DEVELOPING LOW (-)-GOSSYPOL COTTONSEED FOR USE IN BROILER PRODUCTION

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Abstract

Cottonseed cannot be used directly as a feed for non-ruminant animals because it contains the toxin gossypol; gossypol occurs as a mixture of (+)- and (-)-enantiomers. Only the latter shows a high level of toxicity to chickens. We conducted experiments to develop cotton varieties that exhibit a low percentage of (-)-gossypol in seed. Crossing Uzbek cotton varieties with USA accessions that exhibit the low (-)-gossypol seed trait provided recombinants with a low percentage of (-)-gossypol in seed and with good agronomic properties suitable for growing in Uzbekistan. Cottonseed meal (CSM) derived from lines exhibiting the low (-)-gossypol seed trait and from commercial cottonseed meal were incorporated into broiler chicken diets at 5% and 10% in place of soybean meal. These meals were fed to chickens for 21 days, and the birds were then fed a control diet without CSM for 28 days. Birds were also fed a control diet without CSM for 49 days. Birds were weighed weekly, and some birds from each group were selected at random and euthanized after 14, 28 and 42 days, and at the end of the experiment (49 days). The carcasses were subjected to a necropsy, and samples from thighs, breast, gizzard, liver, and blood were collected and freeze dried. The freeze dried samples were analyzed for (+)- and(-)-gossypol. The results indicate that up to 10% commercial CSM could be incorporated into the diet for broilers in place of soybean meal without adversely affecting growth, and that (+)-gossypol is less toxic to chickens than (-)-gossypol. (+)-Gossypol was retained in chicken tissues more efficiently than (-)-gossypol. Very little (-)-gossypol was found in chicken tissues other than the liver. No gossypol was found in feces. The necropsy did not show any abnormalities.

Introduction

Cottonseed is an excellent source of plant derived protein, but it is underutilized because of the presence of a naturally occurring toxin called gossypol. Gossypol occurs as an enantiomeric mixture of (+)-gossypol and (-)-gossypol. The latter is significantly more toxic than the former (Bailey et al., 2000). Uzbekistan scientists are developing germplasm with low levels of (-)-gossypol in the seed (Namazov et al., in press). We now report results of feeding experiments where either cottonseed meal exhibiting the low (-)-gossypol seed trait (L-G-CSM), or commercial cottonseed meal (C-CSM) was incorporated into the diet of broilers. The meals were incorporated into chicken feed at 5% and 10% to replace soybean meal, and these diets were fed to birds for 21 days. Birds were then fed the control growth diet with no CSM until the end of the experiment (49 days).

Materials and Methods

Preparation of Feed

Seed from commercial plants or from cotton plants that exhibit the low (-)-gossypol seed trait were subjected to a "cold press". The resulting cottonseed meal was analyzed for total and percent (+)- and (-)-gossypol by reacting the meal with d-alaninol. This provides a diastereomeric mixture of Schiff bases that were analyzed by capillary electrophoresis as previously described (Vshivkov et al., 2012).

Feeding Protocol

One hundred and fifty newly hatched chicks (Ross 308) were randomly divided into five groups of 30 birds each. One group was selected as the Control and was fed a starter diet for 14 days, a growth diet for 7 days and a finisher diet for 28 days. Treatment groups 1 to 4 were fed the same as the Control birds except part of the soybean meal was replaced with L-G-CSM, cottonseed meal with a low percentage of (-)-gossypol, or commercial cottonseed meal (C-CSM), with a high percentage of (-)-gossypol, for the first 21 days. That is, 5% of the soybean meal was replaced with L-G-CSM to provide Feed I, and 10% soybean meal was replaced by L-G-CSM to provide Feed II. In addition, 5% of the soybean meal was replaced with C-CSM to provide Feed III and 10% soybean meal was replaced with C-CSM to provide Feed IV. At the end of 21 days, all birds were placed on the finisher diet (i.e., no CSM).

Collection of Data

Birds from each group were weighed weekly beginning with day 7. At the end of 14, 28, 42 and 49 days, five to six birds from each group were selected at random and euthanized. The carcasses of all euthanized birds were subjected to a necropsy. Samples of blood, breast muscle, thigh muscle, gizzard and liver were freeze dried and then reacted with d-alaninol to provide a diastereomeric gossypol Schiff base; the latter was analyzed by capillary electrophoresis, to provide the percent (+)- and (-)-gossypol, which together gives the total gossypol.

Results and Discussion

Cottonseed were dehulled and passed through a "cold press" to remove the oil along with some of the gossypol. This CSM was incorporated into the feed for the chickens for the first 21 days at 5% and 10% with a concomitant reduction in the amount of soybean meal. The CSM was derived either from plants exhibiting the low (-)-gossypol seed trait (L-G-CSM) or a commercial CSM (C-CSM). This provided gossypol concentrations at the following levels: Feed I - 0.982gm/kg total gossypol of which 0.131gm/kg was (-)-gossypol; Feed II - 1.883gm/kg total gossypol of which 0.232gm/kg was (-)-gossypol; Feed III - 0.635gm/kg total gossypol of which 0.202gm/kg was (-)-gossypol; Feed IV - 1.59gm/kg total gossypol of which 0.518gm/kg was (-)-gossypol (Figure 1). Five to six birds were euthanized at the end of 14, 28, 42 and 49 days.

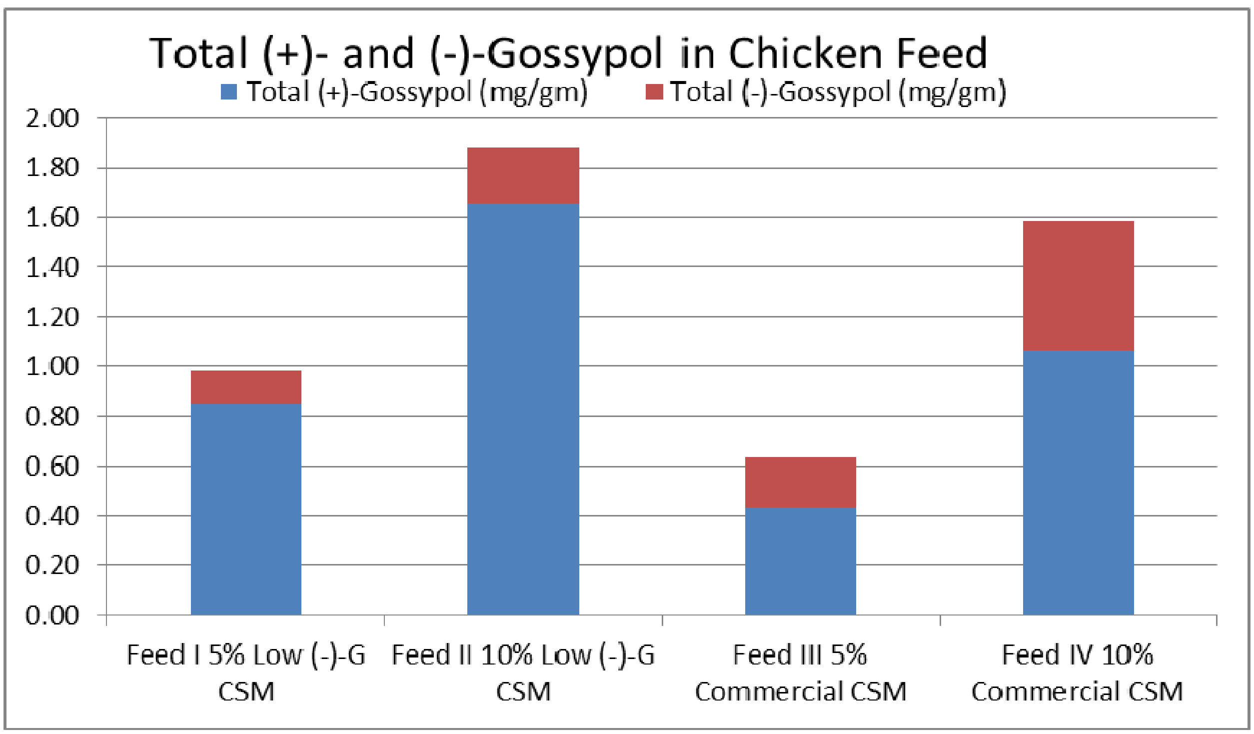

Figure 1. Concentration of (+)- and (-)-gossypol in chicken Feeds I, II, III, IV.

Bird Weights

Birds were weighed weekly, and the weights are shown in Figure 2. After the first week, there was no statistical difference among the weights of the birds in the different groups. However, after 21 days, the birds on the control diet were significantly larger than the birds on the diets containing CSM. The weights of the birds on the CSM diets were not statistically different from each other. After 42 days, the birds on L-(-)-G-CSM Feed I diets were not significantly different than those on the Control diet. The birds fed the other three diets were significantly smaller than those fed the Control diet. This would agree with the earlier findings that showed that (+)-gossypol is less toxic than (-)-gossypol (Bailey et al., 2000). However, at the end of 49 days the weights of birds on Feeds II and IV as well as those on Feed I were not significantly different than those on the Control diet. Only the birds on Feed III were significantly smaller than the birds on the Control diet. Since five to six birds were euthanized at 14, 28, and 42 days, the number of birds remaining to weigh on day 49 were quite small (five birds on each Feed). As a result the standard errors are large in comparison to those on earlier days. This may account for the unexpected discrepancy observed for the weights of birds on Feed III. Nevertheless, the overall results indicate that birds fed up to 10% of a commercial CSM for the first 21 days should perform as well as those fed a diet with no CSM. In addition, the results agree with the earlier study that found (+)-gossypol was less toxic than (-)-gossypol to chickens. No adverse effects were noted during the necropsies that were performed on all euthanized birds.

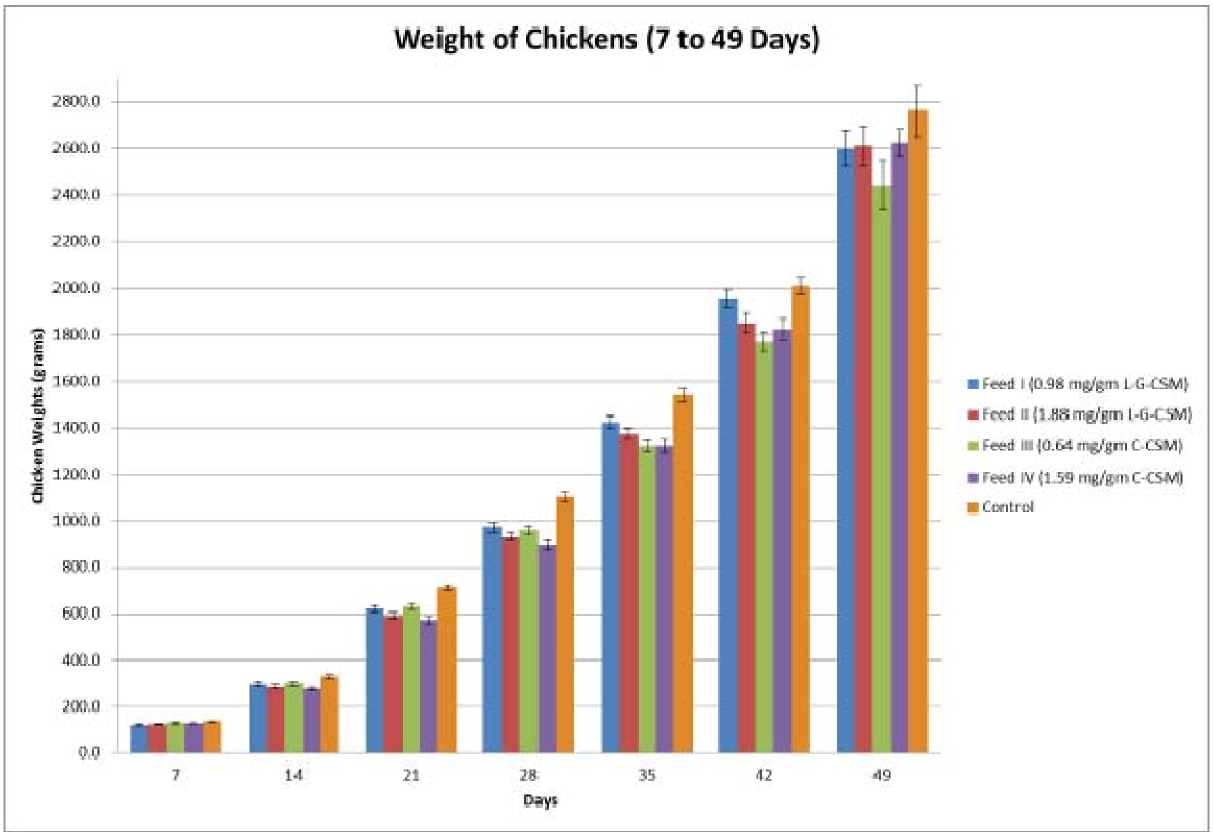

Figure 2. Weights of chickens on Feeds I, II, III and IV. Standard errors bars are shown.

Retention of Gossypol in Chicken Tissues

Freeze dried samples of the thigh, breast, gizzard and liver of all euthanized birds were analyzed for (+)- and (-)-gossypol on days 14, 28, 42 and 49. The concentration of (+)- and (-)-gossypol retained in these tissues are shown in Figure 3. (+)-Gossypol was retained far more efficiently in the animal's body compared to (-)-gossypol. Birds on Feeds I and II, which were fed the L-G-CSM diets and therefore consumed the highest percent of (+)-gossypol, had the highest total gossypol in the thigh muscle throughout the experiment compared to birds fed the 5% and 10% C-CSM. In the breast muscle, the concentration of (+)-gossypol was higher or comparable in the birds on Feed IV compared to birds on Feeds I and II. In the gizzard, only the birds on Feed II had measurable amounts of (+)-gossypol on day 49. The liver had the highest concentration of (+)-gossypol compared to other tissues. Gossypol was not observed in other tissues in birds on Feed III at any time, and it was not found in the liver at 42 and 49 days. (-)-Gossypol occurred sporadically in various tissues thorough out the experiment, but it was at the highest concentrations in the liver.

Blood and fecal material were also analyzed for gossypol. Gossypol was not found in the feces (data not shown). Gossypol was not detected in the blood of any of the birds on Feed III or IV on any day (data not shown). In birds on Feeds I and II, only (+)-gossypol was found in the blood (Table 1). It was not found in any blood samples in 49 day-old birds.

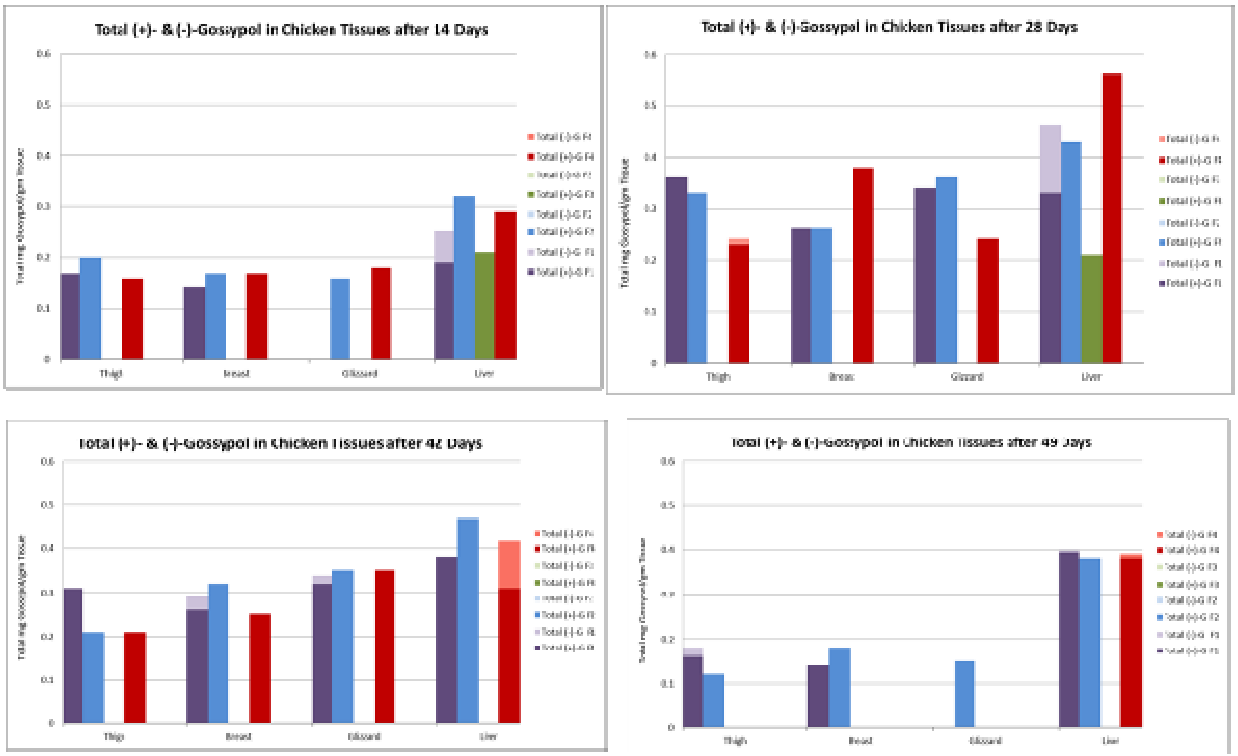

Figure 3. Total and (+)- and (-)-gossypol in chicken tissues of birds raised on Feed I (5% low (-)-gossypol CSM), Feed II (10% low (-)-gossypol CSM), Feed III (5% commercial CSM) and Feed IV (10% commercial CSM). Dark shades indicate the concentration of (+)-gossypol and light shades indicate the concentration of (-)-gossypol in each column.

Table 1. Concentration of (+)-gossypol in blood from chickens on Feed I (5% low (-)-gossypol CSM) and Feed II (10% low (-)-gossypol CSM) for 21 days and the control growth (no CSM) for 28 days.

Day	Feed	(+)-Gossypol (mg/gm)
14	I	0.04
14	II	0.02
28	I	ND*
26	II	0.03
42	I	ND
42	II	0.02
49	I	ND
49	II	ND

* Not detected.

Summary

These experimental results indicate that up to 10% commercial CSM could be incorporated into the feed for broiler chickens for the first 21 days of growth in place of soybean meal without negatively affecting growth [especially with feed derived from low (-)-gossypol seed]. Perhaps even more of the low (-)-gossypol CSM could safely be incorporated. The results also support the hypothesis that (+)-gossypol is less toxic to chickens than (-)-gossypol. However, (+)-gossypol is retained more efficiently than (-)-gossypol in the chicken tissues. That is, very little (-)-gossypol was found in any tissues compared to (+)-gossypol. Lyman et al. (1969) found in their chicken feeding study that most of the radiolabel from ^{14}C-gossypol was in the feces, apparently as a decomposition product. In addition, Lyman et al. report that among body tissues most of radioactivity was in the liver. Based on these observations, it appears that most of the (-)-gossypol is degraded by the liver and eliminated in the feces, while much more of the (+)-gossypol compared to (-)-gossypol passes through the liver and is retained in other tissues. However, other than the liver, much of the (+)- and (-)-gossypol is eliminated from animal tissues over time once the animal is removed from the CSM diet, such that very low levels remain in the muscle tissues at the end of the experiment.

Acknowledgements

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