of weight gained per gram of protein consumed) in a 21-day rat study. The authors suggested that "some other factor within brown grain appeared to cause the reduced PER". In separate experiments with broiler chicks, Nelson and colleagues (Nelson *et al.*, 1975; Kirby *et al.*, 1983) reported significant negative correlations between sorghum tannin content and both metabolizable energy and amino acid digestibilities. However, in each study they observed that several of the high-tannin grains were digested as well as some of the low-tannin varieties. In another study with chicks, Banda-Nyirenda *et al.* (1987) observed significant differences in both apparent nitrogen retention (ANR) and apparent metabolizable energy (AME) among 11 sorghum cultivars (10 low-tannin and 1 high-tannin). Each sorghum comprised ~54% of the diet, with soybean meal and a vitamin and mineral supplement accounting for the remainder of the diet. Interestingly, the one high-tannin variety (1.15% catechin equiv) had the highest ANR and AME values as compared with those of the low-tannin (~0.30% catechin equiv) cultivars. The unexplained lack of a growth depression in chicks consuming two different high-tannin sorghums *vs* low-tannin sorghums has also been observed on several previous occasions in our laboratory (Pearson-Priller, 1985).

Using both *in vitro* and *in vivo* approaches, the present work demonstrates that sorghum cultivars of similar tannin contents may vary greatly in their protein digestibilities. This novel and important finding, which provides an exception to the rule that tannins are always associated with a depression of protein digestibility, suggests that other components besides tannins are responsible for variations in the availability of nutrients in sorghum. One newly emerging candidate, namely the structure of the kafirin-containing protein bodies, has recently been shown to markedly affect *in vitro* protein digestibilities among low-tannin sorghum cultivars (Hamaker *et al.*, 1995b; Oria and Hamaker, 1995). As kafirins are the principal storage proteins of sorghum (Hamaker *et al.*, 1995a), elucidation of the structures of kafirin-containing protein bodies in additional sorghum varieties may help to explain the somewhat inconsistent relationship between tannin content and nutrient digestibilities in this important cereal.

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