Cottonseed with a High (+)- to (−)-Gossypol Enantiomer Ratio Favorable to Broiler Production

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This study was designed to evaluate the relative toxicity of (+)- and (−)-gossypol enantiomers in 0−3-week-old broilers. Treatments consisted of broiler starter diets formulated with either a glandless, which did not contain gossypol, a commercial glanded [62.2% (+)-gossypol], or a glanded moco [83.2% (+)-gossypol] crushed cottonseed (CCS) (six replicates/treatment) plus a soybean meal negative control. Glandless cottonseed was mixed with the moco cottonseed (2.4% free gossypol) so that both the commercial glanded and moco glanded cottonseeds contained equivalent concentrations of free gossypol (2.0%). The cottonseed treatments were added at 5 and 10% of the diet. Body weights and feed conversions were determined weekly. Body weights and feed-to-gain ratios of broilers fed 5 and 10% glandless CCS and 5% moco CCS were not significantly different. Broilers receiving 10% commercial glanded CCS weighed significantly less than those subjected to all other treatments. Feed-to-gain ratios were significantly higher for broilers receiving 10% commercial glanded and 10% moco CCS as compared to 5% moco and glandless CCS, 10% glandless CCS, and control. Relative liver weights of birds receiving 10% moco CCS were significantly less than those of birds receiving 10% commercial CCS. The data clearly showed that broilers fed moco CCS containing a relatively high (+)- to (−)-gossypol enantiomer ratio performed better than broilers receiving commercial CCS with a lower (+)- to (−)-gossypol enantiomer ratio.

Keywords: *Cottonseed; gossypol; gossypol enantiomers; broilers; toxicity*

INTRODUCTION

Gossypol [1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-(2,2′-binaphthalene)-8,8′-dicarboxaldehyde] is a yellow phenolic substance present in various parts of cotton plants. It has two naphthalene rings with identical substituents with restricted rotation about the rings and a molecular formula of $C_{30}H_{30}O_8$. High concentrations of gossypol in poultry diets have been associated with depressed feed intake, feed efficiency, and weight gains and increased mortality (Groschke et al., 1947; Boatner et al., 1948; Lillie and Bird, 1950; Heywang and Bird, 1954; Couch et al., 1955; Heywang et al., 1955; Phelps, 1966). However, Heywang and Bird (1950) reported that increased dietary concentrations of gossypol were not always accompanied by lowered feed efficiency. Free or unbound gossypol seems to be the major biologically active form (Heywang et al., 1952), and this has been the basis for establishing usage levels. Several studies over the past few decades have confirmed these early findings.

Iron was found to protect rabbits from gossypol toxicity when administered in the form of ferric ammonium citrate (Withers and Brewster, 1913). Iron has also been shown to be partially effective in preventing the accumulation of gossypol in porcine liver (Buitrago et al., 1970). It is presumed that ferrous ions can detoxify gossypol by catalyzing its decarboxylation (Abou-Donia and Lyman, 1970). At a conference on the inactivation of gossypol with mineral salts, a panel of nutritionists concluded that, for broilers, a ratio of two parts iron to one part gossypol by weight prevents deleterious effects on growth by cottonseed meals high in free gossypol content (Berardi and Goldblatt, 1980).

Gossypol exists as two enantiomers, with different optical properties because of restricted rotation around the binaphthyl ring [(+)- and (−)-enantiomers]. Matlin and Zhou (1984) were able to use preparative liquid chromatography to resolve gossypol into its two enantiomers. Enantiomer ratios for cottonseeds can vary but are in the approximate range of 50−60% (+)-gossypol in commercial U.S. cotton varieties. In a study of gossypol enantiomers, Cass et al. (1991) used HPLC to assay five different cultivars of *G. hirsutum marie-galante* [moco-3M, moco, moco (CNPA), moco-MF-4, and eparn-2] and found the (+)-gossypol enantiomer to range from 85 to 95% of the total.

Joseph et al. (1986) found the (−)-form to be more cytotoxic than the (+)-form, and the degree of toxicity

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was influenced by the concentration of plasma proteins, presumably due to the binding of gossypol to the free amino groups. Wang et al. (1987) found that rats treated orally with (−)-gossypol at a rate of 30 mg/kg for 1 week had significantly lower body weights, whereas rats treated with an equal dosage of (+)-gossypol showed no effect on body weight. Research in our laboratory with broilers indicates that the (−)-enantiomer may be the more toxic of the two (Gamboa et al., 1997; Gamboa, 1999). Research by Blackstaffe et al. (1997) found melanoma cells to have a 5-fold greater cytotoxic sensitivity to the (−)-enantiomer than to the (+)-enantiomer, suggesting that the (−)-enantiomer may have some potential therapeutic benefits in melanoma patients. The purpose of this study was to further evaluate the performance of 0−3-week-old broilers fed diets differing in (+)- to (−)-gossypol enantiomer ratios.

MATERIALS AND METHODS

Animals and Diets. A total of 240 male broiler chicks were obtained from a local hatchery. These birds were randomly placed into 48 Petersime (Petersime Incubator Co., Gettysburg, OH) brooder pens at a rate of five birds per pen. All diets were based on the National Research Council (1994) requirements for starting broilers. These treatments consisted of a glandless crushed cottonseed (CCS) control containing no gossypol, a commercially grown glanded CCS, or a glanded CCS derived from moco cottons grown in Brazil. Diets were formulated with 5 and 10% CCS to achieve free gossypol concentrations of 0, 0.1, and 0.2%. A treatment with 0% CCS was included as a negative control diet. To decrease iron−gossypol interactions, diets were formulated with a reagent grade phosphorus [calcium phosphate dibasic (USP), N.F. anhydrous, Mallinckrodt Chemical Works) source rather than a feed grade source, which can be relatively high in iron content. Birds were given ad libitum access to feed and water.

Cottonseed Analysis. Cottonseeds were obtained from three different sources (S. R. Oakley, California Planting Cottonseed Distributors, Shafter, CA and R. Vieira, Empresa Brasileira, Paraba, Brazil). These cottonseeds consisted of a glandless, a glanded commercial grown cottonseed, and a glanded cottonseed from moco cottons in Brazil. The cottonseeds were analyzed for free gossypol (Stipanovic *et al.*, 1988) as well as (+)- and (−)-gossypol enantiomers (Kim et al., 1996). Analysis of the moco and commercial cottonseeds revealed the moco sample to have a free gossypol concentration of 2.38% and a (+)- to (−)-enantiomer ratio of 83:17, whereas the commercial sample contained 2.0% free gossypol with a ratio of 62:38.

The glandless cottonseed was mixed with the glanded moco to achieve a free gossypol concentration of 2.0%, equivalent to the assayed concentration of the glanded commercial CCS. Thus, the 5% moco CCS contained 0.083% (+)-enantiomer and 0.017% (−)-enantiomer; the 10% moco CCS contained 0.166% (+)-enantiomer and 0.034% (−)-enantiomer; the 5% commercial CCS contained 0.062% (+)-enantiomer and 0.038% (−)-enantiomer; and the 10% commercial CCS contained 0.124% (+)-enantiomer and 0.076% (−)-enantiomer. Amino acid levels were analyzed for each diet by Degussa Analytical Services (Allendale, NJ).

Data Collection and Analysis. Body weight, feed weight, and feed conversion were determined weekly. At 3 weeks, blood and liver samples were collected from all birds. Relative liver weights (liver weight/live body weight) were calculated to account for differences in the size of birds. Freeze-dried liver samples and serum were analyzed by HPLC to determine the concentration of (+)- and (−)-gossypol enantiomers (Kim et al., 1996). Serum from two randomly selected blood samples per pen was also analyzed with a clinical chemistry analyzer (Gilford Impact 400E, Gilford Systems, Oberlin, OH) for the presence of metabolites and enzymes, which can be indicative of cellular damage. These included aspartate aminotransferase, γ-glutamyltransferase, lactate dehydrogenase, uric acid, blood urea nitrogen, cholesterol, creatine kinase, glucose, alkaline phosphatase, inorganic phosphate, and albumen.

The data obtained in the study were analyzed by ANOVA in a one-way classification using the General Linear Models (GLM) procedure of the SAS Institute (1985). A probability of 0.05% was considered significant. Duncan's multiple-range test was used to compare means when the ANOVA was significant.

Table 1. Mean Cumulative Weight Gain and Cumulative Feed Conversion Ratios of 3-Week-Old Broilers Fed Crushed Cottonseed

CCS treatment[a]	MCWG[b] (g)	CFCR[b] (g/g)
0% negative control	742 ± 20.3[a]	1.58 ± 0.04[c]
5% glandless control	752 ± 44.6[a]	1.63 ± 0.04[bc]
5% moco glanded	731 ± 37.3[a]	1.63 ± 0.02[bc]
5% commercial glanded	601 ± 13.6[b]	1.77 ± 0.06[ab]
10% glandless control	809 ± 19.1[a]	1.50 ± 0.03[c]
10% moco glanded	507 ± 15.4[c]	1.81 ± 0.06[a]
10% commercial glanded	338 ± 19.1[d]	1.91 ± 0.09[a]

[a] There were six pens per treatment. The negative control group had 12 pens. [b] Mean ± standard error. Means with no common superscript letter within a column differ significantly ($p \le 0.05$).

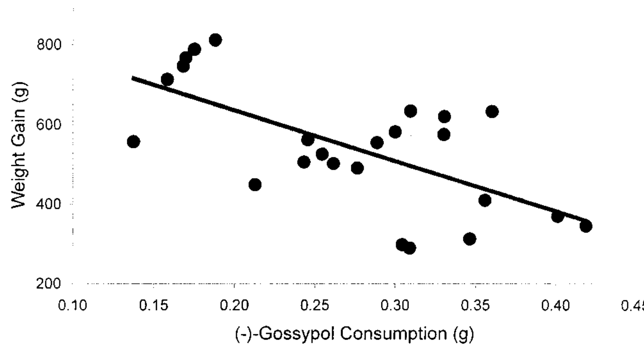

Figure 1. Effect of cumulative (−)-gossypol consumption on 3-week BW gain in broilers fed CCS. The trend line that best describes these data is $y = -1264x + 889$, where y is equal to 3-week BW gain and x is equal to the average total (−)-gossypol consumed per bird over the 3-week growing period. The coefficient of determination is equal to 0.414.

RESULTS AND DISCUSSION

Growth performance, measured as cumulative weight gain, did not differ significantly for broilers fed the control, 5 and 10% glandless CCS, and 5% moco CCS diets (Table 1). The 5 and 10% glanded CCS diets and the 10% moco diets were significantly different from each other and all other diets, with lower weight gains associated with higher CCS levels within the diets. Cumulative weight gains decreased ∼126 g for each 0.1 g increase in (−)-gossypol consumption (Figure 1). For a given concentration of free gossypol, cumulative weight gain increased as the (+)- to (−)-enantiomer ratio increased. Broilers fed a diet consisting of 10% glanded commercial CCS with a (+)- to (−)-gossypol enantiomer of 62:38 resulted in a cumulative weight gain significantly lower than that of all other treatments (Table 1). After 3 weeks of feeding, these birds had consumed a total of 0.36 g of (−)-gossypol and 0.59 g of (+)-gossypol. Broilers receiving 10% moco CCS with a (+)- to (−)-gossypol enantiomer ratio of 83:17 had consumed a total of 0.25 g of (−)-gossypol and 1.22 g of (+)-gossypol, resulting in a cumulative weight gain significantly better than the 10% glanded commercial CCS. Thus, even though broilers fed the 10% moco CCS received 55% more free gossypol than birds consuming the 10% glanded CCS, they weighed significantly more.

Table 2. Gossypol Enantiomers in Freeze-Dried Serum and Liver Tissue of 3-Week-Old Broilers Fed Glanded Crushed Cottonseed

	serum gossypol (ng/mg)			liver gossypol (ng/mg)		
CCS treatment[a]	(+) enantiomer	(−) enantiomer	(+):(−) ratio	(+) enantiomer	(−) enantiomer	(+):(−) ratio
5% moco	439 ± 35	93 ± 6	83:17	684 ± 44	67 ± 5	91:9
10% moco	539 ± 73	109 ± 17	83:17	990 ± 64	98 ± 6	91:9
5% commercial	409 ± 27	155 ± 11	72:28	592 ± 31	123 ± 8	83:17
10% commercial	463 ± 48	164 ± 14	74:26	1132 ± 102	223 ± 14	84:16

[a] There were 30 observations per treatment. The (+):(−) enantiomer ratios in the moco and commercial glanded CCS were 83:17 and 62:38, respectively.

Table 3. Relative Liver Weights and Serum Analysis of 3-Week-Old Broilers Fed Crushed Cottonseed

CCS treatment[a]	relative liver wt[b] (g/g of BW)	γ-glutamyl-transferase[b] (IU/L)	aspartate aminotransferase[b] (IU/L)	cholesterol[b] (IU/L)
0% negative control	3.6 ± 0.1[de]	14.0 ± 0.6[b]	141.1 ± 2.9[bc]	154.9 ± 4.4[c]
5% glandless control	3.4 ± 0.2[e]	13.5 ± 1.1[b]	127.4 ± 9.6[c]	159.4 ± 7.3[bc]
5% moco	4.1 ± 0.1[bc]	14.5 ± 1.2[b]	153.9 ± 3.2[b]	184.6 ± 7.8[b]
5% commercial	4.0 ± 0.1[bcd]	14.6 ± 1.0[b]	147.3 ± 7.3[bc]	213.0 ± 15.3[a]
10% glandless control	3.4 ± 0.3[de]	13.1 ± 1.5[b]	139.7 ± 5.3[bc]	153.8 ± 5.3[c]
10% moco	4.5 ± 0.2[b]	17.8 ± 1.3[a]	192.1 ± 8.0[a]	232.2 ± 13.0[a]
10% commercial	5.3 ± 0.5[a]	18.8 ± 1.3[a]	193.5 ± 12.7[a]	225.4 ± 16.7[a]

[a] For relative liver weight there were 30 observations per treatment. The negative control group represented 60 observations. For the serum analysis there were 12 observations per treatment (24 for the negative control treatment). [b] Means with no common superscript letter within a column differ significantly ($p \leq 0.05$).

The amino acid levels in the diets that gave reduced weight gains (i.e., 10% moco CCS, 5% glanded CCS, and 10% glanded CCS) did not differ at the 95% confidence level from the mean of the amino acid levels in those diets that gave the best weight gains (i.e., control, 5% moco CCS, 5% glandless CCS, and 10% glandless CCS). The highest cumulative weight gain was found in birds fed a diet containing 10% glandless cottonseed. This was not significantly different from the control diet and shows that depressed performance in the other diets was most likely due to gossypol and not the nutritional value of the cottonseed. Our data clearly show that as the (+)- to (−)-gossypol enantiomer ratio decreases, cumulative weight gain also decreases.

Many studies have shown cumulative feed conversion ratios (CFCR) to be negatively affected by gossypol (Boatner et al., 1948; Heywang and Bird, 1954; Couch et al., 1955). This study also showed significant differences in CFCR among treatments (Table 1). The CFCR for the broilers fed the 10% glanded, 5% glanded, and 10% moco diets were not statistically different among themselves, but they were statistically different from the control. CFCR values for the 5% moco and glandless diets were not statistically different from the control. Cumulative feed conversion ratios were highest in the glanded and moco CCS diets fed at the 10% level.

Levels of (+)- and (−)-gossypol in freeze-dried liver tissues and sera were also determined. As expected, the levels of (−)-gossypol were lower in broilers fed the moco CCS (Table 2). The ratio of (+)- and (−)-gossypol in the blood serum did not differ appreciably from that in the CCS itself. However, the (+)-to-(−) ratio was notably higher in the liver. This was not observed in a lamb study in which the ratios in the liver were not different from the dietary ratios (Kim et al., 1996).

Gossypol has been shown to have degenerative effects on the liver (Smith, 1957; Adams et al., 1960; Abou-Donia, 1976). The highest relative liver weights corresponded with the 10% CCS diets, with the commercial glanded CCS diet resulting in relative liver weights significantly greater than found for all other treatments (Table 3). Relative liver weights from birds receiving the glandless CCS were not significantly different from the control. In this study, increasing concentrations of dietary gossypol also corresponded with higher relative liver weights (Table 3). Broilers receiving the highest concentration of (−)-gossypol developed relative liver weights significantly higher than those of all other birds within the study. The relative liver weights of the broilers fed the glandless CCS, which contained no gossypol, were not significantly different from the negative control.

Blood serum samples were assayed for the presence of various metabolites and enzymes. Significant results from the clinical chemistry analyzer are shown in Table 3. Data for other assays are not presented. Concentrations of γ-glutamyltransferase, which can be indicative of liver damage, became higher as gossypol levels increased, with the 10% glanded commercial CCS having the highest values. Aspartate aminotransferase, which can also be indicative of liver damage, was also significantly higher in broilers fed diets consisting of either the commercial or moco cottonseeds when these were formulated at the 10% level. Serum cholesterol also increased in birds fed diets containing gossypol, with the 5 and 10% commercial glanded and 10% moco diets having significantly higher values than the other diets. There were no significant differences in mortality among treatments due to the effect of either type or concentration of CCS.

This study conclusively shows a link between (+)- to (−)-gossypol enantiomer ratios and toxicity and indicates that development of cotton cultivars with a higher (+)- to (−)-gossypol ratio could be useful to the poultry industry with respect to cottonseed meal.

ABBREVIATIONS USED

CCS, crushed cottonseed; CFCR, cumulative feed conversion ratio; MCWG, mean cumulative weight gain; BW, body weight.

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