Tissue Distribution of Gossypol Enantiomers in Broilers Fed Various Cottonseed Meals

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ABSTRACT Plasma and tissue concentrations of total, (+)- and (−)-gossypol were determined in broilers fed cottonseed meals (CSM) from eight oil mills (five expander solvent, two expeller, and one direct solvent). Free gossypol in the meals ranged from 0.033 to 0.180%, and total gossypol ranged from 0.974 to 1.459%. The (+)-enantiomer of gossypol varied from 53.8 to 61.3% of total gossypol. Eight CSM diets containing 28% CSM and a soybean meal control diet were fed to 162 1-d-old male broiler chicks during a 3-wk starter period. Concentrations of free gossypol in the CSM diets ranged from 92 to 504 μg/g, and total gossypol ranged from 2,626 to 4,085 μg/g. All diets were formulated with the same concentrations of digestible lysine and methionine and were fed ad libitum. At 21 d of age, there were no significant differences in body weights, feed conversions, or mortality of birds fed the CSM diets when compared to birds fed the soybean meal diet. Concentrations of (+)- and (−)-gossypol in plasma, liver, kidney, and muscle increased linearly as the level of free gossypol increased in the diets. Liver had the highest concentration of total gossypol (71.4 to 313.6 μg/g DM) followed by kidney (9.2 to 36.3 μg/g DM), plasma (3.0 to 14.6 μg/mL), and muscle (2.1 to 9.8 μg/g DM). The proportion of (−)-gossypol was higher in plasma (26.7%) and kidney (25.6%) than in muscle (19.1%) and liver (16.0%). Performance data from this study indicate CSM can be used successfully in chick starter diets at levels up to 28% when diets are formulated on a digestible amino acid basis.

(*Key words*: broiler, cottonseed meal, gossypol, plasma, enantiomer)

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INTRODUCTION

Cottonseed meal (CSM), a by-product of the cotton industry after extraction of oil from cottonseed, is fed to livestock as a valuable source of protein. It is used primarily in ruminant diets because of their tolerance for gossypol. Its use in poultry diets has been restricted due to fear of gossypol toxicity and concerns about protein quality and fiber content (Phelps, 1966). Gossypol is a pigment produced by plants belonging to the genus *Gossypium* (cotton) of the Malvaceae family. It occurs throughout the cotton plant but is concentrated in the pigment glands of the seeds (Heinstein et al., 1977). It has two naphthalene rings with identical substituents that can partially rotate around the bond connecting the rings, forming two identical structures that have no plane of symmetry and differing optical properties [(+)- and (−)-enantiomers] (Huang et al., 1987).

The gossypol content of cottonseed varies widely and is assumed to be in the free form (Jones, 1981; Calhoun et al., 1995a,b). Its concentration is mainly controlled by the genetics of the cotton plant (Boatner et al., 1949; Stansbury et al., 1956; Percy et al., 1996), but it is also influenced by growing conditions (Pons et al., 1953; Stansbury et al., 1956). High temperatures throughout the development and maturation period depress gossypol content, whereas high rainfall over the maturation period increases gossypol content (Pons et al., 1953; Stansbury et al., 1956), and these effects seem to be interrelated (Pons et al., 1953). The proportion of (+)- and (−)-gossypol is controlled by the genetics of the plant and varies within and between species (Cass et al., 1991; Percy et al., 1996).

During the oil extraction process and production of CSM, pigment glands are ruptured, allowing the aldehyde groups of gossypol to react with free amino groups of protein and peptides, especially the ε-amino group of lysine, resulting in bound gossypol (Lyman et al., 1959; Phelps, 1966). Free gossypol, as determined by the official method of the American Oil Chemists Society (AOCS, 1985a), includes unbound gossypol, gossypol derivatives, and transformation or degradation products of gossypol that can be extracted with aqueous acetone. Total gossy-

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Abbreviation Key: CSM = cottonseed meal.

pol is defined as the sum of bound, free gossypol, and gossypol derivatives (AOCS, 1985b). Bound gossypol is not determined directly but is obtained as the difference between total and free gossypol. Free gossypol concentration is the primary factor used to determine the inclusion level of CSM in animal feeds.

Total gossypol in CSM is determined by the gossypol concentration in the kernel (Pons et al., 1955; Forster and Calhoun, 1995), whereas free gossypol is highly related to temperature, pressure, cooking time, moisture conditions, and all possible combinations of these factors during oil extraction (Pons and Guthrie, 1949; Pons et al., 1955; Calhoun et al., 1995a; Forster and Calhoun, 1995). Currently, commercial types of CSM are screw press (expeller), direct solvent, prepress solvent, and expander solvent (NCPA, 1990). Screw press, prepress, and expander solvent CSM are produced using high temperatures and pressures and usually contain the lowest concentrations of free gossypol. Screw press meals have a higher concentration of residual oil than any of the processes involving solvent extraction, but this fact is not always reflected in the fat content of CSM that are commercially available because of the common practice of adding all of the soapstock produced in the oil refining process back into the CSM (Calhoun et al., 1995a).

Introduction of new cotton varieties, new methodologies for oil extraction (expanders), development of new analytical methodologies for separating (+)- and (–)-gossypol enantiomers in tissues and cottonseed, and suggestions that processing conditions affect gossypol availability (Calk, 1992; Knabe et al., 1995) are factors that may affect recommendations on the use of CSM in poultry feeds. The objective of this experiment was to assess growth and tissue distribution of gossypol enantiomers in broilers fed a variety of CSM produced by different processes from different oil mills and containing different concentrations of free gossypol.

MATERIALS AND METHODS

To provide a wide range of values for free and total gossypol in the diets, eight CSM processed by different methods (five expander solvent, two expeller, and one direct solvent) and differing in free (0.033 to 0.180%) and total gossypol (0.938 to 1.459%) were included at 28% in eight different broiler starter diets (Table 1). A corn-soybean meal-based diet formulated to fulfill the NRC (1994) requirements for total amino acids was used as a control diet. The different CSM diets were formulated to have the same level of digestible lysine and sulfur amino acids as the control (Table 2) with digestibility coefficients of 61.2% for lysine, 74.2% for methionine, and 67.2% for cystine, as per *Heartland Lysine True Digestibility Tables of Essential Amino Acids for Poultry—1995*.[2]

One hundred sixty-two 1-d-old Argo male broiler chicks were placed in heated Petersime battery brooders with nine treatments randomly assigned to pens of six birds each. Each treatment was replicated three times. An air conditioned growing room was set to provide 23 h light:1 h dark. All chicks were provided feed and water ad libitum during a 3-wk growing period. Birds were observed twice daily to assess general condition and record mortality. Body weights and feed intakes were obtained weekly.

At 21 d of age, blood samples were obtained by heart puncture (1.5 mL/bird). Blood from all birds in each pen was pooled within a single, 10-mL vacuum tube containing sodium heparin as an anticoagulant, and the contents were mixed by repeated, gentle inversion. Tubes were kept on ice and protected from light until plasma was separated by centrifugation. Plasma was stored at –20 C until used for gossypol analysis. Birds were killed by cervical dislocation; samples of liver, kidney, and breast muscle were collected. All samples were packed in ice as they were collected and were stored at –20 C until analyzed for gossypol.

Free and total gossypol in CSM was determined by the official methods of the American Oil Chemists Society (AOCS, 1985a,b). The proportions of (+)- and (–)-enantiomers in CSM were determined by HPLC after precolumn derivatization with a chiral amine [(R)-(–)-2-amino-1-propanol] (Hron et al., 1995). The (+)- and (–)-gossypol in lyophilized plasma and tissues were determined by HPLC as described by Kim and Calhoun (1995).

Data Analysis

The experimental design was a completely random arrangement of nine treatments with three replications. The general linear models procedure of SAS software (SAS, 1992) was used to analyze the data. Tukey's means separation test was used to test for differences between means when the *F* test for treatments was significant ($P < 0.05$). Regression analyses were used to test the associations between gossypol intakes, performance criteria, plasma, and tissue concentrations of gossypol.

RESULTS

The (+)-enantiomer of gossypol in the CSM varied from 53.8 to 61.3% of the total gossypol, whereas the (–)-enantiomer varied from 38.7 to 46.2% of the total gossypol (Table 1). Inclusion of CSM at 28% of the total diet gave free gossypol from 92 to 504 μg/g and total gossypol from 2,626 to 4,085 μg/g (Table 1). Estimated digestible lysine in the diets was 1.19%, whereas the estimated digestible methionine plus cystine was 0.81% of the diet (Table 2).

At 21 d of age, there were no significant differences in body weight or feed to gain ratios for birds fed the different CSM diets (Table 3). Also, mortality was not affected by treatments (data not shown). During the second week of the study, there was a significant reduction in cumulative feed to gain ratio for birds receiving the highest concentration of dietary free gossypol.

[2]Heartland Lysine, Inc., Chicago, IL 60607.

TABLE 1. Gossypol content of the eight cottonseed meals obtained by different processes

Process	AOCS Free[1] (%)	AOCS Total[2] (%)	HPLC[3] Total (%)	HPLC[3] (+) (% of total)	HPLC[3] (–) (% of total)	Dietary gossypol[4] Free (μg/g)	Dietary gossypol[4] Total (μg/g)
Expeller	0.033	1.175	0.959	61.3	38.7	92	3,290
Expeller	0.037	0.974	0.688	59.7	40.3	104	2,727
Expander	0.077	1.094	0.799	55.0	45.0	216	3,063
Direct	0.084	0.938	0.719	53.8	46.2	235	2,626
Expander[5]	0.091	1.191	0.995	58.8	41.2	255	3,335
Expander	0.099	1.459	1.088	60.5	39.5	277	4,085
Expander	0.145	1.111	0.782	53.8	46.2	406	3,111
Expander	0.180	1.169	1.006	59.2	40.8	504	3,273

[1]American Oil Chemists Society Official Method Ba 7-58 for determination of free gossypol in cottonseed meats and meal (AOCS, 1985a).

[2]American Oil Chemists Society Official Method Ba 8-78 for determination of total gossypol in cottonseed meats and meal (AOCS, 1985b).

[3](+)- and (–)-gossypol were determined by HPLC as described by Hron et al. (1995).

[4]Estimated levels of total and free gossypol based on the inclusion of 28% CSM in the diets.

[5]This particular cottonseed meal (code 2220) had above average gossypol availability and differed from others in that it did not contain added soapstock.

Tissue gossypol enantiomer concentrations are shown in Table 4. Liver tissue had the highest concentrations of total gossypol (71.4 to 313.6 μg/g DM) followed by kidney (9.2 to 36.3 μg/g DM) and muscle (2.1 to 9.8 μg/g DM).

TABLE 2. Percentage composition and calculated nutritional values for broiler starter diets

Ingredients	0% CSM	28% CSM
Corn	50.86	43.43
Soybean meal (48% CP)	37.86	14.47
Cottonseed meal (CSM)	. . .	28.00
Fat (animal and vegetable blend)	6.92	9.27
Calcium carbonate	1.70	1.80
Mono-dicalcium phosphate[1]	1.66	1.44
L-lysine·HCl (78% Lys)	. . .	0.50
Salt	0.43	0.43
DL-Methionine (98%)	0.19	0.29
Vitamin premix[2]	0.25	0.25
Mineral premix[3]	0.05	0.05
Coban 60®,[4]	0.075	0.075
Calculated nutrient content		
Crude protein, %	23.00	23.00
ME, kcal/kg	3,200	3,200
Crude fat, %	9.07	11.54
Calcium, %	1.00	1.00
Phosphorus (available), %	0.45	0.45
Lysine (total), %	1.32	1.45
Lysine (digestible), %	1.19	1.19
Methionine (total), %	0.55	0.61
Methionine (digestible), %	0.52	0.55
Methionine + cystine (total), %	0.89	0.94
Methionine + cystine (digestible), %	0.81	0.81

[1]Each kilogram provided 15,555 mg of iron.

[2]Supplied per kilogram of diet: vitamin A acetate, 11,023 IU; cholecalciferol, 3,858 IU; vitamin E, 46 IU; menadione (menadione sodium bisulfite complex), 1.47 mg; thiamine (thiamin mononitrate), 2.94 mg; riboflavin, 5.85 mg; niacin, 45.93 mg; choline (choline chloride), 477.67 mg; pantothenic acid (calcium d-pantothenate), 20.21 mg; pyridoxin (pyridoxine hydrochloride), 7.17 mg; vitamin B_{12}, 16.5 μg; d-biotin, 0.55 mg; folic acid, 1.75 mg; antioxidant, 55 mg.

[3]Trace mineral premix provided the following per kilogram of diet: manganese (sulfate or oxide), 68 mg; zinc (oxide), 55 mg; copper (sulfate or oxide), 4.4 mg; iron (ferrous sulfate), 26.4 mg; iodine (calcium iodate), 1.1 mg; selenium (sodium selenite), 0.1 mg.

[4]Each kilogram contained 132 g monensin as monensin sodium.

Plasma concentrations ranged from 3.0 to 14.6 μg/mL. Concentrations of total gossypol in plasma, liver, kidney, and muscle increased as the concentration of free gossypol increased in diets.

After regression analysis, residual analysis (studentized residuals and studentized deleted residuals) indicated the presence of an extreme observation (the CSM containing 255 μg/g free gossypol) that had a high tissue concentration of gossypol enantiomers in relation to the dietary concentration of free gossypol. This observation had a dramatic influence on the goodness of fit. We observed that when this particular treatment was omitted from the regression, r^2 values increased from 0.74 to 0.96 for total plasma gossypol, from 0.68 to 0.94 for liver, from 0.73 to 0.95 for kidney, and from 0.73 to 0.93 for muscle (Table 5).

DISCUSSION

Wide variations in BW and FC responses have been reported when CSM replaced all or part of the soybean meal in poultry diets. This variation in response has been attributed to factors such as gossypol, decreased lysine availability, lower energy concentration, and higher fiber content of CSM compared with soybean meal.

Excellent chick performance in this trial indicated that CSM up to 28% of the starter diet, when diets are formulated on a digestible amino acid basis, can support adequate growth and feed conversion regardless of the oil extraction process (expander, expeller, or direct solvent) or level of free gossypol (92 to 504 μg/g). These results suggest that broilers may tolerate higher concentrations of free gossypol than those widely recognized as safe. Waldroup (1981) reviewed the literature and concluded that free gossypol concentrations up to 100 ppm were acceptable without iron supplementation and up to 400 ppm free gossypol could be fed if iron was supplemented in a ratio of 1 to 2 ppm for each part per million of free gossypol. Our diets were estimated to contain at least 318

TABLE 3. Weekly body weights and feed conversions of broilers fed diets containing 28% cottonseed meal, differing in free gossypol, for 21 d

Dietary free gossypol (μg/g)	Body weight (g/bird)			Cumulative feed to gain ratios (g/g)		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week
0	132.4	366.7	743.3	1.25	1.36[a]	1.47
92	148.8	393.7	785.9	1.13	1.25[ab]	1.40
104	139.0	377.6	741.3	1.14	1.31[ab]	1.45
216	130.1	378.8	766.0	1.17	1.26[ab]	1.38
235	133.9	381.1	754.4	1.15	1.28[ab]	1.45
255	146.0	398.8	779.1	1.13	1.26[ab]	1.39
277	142.0	389.8	776.8	1.24	1.32[ab]	1.44
406	128.6	358.4	721.9	1.22	1.32[ab]	1.44
504	129.6	366.7	721.6	1.16	1.24[b]	1.38
Pooled SEM	8.0	16.4	23.6	0.04	0.02	0.04

[a,b]Means within a column without a common superscript differ ($P < 0.05$).

ppm iron (224 ppm from the mono-dicalcium phosphate and 26 ppm from the trace mineral premix). Good response in performance of broilers fed levels as high as 600 ppm free gossypol was reported by Couch et al. (1955).

Inclusion of 28% CSM resulted in a deficiency of digestible lysine; therefore, lysine•HCL was supplemented to compensate for the lower digestibility. Fernandez et al. (1994) reported that heat used during processing of CSM decreased digestibility of all amino acids except methionine, arginine, and histidine. In unprocessed cottonseed, amino acids were reported to be 85% digestible, whereas processed CSM (expander solvent extracted) was reported to have an average amino acid digestibility of 75%. Digestibility coefficients for individual amino acids in CSM were as follows: lysine, 64%; methionine, 72%; leucine, 74%; isoleucine, 73%; valine, 75%; and histidine, 62%. Fernandez et al. (1995) reported that diets formulated using digestibility coefficients supported adequate growth only when inclusion of CSM was 20%. Watkins et al. (1994) observed no detrimental effect of prepress CSM on BW at 21 d when broiler chickens were fed diets with up to 30% CSM. These authors reported a significant improvement in feed conversion with a coefficient of digestibility of 75% for all essential amino acids.

Accumulation of total gossypol occurs at a faster rate in liver than in plasma or any other tissue. An increment of 1 μg/g of dietary free gossypol resulted in an increment of 0.568 μg/g DM in liver, 0.065 μg/g DM in kidney, 0.018 μg/g DM in muscle, and 0.026 μg/mL in plasma. The same pattern of gossypol accumulation was reported previously by Kim et al. (1996) in lambs and by Knabe et al. (1995) in pigs and chickens. The proportion of (–)-gossypol was higher in plasma (26.7%) and kidney (25.6%) when compared to muscle (19.1%) and liver (16.0%).

In many instances the effects of the (+)- and (–)-isomers of gossypol on biological systems are stereospecific. In studies with small laboratory animals (–)-gossypol was much more toxic than (+)-gossypol and was the isomer responsible for infertility of males (Chen et al., 1987; Lindberg et al., 1987; Yu, 1987; Matlin et al., 1988). The minus isomer was also the more toxic isomer in a study with broiler chickens (Gamboa et al., 1997). Because of these stereospecific effects and the variable amounts of each

TABLE 4. Concentrations of gossypol in the plasma, liver, kidney, and muscle of broilers fed diets containing 28% cottonseed meal, differing in free gossypol, for 21 d

Dietary free gossypol (μg/g)	Gossypol enantiomers							
	Plasma (μg/mL)		Liver (μg/g DM)		Kidney (μg/g DM)		Muscle (μg/g DM)	
	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)
92	2.3[d]	0.7[e]	60.2[d]	11.2[c]	6.3[b]	2.9[b]	2.3[de]	0.4[bc]
104	3.3[d]	0.6[e]	85.7[d]	9.9[c]	9.0[b]	1.9[b]	2.1[e]	0.0[c]
216	3.5[d]	1.6[d]	85.2[d]	19.7[bc]	11.7[ab]	5.3[ab]	2.5[de]	0.7[bc]
235	5.7[c]	2.5[c]	154.8[c]	32.0[b]	17.1[ab]	6.6[ab]	4.0[cd]	1.6[ab]
255[1]	9.3[a]	3.8[ab]	254.9[a]	52.7[a]	25.9[a]	9.1[a]	6.6[ab]	2.3[a]
277	5.6[c]	1.9[cd]	142.8[c]	24.7[bc]	14.1[ab]	4.8[ab]	3.7[de]	0.8[bc]
406	7.6[b]	3.3[b]	201.0[b]	49.8[a]	24.7[a]	6.5[ab]	5.5[bc]	2.3[a]
504	10.5[a]	4.1[a]	266.0[a]	47.7[a]	27.3[a]	9.0[a]	7.4[a]	2.5[a]
Pooled SEM	0.3	0.2	9.3	3.1	4.7	1.7	0.4	0.3

[a–e]Means within a column without a common superscript differ ($P < 0.05$). For the (–)-enantiomer of kidney gossypol $P \leq 0.06$.

[1]This particular cottonseed meal differed from the other meals in that it did not contain added soapstock. Residual analysis indicated it was an outlier.

TABLE 5. Parameters for the regression of tissue gossypol concentrations on dietary free gossypol

Dependent variable[3]	Intercept	β^1	$(r^2)^2$
Total gossypol[4]			
Plasma (μg/mL)	0.61	0.0267*	0.96
Liver (μg/g DM)	21.19	0.5684*	0.94
Kidney (μg/g DM)	4.00	0.0650*	0.95
Muscle (μg/g DM)	0.26	0.0185*	0.93
(+)-Gossypol			
Plasma (μg/mL)	0.71	0.0183*	0.94
Liver (μg/g DM)	19.96	0.4667*	0.93
Kidney (μg/g DM)	2.51	0.0505*	0.94
Muscle (μg/g DM)	0.67	0.0124*	0.93
(–)-Gossypol			
Plasma (μg/mL)	–0.09	0.0084*	0.95
Liver (μg/g DM)	1.23	0.1016*	0.90
Kidney (μg/g DM)	1.50	0.0145*	0.83
Muscle (μg/g DM)	–0.36	0.0059*	0.86

[1]Independent variable is dietary free gossypol (μg/g).

[2]Coefficient of determination.

[3]Regressions do not include data from the CSM containing 255 μg/g free gossypol, which was determined to be an outlier by residual analysis.

[4]Total gossypol represents the sum of the (+)- and (–)-gossypol enantiomers.

*Significantly different from 0 ($P \leq 0.05$).

isomer in cottonseed, depending on species and variety of cotton, recommendations for safe levels of cotton feed products in animal diets need to take into consideration the level of gossypol and the proportions of isomers present.

Gossypol from one CSM (code 2220 containing 255 μg/g free gossypol) appeared to be much more available than from other CSM. This extremely high availability suggested the existence of particular characteristics of the methodology used in the oil extraction process or differences in the additional steps incurred during production of the commercial CSM that are influencing the availability of free gossypol. A particular characteristic of the code 2220 CSM with the extremely high availability of gossypol is that soapstock was not added back during the manufacture of the meal. It has been reported that gossypol from soapstock is not as available as free gossypol from CSM (Curtin and Raper, 1956; Lipstein and Bornstein, 1964a; Garlich et al., 1989), probably due to the alkali and acid treatments used during refining (Curtin and Raper, 1956; Lipstein and Bornstein, 1964a). Also, Lipstein and Bornstein (1964b) reported that gossypol content in acidulated soapstock is positively correlated to the storage time, although this view is not universally accepted.

Starting broilers in this study received diets containing up to 28% CSM and 500 μg/g free gossypol for 21 d without showing a significant depression in performance when diets were formulated using digestibility coefficients for essential amino acids. The diets used in this particular study did not include supplemental iron over that provided by a typical trace mineral premix. Iron present in the mono-dicalcium phosphate and other ingredients also contributed to the total iron content of the diet. The study also suggests differences in cottonseed processing can affect free gossypol concentrations as well as availability.

Finally, it appears there is a relatively good relationship between dietary free gossypol and tissue accumulation of gossypol enantiomers, perhaps depending on the presence or absence of cottonseed soapstock.

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