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Biological Activity of Phenolic Compounds. Hepatic Cytochrome *P*-450, Cytochrome b_5, and NADPH Cytochrome *c* Reductase in Chicks and Rats Fed Phenolic Monomers, Polymers, and Glycosides (42134)

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Abstract. Eight experiments were conducted to determine effects of a phenolic polymer (Kraft wood lignin, Indulin), phenolic glycosides (cane molasses and wood molasses), and phenolic monomers (vanillin, vanillic acid, ferulic acid, and *p*-coumaric acid) on liver cytochromes *P*-450, cytochrome b_5, and NADPH cytochrome *c* reductase in chicks and rats. Chicks fed 6.0% lignin had a higher ($P < 0.01$) cytochromes *P*-450 content than did chicks fed 0% fiber, 6.0% wood cellulose (Solka Floc), or 6.0% arenaceous flour. NADPH cytochrome *c* reductase activity was not affected by treatment. Chicks fed 12.0% wood molasses had a higher ($P < 0.06$) cytochromes *P*-450 level than did chicks fed 0% fiber or 6.0% wood molasses. Cane molasses incorporated at both 6.0 and 12.0% of the diet induced ($P < 0.05$) cytochromes *P*-450 content over those of control-fed birds. Chicks fed 6.0% lignin, with or without antibiotic (bacitracin:neomycin sulfate, 2:1), had a higher ($P < 0.01$) cytochromes *P*-450 level than did chicks fed control diets, with or without antibiotic. Additionally, chicks fed 6.0% lignin had lower ($P < 0.01$) intestinal diaminopimelic acid (DAP) levels than did chicks fed 0% fiber. Rats fed 0% fiber, 6.0% wood cellulose, 6.0% arenaceous flour, or 6.0% lignin exhibited no difference in cytochrome level or activity among treatments. Chicks fed 0.5% vanillin, 0.5% vanillic acid, 0.5% ferulic acid, or 0.5% *p*-coumaric acid had comparable cytochromes level and activity compared with chicks fed no phenolics. Chicks fed 0.5% *p*-coumaric acid had lower ($P < 0.05$) rates of gain than did chicks fed control or other phenolic-containing diets. Rats fed these phenolics had similar cytochromes *P*-450 content among treatments.

Phenol effects vary widely. Some, such as aflatoxin, are potent carcinogens yet others, such as the vitamins E and K, are highly beneficial. Rat LD_{50} values range from <1 to 5000 mg/kg body wt (1). Xenobiotic phenolics ingested by animals appear to be metabolized and detoxified by mechanisms similar to those for essential phenols. Toxic responses elicited from these phenols apparently result, in part, from the consumption of energy in detoxication reactions such as methylation, sulfation, glucuronide formation, and by loss of these functional groups from more useful metabolism (1).

Lignin is a polymerized product of phenylpropanoid alcohols and ferulic and *p*-coumaric acids (2), arising in the plant from the shikimic acid pathway in which the aromatic amino acids, phenylalanine and tyrosine, are major substrates. Lignin, or its constituent phenolic monomers, oligomers, and polymers, represents a significant part of diets of most animals, and, to a lesser degree, that of humans. Dietary lignin has generally been associated with negative effects on the animal, leading to a decrease in utilization of forages and fibrous feedstuffs; however, it has not been known to cause any serious toxic effects. In addition, lignin can serve as an antioxidant and chelate both cholesterol and nitrites (1).

Wood molasses and cane molasses are polymerized forms of carbohydrate with phenolics and are frequently used feed additives. When these substances are used in feed as binders or to improve diet acceptability, they increase the already substantial phenolic load of natural diets.

There is evidence that certain dietary fiber constituents may alter liver metabolic activity. Animals fed natural diets tend to have an increased cytochromes *P*-450 mixed-function oxidase (MFO) level compared to those fed synthetic diets (3). This effect may be due to the presence of foreign residues (e.g., pesti-

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cides or feed additives) and nutrient and(or) nonnutrient constituents of vegetables (4). Efforts have been made to determine the exact component(s) of the natural diet responsible for this increased activity. Since certain nondietary phenolics have been shown to induce cytochromes *P*-450 content (5), the objective of these experiments was to test a Kraft wood lignin (Indulin), phenolic–carbohydrate complexes (cane molasses and wood molasses), and several commonly occurring phenolic monomers, for their cytochromes *P*-450-inducing ability. In addition, an effort was made to distinguish between responses elicited by animal metabolism vs gut microbial metabolism.

Materials and Methods. In experiment 1, 20-day-old New Hampshire × Columbian female chicks (avg init wt, 207 g) were individually wing-banded and allotted in a completely randomized design to three pens of five chicks per treatment. Chicks were housed in a galvanized Petersime brooder with stainless-steel feeders and waterers placed in a temperature-controlled room (23°C). Chicks were placed on one of four diets containing either 0% fiber, 6.0% wood cellulose (Solka Floc, a potentially fermentable fiber control obtained from Brown Co., Berlin, N.H.), or 6.0% Indulin (a Kraft wood lignin obtained from Westvaco Corp., Charleston, S.C.). Composition of the basal diet is shown in Table I. Fiber additions were made at the expense of cornstarch:dextrose (2:1). Ascorbic acid was added to the vitamin mix as a biological antioxidant. A separate control group was maintained to determine *ad libitum* intake of the 0% fiber diet. All chicks were pair-fed four times daily to establish constant feed intakes across treatments. Water was provided *ad libitum*. On Day 10 of the experiment (Day 30 of age), chicks were weighed and sacrificed by cervical dislocation. Livers of the three birds with intermediate weights in each pen were immediately excised, weighed, and subjected to cytochrome b_5 and cytochromes *P*-450 analyses by methods of Omura and Sato (6) as modified by Philpot (7) and NADPH cytochrome *c* reductase analysis by the method of Masters *et al.* (8). Livers were placed in cold 0.15 *M* KCl–0.05 *M* Tris buffer and homogenized in a Glenco glass homogenizer. Samples were then centrifuged at 12,000*g* for 10 min. The supernatant was decanted and centrifuged at 100,000*g* for 60 min. The resulting microsomal pellet was resuspended in the same buffer. Samples containing 0.5 to 1 mg of microsomal protein/ml were saturated with carbon monoxide (CO) for 30 sec. The CO-saturated solution was then placed in both sample and reference cells of a Hewlett Packard 8450A dual-beam spectrophotometer and difference spectroscopy was used to obtain a flat baseline. Cytochrome b_5 was measured by adding 1 to 3 mg of NADH to the sample cell and calculated as the difference between absorbance at 410 and 424 nm. Cytochromes *P*-450 were then measured by adding 1 to 3 mg NADH to the reference cell and 1 to 3 mg sodium dithionite ($Na_2S_2O_4$) to the sample cell and calculated as the difference between absorbance at 450 and 490 nm. Extinction coefficients used for the calculations were

TABLE I. COMPOSITION OF BASAL DIET FED TO CHICKS AND RATS

Diet constituent	Chick diet (%)	Rat diet (%)
Cornstarch:dextrose (2:1)	variable (to 100%)	variable (to 100%)
Casein	23.4	15.1
DL-Methionine	0.35	0.2
Arginine	1.5	—
Glycine	1.0	—
Corn oil	5.0	6.0
Mineral mix[a]	5.4	5.37
Vitamin mix[b]	0.2	0.2
Choline chloride	0.2	0.1
Ethoxyquin	125 mg/kg	—
Sodium bicarbonate	1.5	—
$MgSO_4$	—	0.03

[a] Composed of each of the following (percentage of total diet): $CaCO_3$, 0.3; $Ca_3(PO_4)_2$, 2.8; K_2HPO_4, 0.9; NaCl, 0.9; $MgSO_4 \cdot 7H_2O$, 0.4; $MnSO_4 \cdot H_2O$, 0.07; Fe citrate, 0.05; $ZnCO_3$, 0.01; $CuSO_4 \cdot 5H_2O$, 0.002; H_3BO_3, 0.0009; $Na_2MoO_4 \cdot 2H_2O$, 0.0009; KI, 0.004; $CoSO_4 \cdot 7H_2O$, 0.0001; Na_2SeO_3, 0.00002.

[b] Composed of each of the following (mg/kg total diet): vitamin A palmitate (250,000 IU/g, 40.0); cholecalciferol (400,000 IU/g, 1.5); DL-α-tocopherol acid succinate, 20.0; menadione, 5.0; riboflavin, 16.0; calcium pantothenate, 20.0; niacin, 100.0; vitamin B-12 triturate, 0.02; folic acid, 4.0; biotin, 0.6; ascorbic acid, 250.0; pyridoxine · HCl, 6.0; thiamine · HCl, 100.0; powdered starch, 1334.9.

185 and 91 mM^{-1} cm^{-1} for cytochromes b_5 and *P*-450, respectively.

For NADPH cytochrome *c* reductase determinations, 1.5 mg cytochrome c/ml was added to a solution of 0.05 *M* phosphate buffer (pH 7.7) and 3 m*M* $MgCl_2$. This reaction mixture was placed in both reference and sample cuvettes. The microsomal suspension (10 to 20 μl) was then added to both cuvettes and the baseline was measured at 550 nm at 1-sec intervals for 180 sec. The NADPH (10 to 20 μl of 3 mg/ml Type I NADPH) was then added to the sample cuvette. Absorbance was measured at 550 nm at 1-sec intervals for 180 sec. The extinction coefficient was 18.7 mM^{-1} cm^{-1}. Protein content of the microsomal preparation was determined by the method of Lowry *et al.* (9).

In experiment 2, 20-day-old chicks (average initial wt, 261 g) were placed on one of three diets containing either 0% fiber, 6.0% wood molasses (Laurel Masonex obtained from Masonite Corp., Chicago, Ill.), or 12.0% wood molasses. All other conditions and analyses were as in experiment 1 with the exception that NADPH cytochrome *c* reductase analysis was not performed.

In experiment 3, 20-day-old chicks (avg init wt, 246 g) were placed on one of three diets containing either 0% fiber, 6.0% dried cane molasses (obtained from Kanelass, Mount Pulaski, Ill.), or 12.0% dried cane molasses. All other conditions and analyses were as in experiment 2.

In experiment 4, 19-day-old chicks (avg init wt, 208 g) were allotted in a 2×2 factorial design to three pens of five chicks per treatment. The basal diet and all experimental conditions were as in experiment 1. Chicks were placed on one of four diets containing either 0% fiber, 0% fiber + 0.7% antibiotic, 6.0% lignin, or 6.0% lignin + 0.7% antibiotic. All other conditions and analyses were as in experiment 1.

To measure microbe concentrations, intestinal contents were removed from the large intestine, ceca, and small intestine 1 in. proximal to the cecal–colonic junction and freeze-dried. Samples were then subjected to 2,4-diaminopimelic acid (DAP) analysis similar to the method of Hutton *et al.* (10). Approximately 150 mg of sample was hydrolyzed under nitrogen for 22 hr at 110°C. The hydrolysate was then Millipore-filtered (0.22 μm) and neutralizing buffer was added (NaOH in pH 2.2 citrate buffer to bring the sample pH to 2.0 to 2.5). Samples were then analyzed on a Beckman Model 119 CL amino acid analyzer to determine DAP concentrations.

In experiment 5, weanling, female Sprague–Dawley rats (avg init wt, 75 g; Harlan Industries, Inc., Indianapolis, Ind.) were randomly allotted to individual cages with five rats per treatment. Rats were housed in stainless-steel cages with wire-mesh floors and placed in a temperature-controlled room (23°C). Composition of the basal diet is shown in Table I. Rats were placed on one of four diets containing either 0% fiber, 6.0% wood cellulose, 6.0% arenaceous flour, or 6.0% lignin. Fiber additions were made at the expense of cornstarch:dextrose (2:1). Small porcelain feeders were placed inside glass jars in each cage to minimize feed spillage. Rats were pair-fed to the 0% fiber control diet each evening. Remaining feed was weighed and removed each morning. Water was provided *ad libitum*.

On Day 10 of the experiment, rats were weighed and sacrificed by cervical dislocation. Livers of all rats were immediately excised and subjected to the same analyses as in experiment 1.

In experiment 6, 19-day-old New Hampshire × Columbian female chicks (avg init wt, 164 g) were placed on one of three diets containing either 0% phenolic, 0.5% vanillin, or 0.5% vanillic acid (all phenolics were obtained from Sigma Corp., St. Louis, Mo.). All conditions and analyses were as in experiment 1.

In experiment 7, 18-day-old chicks (avg init wt, 216 g) were placed on one of three diets containing either 0% phenolic, 0.5% ferulic acid, or 0.5% *p*-coumaric acid. All other conditions and analyses were as in experiment 1.

In experiment 8, weanling, female Sprague–Dawley rats (avg init wt, 69 g) were randomly allotted to individual cages with five rats per treatment. Rats were placed on one of five diets containing either 0% phe-

nolic, 0.5% vanillin, 0.5% vanillic acid, 0.5% ferulic acid, or 0.5% *p*-coumaric acid. All conditions and analyses were as in experiment 5.

Data from all experiments were analyzed by the general linear models procedure of the statistical analysis system (11). Means were compared by the least significant difference method for those parameters having a significant *F* test according to the analysis of variance.

Results. Results of experiment 1 are presented in Table II. Inclusion of wood cellulose, arenaceous flour, or lignin in diets of chicks had no effect ($P > 0.05$) on performance, although weight gains and gain:feed ratios were slightly lower for fiber-fed animals. Hepatic weights and microsomal protein levels were also unaffected by treatment, although hepatic weights tended to be slightly lower for lignin-fed birds. Hepatic cytochrome b_5 content was higher ($P < 0.05$) in chicks fed 6% arenaceous flour and 6% lignin. Hepatic cytochromes *P*-450 content was higher ($P < 0.01$) in lignin-fed chicks. NADPH cytochrome *c* reductase activity did not differ among treatments.

Results of experiment 2 are presented in Table III. Weight gain, feed intake, and feed efficiency (gain/feed) were similar among treatments. Livers of birds fed 12.0% wood molasses weighed less ($P < 0.06$) than did those of birds fed 0% fiber or 6.0% wood molasses. Microsomal protein and cytochrome b_5 levels did not differ among treatments. Total hepatic cytochromes *P*-450 content was higher ($P < 0.06$) in birds consuming 12.0% wood molasses compared with control or 6.0% wood molasses-fed birds. Hepatic cytochromes *P*-450 content responded ($P < 0.02$) to higher levels of dietary wood molasses.

Results of experiment 3 are presented in Table III. Performance measurements, as well as liver weight, microsomal protein, and cytochrome b_5 levels, were unaffected by treatment. The cytochromes *P*-450 level was higher ($P < 0.01$) in liver from chicks fed both 6.0 and 12.0% cane molasses as compared with controls.

Results of experiment 4 are presented in Table IV. Weight gains and gain:feed ratios were lower ($P < 0.05$) for animals fed anti-

TABLE II. EFFECTS OF CERTAIN FIBER CONTROLS AND LIGNIN ON PERFORMANCE AND HEPATIC WEIGHT, PROTEIN, CYTOCHROME b_5, CYTOCHROMES *P*-450, AND NADPH CYTOCHROME *c* REDUCTASE IN CHICKS (EXPERIMENT 1)

Treatment	Weight gain	Feed intake	Gain/feed	Hepatic characteristics				
				Weight	Microsomal protein	Cytochrome b_5	Cytochromes *P*-450	NADPH cytochrome *c* reductase
	(g/d)			(g)	(mg)	(nmole/mg prot)		(nmole/min/mg prot)
Control	12.4	22.5	0.56	7.0	80.7	0.1070[a,**]	0.1059[a,**]	58.34
6.0% wood cellulose	11.5	22.7	0.50	6.9	79.2	0.1234[a]	0.1274[a]	64.11
6.0% arenaceous flour	11.8	22.4	0.53	6.8	82.4	0.1478[b]	0.1017[a]	82.38
6.0% lignin	11.6	22.8	0.51	6.5	77.8	0.1393[b]	0.1481[b]	77.53
SEM	0.6	0.2	0.14	0.2	2.5	0.0096	0.0095	9.84

[a,b] Means in the same column with unlike superscripts differ. *$P < 0.05$. **$P < 0.01$.

TABLE III. PERFORMANCE AND HEPATIC WEIGHT, PROTEIN, CYTOCHROME b_5, AND CYTOCHROMES *P*-450 FOR CHICKS FED 6.0 AND 12.0% WOOD MOLASSES (EXPERIMENT 2) OR 6.0 AND 12.0% CANE MOLASSES (EXPERIMENT 3)

Treatment	Weight gain	Feed intake	Gain/feed	Hepatic characteristics: Weight	Microsomal protein	Cytochrome b_5	Cytochromes *P*-450
	(g/d)		(g)	(g)	(mg)	(nmole/mg prot)	
Experiment 2							
Control	9.4	22.5	0.42	7.4[a]	87.64	0.1181	0.1223[a,*]
6.0% wood molasses	9.4	22.5	0.42	7.3[a]	84.57	0.1372	0.1390[a]
12.0% wood molasses	9.2	22.5	0.41	6.8[b]	88.01	0.1352	0.1544[b]
SEM	0.5	0.1	0.01	0.2	1.63	0.0094	0.0090
Experiment 3							
Control	10.4	22.5	0.44	6.9	80.24	0.1326	0.1222[a]
6.0% cane molasses	9.4	22.7	0.41	6.7	79.33	0.1599	0.1632[b]
12.0% cane molasses	9.6	22.6	0.42	6.5	79.74	0.1539	0.1555[b]
SEM	0.6	0.1	0.02	0.2	1.48	0.0099	0.0117

[a,b] Means in the same column, within experiments, with unlike superscripts differ (experiment 2, $P < 0.06$; experiment 3, $P < 0.05$).
* Linear ($P < 0.02$).

biotic. Although results were not significant ($P > 0.05$), feed intakes tended to be lower for antibiotic-fed chicks. As in experiment 1, lignin-fed chicks had slightly lower weight gains and feed efficiencies than did those fed 0% lignin. Hepatic weights were low for both antibiotic ($P < 0.05$) and Indulin-fed ($P < 0.01$) chicks. This effect was additive since animals consuming both lignin and antibiotic had the lowest hepatic weights. Microsomal protein levels were not different among treatments. Cytochrome b_5 levels and NADPH cytochrome *c* reductase activity were not affected by treatment. Cytochromes *P*-450 content was elevated ($P < 0.01$) in liver of lignin-fed chicks, both with and without antibiotic. There was no antibiotic effect, however, as 0% lignin-fed chicks had a similar cytochromes *P*-450 value either with or without inclusion of antibiotic in the diet. Diaminopimelic acid levels of intestinal contents were substantially lower in animals fed antibiotic-containing diets ($P < 0.0001$) as well as in animals fed lignin-containing diets ($P < 0.01$).

In experiment 5, there were no differences in performance among treatments. In addition, all hepatic characteristics measured were similar among treatments. Control values for cytochrome b_5, cytochromes *P*-450, and NADPH cytochrome *c* reductase were 0.1626 nmole/mg protein, 0.2384 nmole/mg protein, and 100.14 nmole/min/mg protein, respectively.

In experiment 6, inclusion of 0.5% vanillin and 0.5% vanillic acid in diets of chicks had no effect on animal performance or hepatic measurements compared with controls. Cytochrome b_5, cytochromes *P*-450, and NADPH cytochrome *c* reductase control values were 0.1180 nmole/mg protein, 0.1066 nmole/mg protein, and 71.98 nmole/min/mg protein, respectively.

In experiment 7, weight gain and feed intake were similar among treatments. However, gain:feed ratios were lower ($P < 0.05$) for animals consuming 0.5% *p*-coumaric (0.45) vs control (0.48) or 0.5% ferulic acid-fed chicks (0.48). Inclusion of 0.5% ferulic acid or 0.5% *p*-coumaric acid in chick diets had no effect on hepatic parameters. Control values for cytochrome b_5, cytochromes *P*-450, and NADPH cytochrome *c* reductase were 0.1235 nmole/mg protein, 0.1410 nmole/mg protein, and 62.68 nmole/min/mg protein, respectively.

TABLE IV. EFFECTS OF LIGNIN AND ANTIBIOTIC (Ab) ON PERFORMANCE AND HEPATIC WEIGHT, PROTEIN, CYTOCHROME b_5, CYTOCHROMES *P*-450, NADPH CYTOCHROME *c* REDUCTASE, AND INTESTINAL DIAMINOPIMELIC ACID (DAP) IN CHICKS (EXPERIMENT 4)

Treatment	Weight gain[a]	Feed intake	Gain/feed[a]	Hepatic characteristics					Intestinal diaminopimelic acid[b,c]
				Weight[a,b]	Microsomal protein	Cytochrome b_5	Cytochromes *P*-450[b]	NADPH cytochrome *c* reductase	
	(g/d)			(g)	(mg)	(nmole/mg prot)		(nmole/min/mg prot)	(mg/g digesta)
Control	9.3	20.1	0.46	6.6	80.9	0.0992	0.1060	49.26	1.67
Control + 0.7% Ab	7.9	19.5	0.41	6.4	79.3	0.1273	0.1361	54.66	0.59
6.0% lignin	8.7	20.1	0.43	6.2	80.0	0.1367	0.1705	56.64	1.25
6.0% lignin + 0.7% Ab	7.4	19.6	0.38	5.9	74.7	0.1214	0.1553	58.17	0.48
SEM	0.4	0.2	0.01	0.2	1.9	0.0095	0.0161	3.96	0.09

[a] Antibiotic effect ($P < 0.05$).
[b] Lignin effect ($P < 0.01$).
[c] Antibiotic effect ($P < 0.0001$).

In experiment 8, performance and all hepatic measurements were similar among treatments. Control levels for cytochrome b_5, cytochromes *P*-450, and NADPH cytochrome *c* reductase were 0.0920 nmole/mg protein, 0.2170 nmole/mg protein, and 84.86 nmole/min/mg protein, respectively.

Discussion. Results of experiment 1 indicate that cytochromes *P*-450 content is induced by addition of lignin to diets of chicks. Cytochromes *P*-450 have been shown to be induced by greater than 300 structurally unrelated compounds (12).

Although many isozymes of cytochromes *P*-450 have been isolated having different spectral, chromatographic, immunologic, and substrate-preference characteristics (13), two major types of cytochromes *P*-450 have been broadly classified as being "phenobarbital-like" or "3-methylcholanthrene-like" based on their respective inducers (12, 14). NADPH cytochrome *c* reductase activity parallels the increase in cytochromes *P*-450 content in animals that have been administered phenobarbital (12). In our studies, NADPH cytochrome *c* reductase activity did not increase concomitant with the increase in cytochromes *P*-450 content, implying that induction was not "phenobarbital-like."

Cytochrome b_5 levels may or may not be affected relative to changes in cytochromes *P*-450. Cytochrome b_5 is not required in the cytochromes *P*-450-dependent microsomal mixed-function oxidase system based on studies of activity in reconstituted systems; however, cytochrome b_5 may serve as a carrier for a second electron donation from NADH (15). In experiment 1, cytochrome b_5 levels were higher for chicks fed both 6.0% arenaceous flour and 6.0% lignin diets; however, we are uncertain as to the biological significance of this observation, especially since we did not observe this response in subsequent experiments.

Since cytochromes *P*-450 can be influenced by both quantity and quality of dietary protein (16), as well as by changes in concentrations of other macronutrients, it is very important to obtain equivalent feed intakes when attempting to establish effects of certain chemicals on the xenobiotic-metabolizing enzyme systems (4). In our experiments, feed intake was maintained at a constant level

among treatments. Our diets were not isocaloric to avoid varying other diet constituents; however, we attempted to examine effects of lower caloric density by including two positive controls in our experiments (wood cellulose, a potentially fermentable fiber, and arenaceous flour, a relatively inert bulking agent). Although animals fed the three fiber sources tended to gain less weight and had lower feed efficiencies (gain:feed ratios) compared to the 0% fiber control, this was expected due to the nonisocaloric nature of diets.

Wood molasses (Masonex) is a wood byproduct formed during the production of hardboard without the use of acids, alkalis, or salts and is frequently used as a pellet binder for animal feed and supplements. It contains high levels of soluble phenolics, generally in the form of phenolic–carbohydrate complexes. Carbohydrates (pentoses and hexoses) comprise greater than 55% of wood molasses dry matter (17). Because wood molasses is approximately 45% phenolics and lignin is approximately 97% phenolics, we chose, in experiment 2, to include wood molasses in diets at both the 6.0 and 12.0% levels to achieve approximate isophenolic levels compared with lignin in experiment 1. Fahey (17) found that wood molasses fed to chicks resulted in a significant increase in feed intake and weight gain over controls. We did not observe increased weight gains with inclusion of wood molasses in our diets but this was to be expected due to pair-feeding of non-isocaloric diets. In this experiment, 12.0% wood molasses depressed ($P < 0.06$) liver weights, probably due to caloric restriction rather than to any treatment effect, per se. Cytochromes *P*-450 content was increased ($P < 0.06$) in livers of birds fed 12.0% wood molasses. Since the 12.0% wood molasses diet is approximately isophenolic to the 6.0% lignin-containing diet of experiment 1, we hypothesize that the phenolic monomers or oligomers responsible for induction of cytochromes *P*-450 are equally available from wood molasses and lignin.

Cane molasses is another naturally occurring phenolic-containing substance utilized as a feed additive to improve diet acceptability and reduce dustiness (18). Cane molasses has been fractionated to yield lignin, hemicelluloses, and carbohydrate-bound phenolics (19, 20). Cytochromes *P*-450 level was induced at both the 6.0 and 12.0% levels of dietary cane molasses. Apparently, the phenolic(s) responsible for this induction is(are) distributed in different types of feed ingredients.

Response of nonruminant animals to fibrous components of the diet is markedly affected by the anaerobic microbial population located in the hindgut. Antibiotics such as bacitracin:neomycin sulfate (2:1) have been used experimentally to decrease numbers of intestinal microbes, thus affecting various digestive processes (21–23). Experiment 4 was designed to determine if a reduction in the intestinal microbial population affected the ability of lignin to induce cytochromes *P*-450 level. The high level of antibiotic used in this experiment would be expected to lower performance due to its inherent toxicity. Lower liver weights observed are probably a reflection of lower total body weight. Results indicate that manipulation of this phenolic polymer by gastrointestinal tract microflora is apparently unnecessary for cytochromes *P*-450 induction to occur. Therefore, some phenolics apparently are absorbed intact in sufficient quantities for cytochromes *P*-450 induction. It is conceivable, however, that certain populations of microorganisms remained active in the gut and subsequent metabolic endproducts from bacterial fermentation of lignin were absorbed and, in turn, increased cytochromes *P*-450 content. Although this possibility cannot be completely ruled out from data collected in this experiment, we believe that these results provide further evidence that lignin effects are of a direct rather than of an indirect nature.

Diaminopimelic acid is present in bacterial cells and is essentially absent in protozoa and plant material. Therefore, it is frequently used to estimate the bacterial content of the gut. As expected, DAP levels of intestinal contents were greatly reduced by inclusion of antibiotic in the diet demonstrating marked sterilization of the gut. Also, DAP levels were somewhat depressed for animals consuming lignin. It appears as if lignin might have certain antibiotic-like properties. This is consistent with work of Zemek *et al.* (24) who studied 11 phenolic components representative of those contained in wood lignin and

found them to have inhibitory effects on various microorganisms. Johanning *et al.* (25) also found that cell wall material from cabbage and alfalfa had antibacterial activity. The fraction which promoted guinea pig growth also inhibited growth of an anaerobic, cellulolytic bacterium isolated from guinea pig cecum. When this fraction was treated with alkali, resulting in the loss of caffeic and *p*-coumaric acids (two phenolic acids which are components of lignin), antibacterial activity was lost.

In experiment 5, we examined effects of the various fibers tested in experiment 1 on a mammalian model, the rat. In contrast to the chick, rat hepatic cytochromes *P*-450 did not respond to lignin. There are several possible explanations for this species difference. It is recognized that specific cytochrome *P*-450 isozymes exist and that their relative activities may differ (26, 27). It is possible that the particular isozyme(s) in chick liver that is(are) affected by lignin is(are) not present in the rat, or that one isozyme in the rat may increase while another decreases, allowing total cytochrome *P*-450 level to remain relatively constant. Alternatively, since cytochrome *P*-450 content is several times higher in the rat than in the chick, its level may be more difficult to change (requiring more inducer) or differences may be more difficult to detect. Also, rats may have more detoxification capacity than do poultry.

Another reason for the species difference may have to do with the evolutionary origin of mammals vs poultry (1). Mammals, in general, appear to be adapted to angiosperms as their diet. Angiosperms gained ascendancy over gymnosperms and more primitive plants at about the same time that mammals were replacing reptilians. Reptilian species are seldom plant eaters and those surviving reptilian species are quite sensitive to certain plant phenolics. Thus, the especially high toxicity of certain phenolics like aflatoxin to birds may be related to their reptilian connection. However, Smith *et al.* (28) noted that hepatic enzymatic capabilities toward xenobiotics do not parallel phylogenetic classifications, indicating that the comparative data available today are more descriptive than predictive of relationships among species.

The question remains as to what specific moieties of the complex oligomers and polymers studied are responsible for eliciting changes in cytochromes *P*-450. As phenolic monomers have been shown to be absorbed by the mammalian gut (29), it is likely that one or more monomers may act alone or in concert to orchestrate these alterations. The final three experiments tested four common dietary phenolic monomers for the same cytochromes *P*-450-inducing ability already demonstrated by complex phenolic substances.

Vanillin and vanillic acid comprise the largest percentage of alkali-labile phenolic monomers in lignin (29). Consequently, we felt that if phenolic monomers were responsible for the cytochromes *P*-450-inducing ability of 6.0% dietary lignin, these would be likely candidates and that additions at the 0.5% level would be more than adequate to elicit the changes (experiment 6). However, no changes occurred using the rat as the model.

Ferulic and *p*-coumaric acids are two phenolic monomers common in many plant feedstuffs (29). Therefore, we tested these compounds as possible cytochromes *P*-450 inducers in chicks in experiment 7. Once again, these phenolic monomers had no effect on cytochromes *P*-450. *p*-Coumaric acid had a detrimental effect on weight gain ($P < 0.05$); however, no other phenolic monomer tested elicited this response at the 0.5% level.

Although cytochromes *P*-450 content was not changed in rats fed 6.0% lignin (experiment 5), we did not know whether this result was due to the inability of rats to release and subsequently absorb phenolics from lignin or whether there were some other inherent metabolic differences between rats and chicks. Therefore, we tested the same phenolic monomers for their cytochromes *P*-450-altering effects in rats as we did in chicks (experiment 8). Once again, as with chicks, no change in cytochromes *P*-450 content was noted when ferulic, *p*-coumaric and vanillic acids, as well as vanillin, were fed to rats.

Previously, it was believed that large dietary phenolic compounds such as lignin were biologically inert, partially because they were not degraded enzymatically by the animal.

However, our results indicate that at least some large phenolic complexes (lignin, wood, and cane molasses) have effects outside the gastrointestinal tract. Furthermore, these effects are not due to major monomeric forms associated with these complexes. Although we did not test all dietary phenolic monomers thereby ruling out their effect on cytochromes *P*-450 levels, we suggest that larger forms might be the biologically active component. Relative rates of penetration into the body should increase with poly-aromatic compounds due to their increased partition coefficients (solubility in organic phase/solubility in water) (30), potentially increasing availability to the animal. Further work elucidating the mode of action of these oligomers and polymers as well as isolating isomeric form(s) of cytochromes *P*-450 induced by these compounds is warranted.

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