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Effects of Sorghum Grain Tannins and Dietary Protein on the Activity of Liver UDP-Glucuronyltransferase[1] (41709)

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Abstract. The activity of liver microsomal UDP-glucuronyltransferase (EC 2.4.1.17), an enzyme known to detoxify phenolic compounds, was measured in chicks and rats fed high- (HTS) and low-tannin sorghums (LTS). In an initial investigation, activity was significantly elevated in chicks fed HTS–soybean meal diets over those fed the LTS control diet. Other studies were designed to differentiate between the effects due to tannin and those resulting from a protein deficiency which had previously been reported to increase the activity of this enzyme. In general, only a relatively small part of the increased activity observed by feeding HTS to chicks could be attributed to a tannin-induced protein deficiency. The same phenomenon of elevated activity produced by feeding HTS to chicks was not observed in the rat. These results would suggest that sorghum tannins, or their breakdown products, are absorbed and activating UDP-glucuronyltransferase in the chick, but not the rat.

Previous investigations in our laboratory (1–3) have shown a significant increase in the incidence of a leg abnormality characterized by an outward bowing of the legs with an enlargement of the hock joint in broiler chicks fed high-tannin sorghum (HTS) diets. Since results indicated that tannins were not interfering with bone mineralization (3, 4), it was suggested that tannins were being absorbed and were functioning *in vivo* as they do *in vitro*, by increasing the amount of crosslinking in collagen molecules, thereby altering the organic matrix of the bone (3). In a preliminary study, Giles (4) demonstrated a higher specific activity of hepatic microsomal UDP-glucuronyltransferase (EC 2.4.1.17) in HTS-fed chicks compared with low-tannin sorghum (LTS)-fed controls. Since UDP-glucuronyltransferase (GT) functions as an important enzyme in phenolic detoxification (5), the results of Giles (4) suggested that tannins or their phenolic breakdown products were being absorbed in the chick and thus activating the enzyme. However, GT is noted to be highly sensitive to diet-induced changes in the lipoidal microenvironment of the endoplasmic reticular membrane where the enzyme is embedded (6). Previous studies have shown that a protein deficiency will substantially elevate the level of activity of this enzyme in rat liver (7–9).

The present studies were undertaken in an attempt to: (1) verify the preliminary findings of increased enzymatic activity by feeding HTS; (2) determine if a tannin-induced protein deficiency explained the observed elevation in HTS-fed chicks; (3) investigate whether the same elevation could be reproduced with rats fed similar diets.

Materials and Methods. *Animals.* Four pens, containing seven, day-old White Mountain broiler-type cockerels[2] each, were fed sorghum-containing diets for a 19- or 20-day period in Experiments 1 and 2, while 10 chicks of the same strain and sex were divided among four pens and fed experimental diets for 20 days in Experiment 3. In a final study (Experiment 4), male weanling rats of the Sprague–Dawley strain[3] were individually caged and fed a commercial laboratory diet for a 3-day adjustment period followed by feeding sorghum-containing experimental diets for 29 days. There were 10 rats per treatment. Chicks were assigned randomly to treatments while rats were assigned to their respective treatments such that the average initial weights of rats on each treatment were

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[2] Fairview Farms, Inc., Remington, Ind.

[3] Murphy Breeding Lab. Inc., Plainfield, Ind.

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TABLE I. EFFECTS OF HIGH- AND LOW-TANNIN SORGHUM–SOYBEAN MEAL DIETS ON 20-DAY CHICK WEIGHT GAIN, FEED CONVERSION, AND HEPATIC MICROSOMAL UDP-GLUCURONYLTRANSFERASE ACTIVITY (EXPERIMENT 1)

	Diets		
	1	2	3
Ingredients (%)			
RS610 (10.3[a]; <0.1[b])	70.69	—	—
Savanna (10.6[a]; 11.8[b])	—	68.70	—
BR64 (9.2[a]; 15.1[b])	—	—	67.12
Soybean meal (49.3[a])	17.28	17.28	19.57
Corn oil	4.00	4.00	4.00
Vitamin and mineral premix[c]	4.41	4.41	4.41
Glucose monohydrate	3.62	5.61	4.90
Weight gain (g)[d]	263[g]	148[h]	137[h]
Gain (g)/feed (g)[d]	0.57[g]	0.39[h]	0.39[h]
UDP-glucuronyltransferase activity[e,f]			
nmole/mg protein	572[h]	946[g]	923[g]
μmole/100 g body wt	11[h]	19[g]	21[g]

[a] Crude protein content (%).
[b] Tannin content expressed as catechin equivalents (%).
[c] The vitamin and mineral premix contributed the following to each kilogram of diet: dicalcium phosphate, 22.5 g; limestone, 10.0 g; sodium chloride (iodized), 4.5 g; manganese sulfate, 0.35 g; zinc oxide, 0.06 g; sodium selenite, 0.22 mg; butylated hydroxy toluene, 0.125 g; choline chloride, 1.3 g; retinyl palmitate, 5000 IU; cholecalciferol, 2400 ICU; *d*-alpha tocopherol acetate, 22 IU; menadione sodium bisulfite, 1.23 mg; riboflavin, 8.8 mg; calcium pantothenate, 17.6 mg; niacin, 39.6 mg; biotin, 0.15 mg; vitamin B_{12}, 11.1 μg.
[d] Means of four replicates of seven chicks each. Pooled SEM for gains = 9 and for gain/feed = 0.01.
[e] Means of individual microsomal preparations from 10 chicks per diet. Figures represent the amount of naphthol conjugated in 10 min at 37°C.
[f] Pooled SEM for nmole/mg protein = 88 and for μmole/100 g body wt = 2.
[g,h] Row means with different superscripts are significantly different ($P < 0.05$).

approximately equal. All experiments were conducted in a temperature-controlled room (22°C) with continuous artificial light for all chicks studies and a fixed 12-hr artificial light–dark cycle for the rat study. During each experiment, feed and water were provided *ad libitum*. Weight gain was determined on an individual basis in all studies while feed consumption was determined on a group basis for the chick and an individual basis for the rat.

Diets. Sorghum-containing diets for chicks (Experiments 1–3) or rats (Experiment 4) were formulated with one of three varieties of grain sorghum (low-tannin RS610, high-tannin Savanna, or high-tannin BR64) and supplemented with enough soybean meal (Experiments 1 and 2) or isolated soy protein (Experiments 3 and 4) to provide the desired level of dietary protein. Crude protein contents of sorghums, soybean meal, and isolated soy protein were determined by the Kjeldahl procedure (10). Sorghum tannin concentrations were estimated as catechin equivalents based on the method of Price *et al.* (11). In all experiments, minerals and vitamins were provided to meet the requirements for the chick (12) or the rat (13).

The diets used in Experiment 1 (Table I) consisted of either RS610, Savanna, or BR64 sorghums and supplemented with enough soybean meal to provide 16% protein. The sorghums used in this particular experiment were harvested at an immature stage (30 days past half-anthesis), and therefore, the tannin concentrations (Table I) in the HTS varieties were much higher than those of the mature sorghums used in all other experiments. This finding agrees with other studies in our laboratory[4] and studies of other investigators (14, 15) which indicated that immature HTS is

[4] Rogler *et al.*, unpublished results.

higher in tannin than mature HTS. Since the difference in protein content between RS610 and Savanna was small, diets containing these sorghums were made isonitrogenous with respect to both sorghum and soybean protein by the substitution of glucose monohydrate for some of the higher protein sorghum. However, the protein content of BR64 was considerably lower than the other two grains and rather than dilute the Savanna and RS610 down to the protein level of BR64, the protein content of the BR64 diet was increased up to the level of the other diets by the addition of 2.29% soybean meal.

Three different LTS–soybean meal diets were formulated in Experiment 2 (Table II) to provide varying levels of protein (16, 14.5, and 13%). These three diets in turn were compared with a HTS–soybean meal diet containing 15.8% protein. The 16% protein RS610 diet was not made isonitrogenous to the Savanna diet; however, this small difference in protein content between these two diets should not have affected the results since they were still isonitrogenous with respect to soybean meal.

Dietary treatments of Experiment 3 (Table III) consisted of RS610 or Savanna sorghums and formulated with isolated soybean protein (ISP) to 15.1 or 24.2% total crude protein. The higher-protein diets contained twice the level of ISP as the lower-protein diets. All four diets were made isonitrogenous with respect to sorghum and with respect to ISP within a protein level by the addition of glucose monohydrate.

Diets for rats used in Experiment 4 are presented in Table IV. Two of the three diets contained 9.9% protein and were formulated with RS610 or Savanna sorghums, but no other protein source. Glucose monohydrate was substituted for some of the higher-protein sorghum to make the diets isonitrogenous. Lysine was supplemented at a level of 0.75% (16). Comparison of the first two diets was made with a third diet formulated with LTS (RS610) and ISP to provide 16% protein and supplemented with L-lysine and DL-methionine to a level of adequacy based on calculated diet composition and amino acid requirements (13).

Enzyme assay. At the end of each growth study, 10 chicks or rats from each treatment were randomly selected and killed either by cervical dislocation (chick) or by decapitation (rat). Livers were immediately removed,

TABLE II. EFFECTS OF HIGH- AND LOW-TANNIN SORGHUM–SOYBEAN MEAL DIETS VARYING IN PROTEIN LEVEL ON 19-DAY CHICK WEIGHT GAIN, FEED CONVERSION, AND HEPATIC MICROSOMAL UDP-GLUCURONYLTRANSFERASE ACTIVITY (EXPERIMENT 2)

	Diets			
	1	2	3	4
Ingredients (%)				
RS610 (10.0[a]; <0.1[b])	74.09	74.09	74.09	—
Savanna (9.7[a]; 5.5[b])	—	—	—	74.09
Soybean meal (49.3[a])	17.50	14.44	11.38	17.50
Soybean oil	4.00	4.00	4.00	4.00
Vitamin and mineral premix[c]	4.41	4.41	4.41	4.41
Glucose monohydrate	—	3.06	6.12	—
Crude protein (%)	16.0	14.5	13.0	15.8
Weight gain (g)[d]	303[g]	201[i]	144[j]	235[h]
Gain (g)/feed (g)[d]	0.58[g]	0.53[h]	0.46[i]	0.45[i]
UDP-glucuronyltransferase activty[e,f]				
nmole/mg protein	412[i]	515[h]	504[h]	760[g]
μmole/100 g body wt	13[i]	18[h]	19[h]	30[g]

[a,b,c,e] See Table I.
[d] Means of four replicates of seven chicks each. Pooled SEM for gains = 10 and for gain/feed = 0.01.
[f] Pooled SEM for nmole/mg protein = 34 and for μmole/100 g body wt = 2.
[g,h,i,j] Row means with different superscripts are significantly different ($P < 0.05$).

TABLE III. EFFECTS OF PROTEIN LEVELS OF 15.1 AND 24.2% IN HIGH- AND LOW-TANNIN SORGHUM—ISP DIETS ON 20-DAY CHICK WEIGHT GAIN, FEED CONVERSION, AND HEPATIC MICROSOMAL UDP-GLUCURONYLTRANSFERASE ACTIVITY (EXPERIMENT 3)

	Diets			
	1	2	3	4
Ingredients (%)				
RS610 (9.8[a]; <0.1[b])	60.92	—	60.92	—
Savanna (8.5[a]; 7.3[b])	—	70.25	—	70.25
Isolated soybean protein[c] (88.6[a])	10.29	10.29	20.58	20.58
Corn oil	3.00	3.00	3.00	3.00
Vitamin and mineral premix[d]	4.84	4.84	4.84	4.84
Glucose monohydrate	20.95	11.62	10.66	1.33
Crude protein (%)	15.1	15.1	24.2	24.2
Weight gain (g)[e]	246[h]	163[i]	463[g]	418[g]
Gain (g)/feed (g)[e]	0.54[h,i]	0.43[i]	0.70[g]	0.60[g,h]
UDP-glucuronyltransferase activity[f]				
nmole/mg protein	578[h]	889[g]	230[i]	509[h]
μmole/100 g body wt	13[h]	20[g]	6[i]	12[h]

[a,b] See Table I.

[c] Ralston Purina Company, St. Louis, Mo.

[d] See footnote *c*, Table I. In addition, the premix also contributed the following to each kilogram of diet: magnesium carbonate, 1.5 g; ferrous suflate, 0.16 g; potassium phosphate (dibasic), 2.5 g; inositol, 1 g; thiamin, 60 mg; pyridoxine, 80 mg; folic acid, 20 mg.

[e] Means of 10 chicks each. Pooled SEM for gain = 20 and for gain/feed = 0.04.

[f] See footnote *e*, Table I. Pooled SEM for nmole/mg protein = 41 and for μmole/100 g body wt = 1.

[g,h,i,j] Row means with different superscripts are significantly different ($P < 0.05$).

weighed, and frozen in liquid nitrogen. Each liver was later thawed and a 10% homogenate was prepared in cold 0.05 M Tris buffer at pH 7.4 by initially homogenizing the whole liver in an Omni-Mixer[5] followed by rehomogenizing an aliquot in a Potter–Elvehjem type glass–Teflon homogenizer. Each microsomal suspension was obtained by centrifugation of the homogenate at 12,000*g* for 20 min at 4°C followed by recentrifugation of the obtained supernate at 105,000*g* for 1 hr at the same temperature. The pellet was weighed and resuspended in Tris buffer at a concentration of 0.05 g of pellet/ml of buffer by use of the Potter–Elvehjem homogenizer. All suspensions were refrigerated or kept on ice at all times. Protein content of each microsomal suspension was assayed according to Lowry *et al.* (17) using bovine serum albumin as the standard.

Activity of GT was measured according to the method of Lucier *et al.* (18) as modified by Lucier (19). The method involved incubation of α-1-^{14}C-naphthol[6], α-naphthol[7], UDP-glucuronic acid[7] (sodium salt) and either 50 or 100 μl of the prepared microsomal suspension in a total volume of 1.3 ml. The final specific activity of the naphthol was 0.21 μCi/μmole. All incubations were carried out for 10 min at 37°C in 20-ml scintillation vials after which the reaction was stopped by the addition of 10 ml of toluene nonaqueous-based scintillation cocktail (20). The vials were counted in a Tri-Carb liquid scintillation counter[8] equipped with an automatic analyzer for quench correction.

A preliminary investigation showed that the activity response of the enzyme was nonlinear. In general, with increasing amounts of microsomal protein, less naphthol was converted per unit of protein. Therefore, in an attempt

[5] Sorvall, Inc., Norwalk, Conn.

[6] New England Nuclear, Boston, Mass.

[7] Sigma Chemical Co., St. Louis, Mo.

[8] Model 460C, Packard Instrument Co., Inc., Downers Grove, Ill.

TABLE IV. EFFECTS OF PROTEIN LEVELS IN SORGHUM-CONTAINING DIETS VARYING IN TANNIN CONTENT ON 29-DAY RAT WEIGHT GAIN, FEED CONVERSION, AND HEPATIC MICROSOMAL UDP-GLUCURONYLTRANSFERASE ACTIVITY (EXPERIMENT 4)

	Diets		
	1	2	3
Ingredients (%)			
RS610 (10.7[a]; <0.1[b])	92.46	—	84.56
Savanna (11.1[a]; 5.0[b])	—	88.88	—
Isolated soybean protein[c] (87.0[a])	—	—	8.03
Soybean oil	2.00	2.00	2.00
Vitamin and mineral premix[d]	4.79	4.79	4.79
L-Lysine-HCl	0.75	0.75	0.42
DL-Methionine	—	—	0.20
Glucose monohydrate	—	3.58	—
Crude protein (%)	9.9	9.9	16.0
Weight gain (g)[e]	138[i]	88[j]	202[h]
Gain (g)/feed (g)[e]	0.26[i]	0.18[j]	0.37[h]
UDP-glucuronyltransferase activity[f,g]			
nmole/mg protein	575[h]	616[h]	207[i]
μmole/100 g body wt	66[h]	66[h]	30[i]

[a,b] See Table I.
[c] See Table III.
[d] The premix contributed the following to each kilogram of diet: vitamin mixture, 10 g; mineral mixture, 35 g (American Institute of Nutrition AIN-76 vitamin and mineral mixtures for rats, ICN Nutritional Biochemicals, Cleveland, Ohio); choline chloride, 0.14 g; butylated hydroxy toluene, 0.125 g.
[e] Means of 10 rats per diet. Pooled SEM for gains = 8 and for gain/feed = 0.01.
[f] Means of individual microsomal preparations from 10 rats per diet. Figures represent the amount of naphthol conjugated in 10 min at 37°C.
[g] Pooled SEM for nmole/mg protein = 24 and for μmole/100 g body wt = 3.
[h,i,j] Row means with different superscripts are significantly different ($P < 0.05$).

to correct this problem of nonlinearity of the assay, the protein content of each microsomal suspension was determined and, in turn, the protein content of all suspensions within an experiment was made equal by dilution with buffer. Equality of protein content was verified by reassay for protein of each suspension after dilution. Since the assay response was more linear with lower as compared with higher protein concentrations, the amount of suspension that was used in the assays was such that the amount of protein added per vial[9] was within the more linear portion of the response curve.

Calculations of enzymatic activity were made in a similar manner to that of Lucier (19). In all experiments, enzyme activities represent the amount of naphthol conjugated to naphthyl glucuronide in 10 min at 37°C expressed either on a milligram microsomal protein (specific activity) or 100 g body weight basis. The latter method is thought to be a more accurate expression of the capacity of the animal to metabolize compounds since it takes into consideration effects of treatments on organ size relative to body weight as well as enzyme specific activity (21).

Statistical methods. The data were statistically analyzed by analysis of variance (22). Individual treatment differences were tested by the Newman-Keuls multiple-range test (22).

Results and Discussion. The results of Experiment 1 (Table I) showed that 20-day weight gains and feed conversions were both severely depressed ($P < 0.001$) in chicks fed HTS diets (Savanna and BR64) as compared with chicks fed the LTS control diet (RS610).

[9] The amount of protein added per vial, expressed as milligrams protein ± SEM, for Experiments 1–4 was 0.133 ± 0.006, 0.223 ± 0.002, 0.190 ± 0.002, and 0.219 ± 0.001, respectively.

Chicks fed Savanna or BR64 showed equivalent ($P > 0.05$) weight gains and feed conversions. These observations would agree with previous studies on the detrimental effects of HTS on growth and feed efficiency (1–4, 16, 23, 29).

The activity of hepatic microsomal GT, expressed either as nanomoles naphthol converted per milligram microsomal protein or micromoles naphthol converted per 100 g body weight, was significantly ($P < 0.01$) elevated in the two HTS treatments as compared with the LTS control treatment. Similar to growth responses, enzyme activity in chicks fed Savanna and BR64 was essentially equivalent. These results agree with the preliminary studies in our laboratory by Giles (4) who also observed elevated GT activity by feeding HTS to chicks.

It has been reported that when rats were fed a protein-free (7–9) or a protein-deficient 5% casein diet (9), there was a substantial elevation of the specific activity of this enzyme compared with protein-satiated control rats fed an 18% casein diet. Activity of GT was reported to be highly dependent on phospholipid content, and thus on the structural integrity of the microsomal membrane where this enzyme is embedded (24, 25). A protein deficiency was found to profoundly alter the phospholipid composition of the microsomal membrane causing an increased content of lysophosphatidylcholine and lysophosphatidylethanolamine, whereas little or none of these lysophosphatides were found in control preparations (9).

Since tannin has the ability to interact with protein (26–28) and thereby reduce digestibility (16), an explanation for the observed elevation of activity of GT in chicks fed HTS might be explained by a tannin-induced protein deficiency. In a preliminary study[10], it was found that the phospholipid composition of hepatic microsomal suspensions from chicks fed LTS and HTS diets in Experiment 1 was equivalent, which would nonconclusively indicate that the protein effect was unimportant in explaining the observed increase in HTS treatments. However, a protein deficiency induced by tannin would be a mild one for chicks compared with the severe protein deficiency induced by feeding either a protein-free or a 5% casein diet to rats (7–9) where the significant alteration in phospholipid composition of microsomal membranes was noted (9). Furthermore, the comparison of Graham *et al.*, (9) was between diets that were adequate (18% casein) and deficient (5% casein) in protein for rats while the comparison in Experiment 1 was among diets that were all protein deficient (16% protein) for chicks, but to a much lesser extent. Therefore, the purpose of Experiment 2 was to differentiate the effect due to a protein deficiency from that due to tannin on the activity of GT in chick liver.

The three dietary levels of protein used in the LTS diets of Experiment 2 were intentionally chosen in order to make a relevant comparison to the first experiment and at the same time to differentiate between protein and tannin effects. Diets containing 16% protein are suboptimal for the chick (12). However, this approximate level of protein has been used in sorghum studies by previous investigators in our laboratory (1–4, 16, 29) and is purposely used to maximize any effect of tannin since increasing the level of protein has been reported to reduce or alleviate the detrimental effects of tannin (29–31). Therefore, in order to approximate the growth depression caused by feeding HTS relative to the 16% protein LTS control diet, total protein contents of 14.5 and 13% were used in two other LTS diets of this experiment (Table II).

Chick response to sorghum–soybean meal diets varying in protein and tannin content showed that both 19-day weight gains and feed conversions were progressively depressed by each incremental reduction in protein for the LTS treatments (Table II). Both parameters were significantly ($P < 0.001$) poorer for the 14.5 and 13% protein LTS treatments as compared with the 16% protein LTS treatment. Similarly, both gain ($P < 0.01$) and feed conversion ($P < 0.001$) were significantly poorer for the 13% protein LTS as compared with the 14.5% protein LTS treatment. Weight gain for the HTS treatment was significantly ($P < 0.01$) poorer than the 16% protein LTS, but significantly greater than both the 14.5% protein LTS ($P < 0.05$) and the 13% protein LTS ($P < 0.001$) treatments. Feed conversion of

[10] Sell and Rogler, unpublished results.

the HTS treatment was significantly poorer than both the 16 and 14.5% protein LTS treatments, but statistically ($P > 0.05$) equivalent to the 13% protein LTS treatment.

Regardless of method of expression, activity of GT was significantly ($P < 0.05$) elevated in chicks fed either the 14.5 or 13% protein LTS diets over those fed the 16% protein LTS diet (Table II). Mean activity of the 13% protein LTS diet was slightly greater than that of the 14.5% protein LTS diet; however, the difference was not statistically significant ($P > 0.05$), unlike weight gain which was significantly ($P < 0.01$) different between these two treatments. In comparison, mean activity of the HTS treatment was significantly ($P < 0.001$) greater when expressed both ways than the mean activities of all LTS treatments even though the average weight gain of this treatment was significantly greater than either the 13% ($P < 0.001$) or 14.5% ($P < 0.05$) protein LTS treatments. These results suggest that only a relatively small part of the increased GT activity observed by feeding HTS can be attributed to a tannin-induced protein deficiency.

Since the diets of the previous two experiments contained a deficient level of protein, Experiment 3 was designed to determine the effects of a higher level of dietary protein on the activity of GT and to see if the same relationship between HTS and LTS held at this higher level. The experimental design was a 2×2 factorial consisting of variety of sorghum (LTS and HTS) versus level of protein (15.1 and 24.2%).

The results showed that 20-day weight gains (Table III) were adversely affected by both the lower protein ($P < 0.001$) and HTS ($P < 0.005$). Chicks fed the 24.2% protein LTS diet demonstrated the greatest weight gains; however, these gains were nonsignificantly ($P > 0.05$) different from gains of chicks fed the 24.2% protein HTS diet. In contrast, average weight gains for the 15.1% protein LTS treatment were significantly ($P < 0.01$) greater than that of the 15.1% protein HTS treatment.

Dietary trends for feed conversions among diets paralleled closely that of weight gains (Table III). Significant main effects were noted for both protein ($P < 0.001$) and tannin ($P < 0.025$) in that feed conversions were negatively affected by the lower protein and HTS. Tannin tended to have less adverse effect on growth and feed conversion at the higher level of protein, which would agree with previous investigations (29–31) where protein was found to alleviate the depressive effects of tannin; however, in this case, the tannin by protein interaction was not significant ($P > 0.05$).

When the dietary protein level was increased to 24.2% in the LTS diet, activity of GT was significantly ($P < 0.001$) reduced compared with activity of the 15.1% protein LTS control treatment (Table III). This observation is in agreement with the findings of Graham *et al.* (9) who reported that decreasing the protein from an optimal to a deficient level significantly activated GT in rat liver, and also that of Experiment 2 (Table II) where lowering the protein from 16 to 14.5 and 13% significantly ($P < 0.05$) activated this enzyme in chick liver. Similarly, activity was also depressed ($P < 0.001$) by feeding 24.2% protein as opposed to 15.1% protein in the HTS diets, but the relative magnitude of the depression was less that that noted between the LTS diets. As in the previous experiments (Tables I and II), GT was significantly ($P < 0.001$) activated by HTS in chicks fed 15.1% protein. HTS had a similar effect at the 24.2% protein level and actually increased the difference in activities between HTS and LTS. Activity of the 24.2% protein HTS treatment remained significantly ($P < 0.001$) elevated over that of the 24.2% protein LTS, even though weight gain between these two treatments was not significantly different ($P > 0.05$). However, activity of the 24.2% protein HTS treatment was not significantly ($P > 0.05$) greater than the 15.1% protein LTS treatment. Therefore, the results of this experiment would also indicate that a tannin-induced protein deficiency was not totally explaining the activation of this enzyme in HTS-fed chicks.

Experiment 4 examined the effects of feeding HTS and LTS to rats on the activity of GT. Rats were fed either HTS or LTS diets containing 9.9% protein contributed only by sorghum or a LTS–ISP diet containing 16% protein. The results (Table IV) showed that 29-day weight gains and feed conversions were both significantly ($P < 0.001$) reduced by feeding HTS as compared with both LTS and LTS–ISP diets. Both parameters for the LTS–ISP diet were significantly ($P < 0.001$) superior

to those of the 9.9% protein LTS diet. In agreement with the results of the previous experiments with chicks, GT was significantly ($P < 0.001$) elevated in rats fed the lower-protein LTS or HTS diets compared with the higher-protein LTS–ISP diet. However, unlike the results of the experiments with chicks, activity expressed by two methods was not significantly ($P > 0.05$) different between HTS and LTS treatments. These results agree with a preliminary study with a limited number of rats[10] which showed that GT activity was not significantly ($P > 0.05$) greater for rats fed two HTS varieties (BR64 and Savanna) for 73 days over those fed the LTS (RS610) control diet. However, feeding a commercial type of diet for the same length of time significantly ($P < 0.001$) reduced the activity of this enzyme.

Thus, the results of these experiments suggest that only a relatively small part of the noted increased GT activity in HTS-fed chicks can be attributed to a sorghum tannin effect on protein digestibility. Furthermore, the results indicate that condensed tannins, or their breakdown products, are being absorbed and activating the hepatic GT system in the chick, but not the rat. Tannins found in grain sorghum are of the condensed type (32, 33) and have been proposed to be oligomers of 6 to 7 flavan-3-ol units (33). Although mono- and dimeric flavan-3-ols have been detected in immature sorghum grain, little to none have been identified in the mature grain where the polymeric form is the principal and commonly the sole tannin (33). Although there is some evidence that suggests condensed tannins may undergo microbial degradation (34), there is essentially no evidence that indicates their degradation and absorption in the gastrointestinal tract. The monomeric form of condensed tannin, catechin, or flavan-3-ol, when given orally has been reported to be absorbed and eliminated as one of several glucuronide conjugated metabolites (35–37). The fact that HTS increased GT in the chick and had little effect in the rat suggests a true species difference either in the absorption of intact tannins or in the intestinal degradation of the oligomers and subsequent absorption of the breakdown products.

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