Absorption and Distribution of ^{14}C-Labeled Condensed Tannins and Related Sorghum Phenolics in Chickens†

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Condensed tannins from sorghum [*Sorghum bicolor* (L.) Moench.] seeds are the only type of tannins found in this plant and are commonly associated with toxic effects in animals fed diets based on high-tannin sorghum grains. However, it is still not certain whether those polyphenolic polymers are absorbed from the digestive tract. Condensed tannin and other related phenolic fractions from sorghum seeds were metabolically radiolabeled with ^{14}C and separately fed to Hubbard White Mountain chicks. Their tissues were examined to determine the absorption and distribution of the polyphenolic fractions. Immature high-tannin sorghum seeds (Dekalb BR-64) were radiolabeled with $NaH^{14}CO_3$ or $^{14}CO_2$, and various phenolic compounds were purified according to the method of Reddy and Butler (*J. Agric. Food Chem.* **1989**, *37*, 383–384). Radiolabeled condensed tannin and non-tannin phenolic fractions were lyophilized and separately placed into starch capsules, and each capsule was placed into the crop of chicks. Eight hours later, blood samples were taken by cardiac puncture, the birds were killed by excess CO_2 inhalation, tissue samples were excised, and total excreta were collected. Analyses of ^{14}C distribution in chick tissues and excreta suggest that radiolabeled condensed tannins from sorhum grain were not absorbed from the digestive tract of chickens. However, ^{14}C from non-tannin fractions was absorbed and distributed in various tissues. It is proposed that low molecular weight polyphenols present in these fractions may be partially responsible for the toxic effects seen in chickens fed high-tannin sorghum diets.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench.] cultivars with high levels of condensed tannins are widely cultivated around the world because they are resistant to bird depredation, preharvest germination, mold production, and weathering (Butler, 1989; Salunkhe *et al.*, 1990). Unfortunately, when these grains are fed to animals, toxic effects are commonly observed. Reduced growth rate, poor feed efficiency, and decreased dry matter, protein, and amino acid digestibilities are the primary effects reported (Butler, 1989; Salunkhe *et al.*, 1990).

Some studies suggested that these negative effects arise mainly because the digestion of dietary protein is inhibited (Rostagno *et al.*, 1973; Cousins *et al.*, 1981). However, the growth depression seen in chicks fed high-tannin sorghum diets could be overcome without affecting the apparent digestibility of dietary protein or dry matter utilization (Elkin *et al.*, 1978a). In addition, some toxic effects observed in chicks fed high-tannin sorghum diets suggest that polyphenolic compounds are absorbed from the digestive tract. These include an increased incidence of leg anomalies in chicks fed high-tannin sorghum as compared with those fed low-tannin sorghum diets (Rostagno *et al.*, 1973; Armstrong *et al.*, 1973; Elkin *et al.*, 1978b) and increased hepatic UDPglucuronyltransferase activity in chickens fed high-tannin sorghum vs low-tannin sorghum diets (Sell and Rogler, 1983). Since UDPglucuronyltransferase participates in the detoxification of phenolics, an increase in its activity would be expected if condensed tannin or other phenolic compounds were absorbed. Further evidence for possible absorption of condensed tannins was provided by the finding of radioactivity in blood, liver, and kidneys of rats fed ^{125}I-labeled polyphenolics from Quebracho (*Schinopsis* spp.) (Rogler and Butler, 1985). Whether the iodination process changed the properties or structure of these compounds is not known. More conclusive evidence that condensed tannins may be absorbed was provided by Laparra *et al.* (1977). When ^{14}C-labeled grape (*Vitis vinifera*) procyanidins were fed to mice, radioactivity was detected in blood, liver, kidney, and other tissues; however, the ^{14}C oligomers used were basically dimeric procyanidins.

Whether polymeric condensed tannins are digested and/or absorbed from the digestive tract of birds and small mammals is still unclear. The purpose of this study was to determine whether condensed tannins and related phenolics from high-tannin sorghum seeds are absorbed from the digestive tract of chickens when ^{14}C-labeled condensed tannins and non-tannin phenolic fractions are orally administered.

MATERIALS AND METHODS

Radiolabeling of High-Tannin Sorghum Seeds. Two procedures were followed: (1) immersion of immature high-tannin sorghum seeds in buffer solution containing $NaH^{14}CO_3$, and (2) exposure of the seeds to $^{14}CO_2$. The first procedure was repeated four times as follows: Thirty grams of fresh immature seeds (4–5 days after half-anthesis) from Dekalb BR-64 were placed in a 10-cm Petri dish and immersed in 50 mL of either 10 or 100 mM HEPES buffer (1 or 10 mM $CaCl_2$ plus 10 or 100 mM HEPES plus 0.1 or 1.0% BSA, respectively) and adjusted with KOH to a pH of 7.2. A mixture of cool-white fluorescent and tungsten filament incandescent lamps provided a photosynthetic photon flux density of 300 microeinsteins·m^{-2}·s^{-1}. Eighty microcuries of $NaH^{14}CO_3$ was mixed into the buffer. After 4 h, the seeds were taken out of the dish and allowed to dry in a hood at room temperature for 4 days. The second procedure used two to three

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freshly cut immature panicles (5–6 days after half-anthesis) and was repeated four times. The stems were cut 10 cm long and put in a jar with distilled water. The jar was sealed in a 3.5-L closed system. The same photosynthetic photon flux density as before was provided, and the $^{14}CO_2$ was generated by reacting 80 μCi of $NaH^{14}CO_3$ with 2.0 mL of 1 M lactic acid. Air contained in the system and the $^{14}CO_2$ generated circulated throughout the system at a rate of 1 L·min^{-1}. After 7 h, the remaining $^{14}CO_2$ was trapped in 0.5 N NaOH. The panicles were taken out of the chamber and allowed to dry in a hood at room temperature for 4 days. After that drying period, an increase is expected in the concentration of condensed tannins, by increasing the polymerization of the proanthocyanidins present in the early immature sorghum seeds, as is an increase in tannin specific activity (Butler, 1982; Reddy and Butler, 1989).

Extraction and Purification of the Radiolabeled Sorghum Tannins and Non-Tannin Related Phenolics. The procedure was based on that of Reddy and Butler (1989). Four ^{14}C-labeled fractions were obtained: (a) three non-tannin polyphenolic fractions, (1) aqueous, (2) ethanol wash, and (3) ethanol eluate; and (b) one condensed tannin fraction, (4) acetone eluate. Fractions 1 and 2 were originated from washing the Sephadex LH-20 slurry with distilled water and absolute ethanol, respectively. Fractions 3 and 4 came from the elution of a 4 × 30 cm Sephadex LH-20 column with absolute ethanol and combined 60% and 80% acetone, respectively. The organic solvents were evaporated under reduced pressure; the residues were dissolved in a small volume of distilled water and then lyophilized. The aqueous fraction remained in a liquid phase due to the inability to completely lyophilize it.

The replications of each phenolic fraction obtained by either exposing the seeds to $^{14}CO_2$ or soaking in $NaH^{14}CO_3$ were separately combined and lyophilized to obtain sufficient radiolabeled phenolics to run the chick experiment. Samples of each lyophilized fraction were combusted for 1.5 min in a sample oxidizer (Packard Model 306), with the resultant $^{14}CO_2$ trapped in 10 mL of Carbo-sorb (Packard Instrument Co.) mixed with 10 mL of Permafluor V (Packard). Liquid samples from the aqueous fraction were pipetted onto cotton pads placed into Combust-cones (Packard), and the same volume of Combust-aid (Packard) was added. The radioactivity was measured in a liquid scintillation spectrometer (Packard Model 460C). Each sample was counted twice for periods of 10 min each. The counts were corrected for background, quenching, and counter efficiency. The results were expressed as disintegrations per minute (dpm).

Characterization of the ^{14}C-Labeled Phenolic Fractions. The radiolabeled phenolic fractions were chromatographed on thin-layer chromatography (TLC) AVICEL microcrystalline cellulose plates (20 × 20 cm in size, 0.250 mm thick, Analtech, Inc.) at room temperature. The plates were developed in 1-butanol–acetic acid–water (BAW) (4:1:5 v/v/v) and in 6% acetic acid (v/v with distilled water) (Van Sumere, 1989) until the solvent front was 15 cm from the origin. After drying at room temperature, the plates were observed under long-wave UV light. The plates were then sprayed with vanillin reagent (vanillin–HCl, 1:10 w/v) to detect flavan-3-ols or a mixture of equal volumes of 2% $K_3Fe(CN)_6$ and 2% $FeCl_3$ (w/v in distilled water) to detect phenolics. Radiolabeled compounds were located by exposing X-ray films (XAR-5, Kodak Diagnostic Films) to the plates for 7 days.

Aqueous and ethanol wash fractions (treatments 2 and 3) were subjected to an acid hydrolysis according to the method of Harborne (1984) to detect the presence of glycosides through their sugars and aglycon groups. Ten microliters of aqueous fraction or 7 mg of the ethanol wash fraction was subjected to the assay. Three different subfractions were obtained from each hydrolysis: ethyl acetate, expected to contain the aglycon groups except for anthocyanidins; amyl alcohol, expected to contain anthocyanidins; and the aqueous fraction, expected to contain sugars. After being dried under reduced pressure, the subfractions were dissolved in 0.2 mL of absolute ethanol, 1% HCl–methanol (1:99 v/v), or distilled water, respectively, for chromatography. Those three subfractions from each hydrolyzed ^{14}C-labeled phenolic fraction were chromatographed along with a ^{14}C-labeled glucose on an AVICEL microcrystalline cellulose plate, as before, at room temperature. The plate was developed in Forestal solvent (acetic acid–HCl–water, 30:3:10 v/v/v; Harborne, 1984) until the solvent front was 15 cm from the origin. After drying at room temperature, the plate was sprayed with a mixture of equal volumes of 1.8% aniline in absolute ethanol and 1.8% oxalic acid in distilled water and dried at 100 °C for 10 min (Partridge, 1949) to detect sugars. Radiolabeled compounds were detected by autoradiography, as explained previously.

Table 1. Composition of the Experimental Diet

ingredient	%
sorghum Northrup King Savanna III (10.6)[a] (4.6)[b]	80.20
soybean meal (48.5)[a]	13.40
iodized NaCl	0.30
dicalcium phosphate	1.84
limestone	0.63
vitamin and trace mineral mix[c]	1.00
corn oil	2.00
L-lysine hydrochloride	0.18
L-threonine	0.05
L-arginine	0.20
glycine	0.20
total	100.00

[a] Percent protein of the grain. The crude protein of the diet was 15.0%. [b] Percent catechin equivalent. [c] Contains, per kg of diet: choline chloride, 1.3 g; retinyl palmitate, 5000 IU; cholecalciferol, 2500 ICU; *d*-α-tocopheryl acetate, 22 IU; menadione sodium bisulfite, 1.23 mg; riboflavin, 8.8 mg; calcium pantothenate, 17.6 mg; niacin, 39.6 mg; D-biotin, 0.15 mg; vitamin B_{12}, 84 μg; sodium selenite, 0.33 mg; manganese sulfate, 0.5 g; zinc oxide, 60 mg; butylated hydroxytoluene, 0.125 g.

Animals and Treatments. Fifty-two day-old male Hubbard White Mountain chicks were weighed, wing-banded, and placed in electrically heated battery brooders with raised wire floors. Commercial feed and water were provided *ad libitum* during a 19-day period. On the last day, the birds were weighed and a group of nineteen chicks with similar body weights were randomly allotted to the different treatments. The treatments consisted of the different radiolabeled phenolic fractions given to the chickens, as follows: treatment 1, control (no ^{14}C); treatment 2, aqueous fraction; treatment 3, ethanol wash fraction; treatment 4, ethanol eluate fraction; treatment 5, condensed tannin fraction. Each bird was individually placed in a stainless steel rat metabolism cage, with four replicates per treatment. Treatment 3 (ethanol eluate) had only three replicates due to insufficient amounts of this fraction. During a 4-day adjustment period, the chicks received a high-tannin sorghum-based diet (Northrup King Savanna III) containing 15% crude protein (Table 1). Crude protein contents of the sorghum grain and soybean (*Glycine max* L.) meal were determined by the Kjeldahl procedure (AOAC, 1980), and the sorghum condensed tannin concentration was estimated as catechin equivalent according to the method of Price *et al.* (1978).

Preparation of the Starch Capsules. The ^{14}C-labeled fractions were weighed, placed into 12 × 7 mm corn (*Zea mays* L.) starch capsules, and sealed. The aqueous fraction (treatment 2) was pipetted into the capsules previously lined with corn starch. This was done shortly before administration to the chicks to avoid disintegration of the capsules. Capsules for the control treatment contained only corn starch. The approximate amounts of radioactivity provided to each bird in treatments 2–5 were, respectively, 0.34, 0.27, 0.15, and 0.22 μCi.

Administration of the Radiolabeled Fractions and Collection of the Samples. A capsule containing one of the ^{14}C-labeled phenolic fractions or unlabeled control was placed into the crop of each chicken. Eight hours later, blood samples were taken by cardiac puncture with a heparinized needle and syringe. Blood was stored in test tubes on ice until centrifugation at 4 °C for 10 min at 10000*g*. The plasma obtained following centrifugation was frozen for later analyses. The birds were killed by excess CO_2 inhalation, and tissue samples were immediately excised and frozen. The entire digestive tract was sectioned. The contents of the stomach, crop, and gizzard were combined, and the colon content comprised a separate sample. The other

Table 2. Activity of the Phenolic Fractions from High-Tannin Sorghum Seeds Labeled with ^{14}C[a]

treatment	description	specific activity, dpm·mL^{-1}
	Non-Tannin Fractions	
2	aqueous	2193[b]
3	ethanol wash	3648
4	ethanol eluate	3273
	Condensed Tannin Fraction	
5	acetone	6025

[a] Combined phenolic fractions labeled either by immersion in HEPES buffer with 80 μCi of $NaH^{14}CO_3$ for 4 h or by exposure to $^{14}CO_2$ for 7 h (80 μCi of $NaH^{14}CO_3$ + 2 mL of 1 M lactic acid). [b] Specific activity was unknown due to an inability to completely dry the material.

sections of the digestive tract remained with their respective digesta contents. Entire livers, kidneys, and left femurs were also removed. Excreta from the 8-h test period were air-dried.

Sample Preparation, Oxidation, and Radioactivity Measurements. The various frozen tissues, digesta, and excreta were weighed, lyophilized, and ground and their weights recorded. Duplicate samples (0.20 g each) were oxidized for 1.5 min in a sample oxidizer, and the radioactivity was measured as previously described. Plasma samples (0.5 mL) were pipetted into Combustcones lined with cotton pads, and the same volume of Combustaid was added. The total volume of plasma for each bird was estimated to be approximately 7% of the chicken's body weight (Sturkie, 1986).

The recovery of the oxidation products was determined by adding known amounts of a [^{14}C]leucine standard solution to triplicates of three different kinds of tissue before and after oxidation. The counts per minute (cpm) obtained were corrected for background, quenching, and counter efficiency and the results expressed as dpm. The average recovery was 103% (data not shown).

Statistical Analysis. Data were analyzed by ANOVA using the General Linear Models procedure (SAS Institute, 1982). The model included terms for replicate, treatment, and experimental error. Treatment means were compared by Newman–Keuls multiple-range test (Steel and Torrie, 1980). A probability level of $\alpha = 0.05$ was used in testing the statistical significance of all experimental data. All data were subjected to log transformation after Bartlett's test was applied to test the homogeneity of the variances ($\alpha = 0.0001$) (Anderson and McClean, 1974).

RESULTS AND DISCUSSION

Radiolabeled Phenolic Fractions. Radiolabel was incorporated into phenolic compounds after exposure of the immature seeds to either $NaH^{14}CO_3$ or $^{14}CO_2$. More radioactivity was incorporated into the condensed tannin fraction from seeds exposed to $^{14}CO_2$ (data not shown). In contrast, the incorporation by the non-tannin fractions was similar or greater in the more diluted HEPES buffer (10 mM) containing $NaH^{14}CO_3$ than in $^{14}CO_2$ (data not shown). Combined fractions of condensed tannin gave the highest specific activity (Table 2).

Using $NaH^{14}CO_3$ or $^{14}CO_2$ as radiolabels gave lower values of radioactivity in the ethanol and condensed tannin fractions than those reported by Reddy and Butler (1989). This may be explained in part by the different cultivars used (Dekalb BR-64 vs IS 8768 and IS 6881) but more likely by the nature of the radiolabel provided ($NaH^{14}CO_3$ or $^{14}CO_2$ vs [^{14}C]phenylalanine). Radiolabel incorporated from the former may be introduced in many different molecules, including sugars, while phenylalanine is much more specific, since it is a precursor of flavonoids through the phenylpropanoid pathway (Stafford, 1989).

On the basis of R_f values, chromatograms from the TLC plates developed with either BAW or 6% acetic acid showed that both ethanol fractions (treatments 3 and 4) had similar compositions, but they differed from the aqueous fraction (treatment 2) (Table 3). The condensed tannin fraction (treatment 5) behaved differently from the non-tannin fractions, with most of the sample remaining at the origin but also giving a long brown smear. It was not possible to precisely characterize the compounds present in each fraction because of the poor resolution and the smears due to mixtures of oligomeric forms (Markham, 1989).

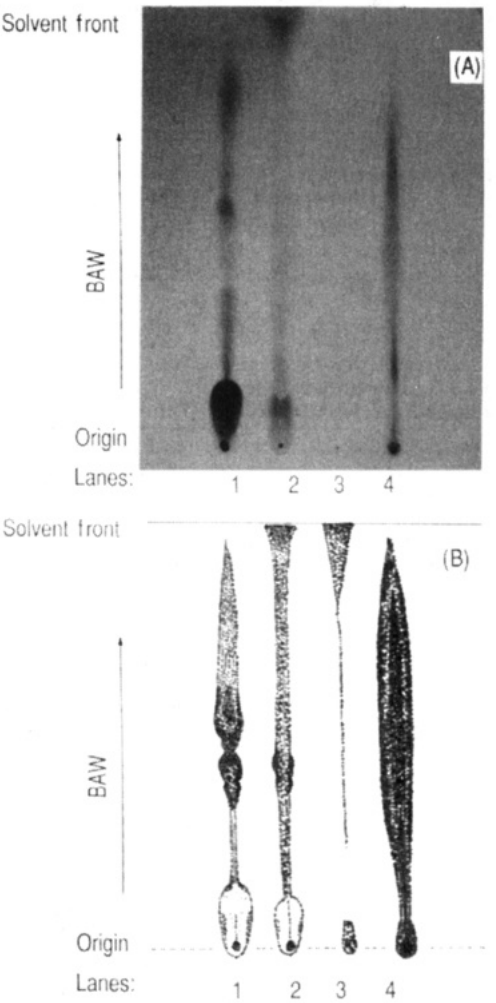

Figure 1. (A) Autoradiogram of the TLC plate of the radiolabeled sorghum phenolic fractions, developed in 1-butanol–acetic acid–water (4:1:5 v/v/v) solvent system. (B) Reproduction of the reaction of the radiolabeled sorghum phenolic fractions to 2% $FeCl_3$/2% $K_3Fe(CN)_6$ (w/v of water) spray reagent. Lanes: (1) aqueous fraction; (2) ethanol wash fraction; (3) ethanol eluate fraction; (4) acetone fraction.

Autoradiograms from TLC plates developed in Forestal solvent are presented in Figure 1A. On TLC plates, only the condensed tannin fraction reacted positively with the vanillin reagent, indicating the presence of flavan-3-ols or their polymers (condensed tannins). All fractions gave a positive reaction with $FeCl_3/K_3Fe(CN)_6$ spray reagent, suggesting the presence of phenols (Figure 1B). Other types of compounds may have been labeled with ^{14}C. In fact, the autoradiogram from the TLC plate developed in Forestal solvent showed that both the hydrolyzed and nonhydrolyzed samples from the aqueous fraction had radiolabeled glucose in high levels ($R_f = 0.71$). Hydrolyzed and nonhydrolyzed samples from both ethanol fractions also had a spot with R_f equal to the [^{14}C]glucose standard ($R_f = 0.71$). Light brown colors were observed on those spots after the plate was sprayed with the aniline/oxalic reagent, consistent with the presence of hexoses (data not shown). None of the hydrolyzed samples showed a positive

Table 3. R_f Values and Smearing from Thin-Layer Chromatograms of Phenolic Fractions Developed in AVICEL Microcrystalline Cellulose Plates in 6% Acetic Acid and in BAW:[a] Observed under Daylight and Long-Wave UV

		R_f in 6% HOAc		R_f in BAW	
treatment	description	daylight	UV	daylight	UV
		Non-Tannin Fractions			
2	aqueous	brown origin and smear; 0.62	0.57	0.58	0.13, 0.50, 0.57
3	ethanol wash	pinkish smear; 0.97	0.35, 0.72	0.57, 0.97	0.13, 0.57
4	ethanol eluate	0.97	0.36, 0.73	0.57, 0.97	0.50, 0.57
		Condensed Tannin Fraction			
5	acetone eluate	brown origin and smear	smear	brown origin and smear	smear

[a] 4:1:5 v/v/v.

Table 4. Distribution of ^{14}C in Various Tissues of Chickens 8 h after an Oral Dose[a] of ^{14}C-Labeled Polyphenolic Fractions from High-Tannin Sorghum Seeds

	% of dose for treatment[b]			
tissue	2	3	4	5
liver	3.91[a]	1.78[b]	2.43[ab]	0.07[c]
crop, stomach, gizzard, and contents	1.25[a]	0.97[a]	2.98[a]	1.56[a]
intestines, ceca, colon and contents	8.06[b]	11.27[b]	16.61[b]	24.94[a]
excreta	8.83[d]	50.09[b]	36.57[c]	73.58[a]
femur	0.14[a]	0.09[b]	0.10[b]	0.00[c]
plasma	0.84[a]	0.53[b]	0.81[a]	0.00[c]
total recovery	23.39[d]	64.93[b]	59.84[c]	100.39[a]

[a] Each bird from treatments 2–5 received, respectively. 0.34, 0.27, 0.15, or 0.22 μCi. [b] Treatments 2–5 correspond to the fractions as follows: aqueous, ethanol wash, ethanol eluate, and acetone eluate, respectively. Means within rows with no common superscripts are significantly different ($P < 0.05$), as determined by the Newman–Keuls multiple-range test (Steel and Torrie, 1980).

response with the vanillin spray reagent. No other radioactive spots besides glucose were observed in the autoradiogram. This may be due to the small amount of radiolabeled sample used in the hydrolysis. When bigger samples were used in another experiment (data not shown), phenolics were detected from similar though nonradiolabeled hydrolyzed phenolic fractions from sorghum grain.

Chicken Experiment. Virtually no radioactivity was found in the various tissues of chickens fed the control treatment (treatment 1). Radioactivity was detected, however, in all tissues, plasma, and excreta from chickens receiving treatments 2–4 (Table 4). In general, the amount of activity recovered in the nondigestive tract tissues was higher for the aqueous fraction than for the ethanol fractions. Most of the ^{14}C administered to the chickens was recovered in the excreta, with the next greatest amount found in the digestive tract and its contents. Significantly, no radioactivity was detected in plasma or tissue samples from birds receiving the condensed tannin fraction (treatment 5). In fact, all of the radioactivity from this fraction was recovered in the excreta and in the gastrointestinal tract and its contents. These results indicated that condensed tannins were not absorbed from the digestive tract. On the other hand, the results obtained from treatments 2–4 showed that radiolabeled compounds present in those non-tannin fractions were absorbed by various tissues.

The ethanol fractions (treatments 3 and 4) gave similar TLC chromatograms (Table 3). The phenolic compounds from those treatments likely include catechin, a major component of sorghum condensed tannins, and its proanthocyanidin dimers and derivatives (Gujer *et al.*, 1986). Those phenolics are small enough to be absorbed as evidenced by the studies of Rao and Rao (1980), who reported that orally administered (+)-catechin was absorbed from the digestive tract of rats. Radioactivity detected in the various tissues may have originated mostly from ^{14}C-labeled sugars present in the non-tannin fractions as well as from radiolabeled phenolics (Figure 1). Phenolics could not be detected in chick tissues by TLC chromatography, probably due to their low concentration. The aqueous fraction (treatment 2) may consist of relatively polar flavonoids plus glycosides and/or free sugars. The presence of glycosides in sorghum grains had been reported (Nip and Burns, 1969).

The present results are in agreement with those of Laparra *et al.* (1977), who labeled phenolic compounds in grapes by exposing the plants to $^{14}CO_2$. The ^{14}C-labeled monomers and dimeric procyanidins were fed to mice, and radioactivity was recovered in the plasma and various other tissues, as well as in expired $^{14}CO_2$. Moreover, $^{14}CO_2$ was detected in large amounts when (+)-[*ring A*-^{14}C]-catechin was orally adminstered to rats and guinea pigs (Das and Griffiths, 1969). Furthermore, these investigators also found low though significant levels of $^{14}CO_2$ when (+)-[U-^{14}C]catechin was used. Although we did not measure expired $^{14}CO_2$, it is likely that the low total recoveries of the radioactivity from the tissues and excreta of birds administered the non-tannin fractions may be due to conversion of ^{14}C-labeled compounds to $^{14}CO_2$ (Table 4). Some radioactivity not accounted for may have also been present in other tissues and organs that were not sampled.

Tannin and non-tannin fractions are presently being characterized by RP-HPLC, and the sugars present in the latter are being identified and quantified by GC–EIMS. The ultimate goal of this work would be the improvement of the nutritional value of high-tannin sorghum feeds.

Conclusions. Polymeric ^{14}C-labeled condensed tannins extracted from sorghum grains were not absorbed from the intestinal tract of chickens. Manipulation of condensed tannins during the extraction and purification procedures may have affected the original structure of the tannin molecules, which could have influenced their absorbability. If polymeric condensed tannins were not absorbed, then the toxic effects observed in birds consuming high-tannin sorghum diets may be due to intraluminal effects of condensed tannins or to other toxic compounds present in sorghum grains that can be absorbed from the intestinal tract of birds. At least in *in vitro* conditions, the presence of some phenolic acids or catechin (condensed tannin monomer) reduced Na^+-dependent D-glucose uptake (Welsch *et al.*, 1989a) and inhibited intestinal sucrase (EC 3.2.1.48) activity in brush border membrane vesicles of rat intestines (Welsch *et al.*, 1989b).

ABBREVIATIONS USED

TLC; thin-layer chromatography; BAW; 1-butanol–acetic acid–water; RP-HPLC; reversed-phase high-performance liquid chromatography; GC–EIMS, gas chromatography–electron impact mass spectrometry.

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