Effects of dietary gossypol concentration on growth performance, blood profiles, and hepatic histopathology in meat ducks[1]

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ABSTRACT The objective of this study was to determine the effects of gossypol from cottonseed meal (CSM) on growth performance, blood biochemical profiles, and liver histopathology of ducks. A total of 900 1-d-old ducks were randomly allocated to 5 treatments with 12 pens/treatment and 15 ducks/pen. The 5 experimental diets were formulated in such a way that 0% (a corn-soybean meal basal diet, diet 1), 25% (diet 2), 50% (diet 3), 75% (diet 4), and 100% (diet 5) of protein from soybean meal were replaced with that from CSM. All diets were formulated on a digestible amino acid basis. The experiment included 2 phases, the starter phase (1 to 3 wk) where the test diets contained graded levels of CSM and the growth phase (4 to 5 wk) where birds were fed a corn-soybean basal diet to examine the recovery of ducks after CSM withdrawal. Dietary CSM and gossypol linearly ($P < 0.01$) and quadratically ($P < 0.01$) decreased ADG and ADFI during d 1 to 14. The threshold of daily total gossypol (TG) and free gossypol (FG) intake based on ADG on d 1 to 7 and d 7 to 14 were 32.20 and 2.64 mg/d, and 92.12 and 9.62 mg/d, respectively. Serum alanine aminotransferase increased ($P < 0.05$) linearly with increasing level of gossypol in the diets (d 7), whereas aspartate aminotransferase increased ($P < 0.05$) linearly and quadratically (d 14). Serum albumin concentration decreased ($P < 0.05$) quadratically with increasing dietary CSM concentrations on d 21. The degree of damage to the liver increased markedly with increasing dietary CSM and gossypol content and the length of CSM and gossypol intake. On d 35, there was no difference on BW and blood profiles of ducks among all treatments. These results suggest that meat ducks' dietary TG and FG concentration should be lower than 928.9 and 77.2 mg/kg, respectively, during d 1 to 21 of age and that a 2-wk withdrawal of diets containing gossypol should be considered.

Key words: duck, gossypol, growth performance, hepatic histopathology, serum biochemical profile

INTRODUCTION

In China, cottonseed meal (**CSM**) is an important feedstuff for meat duck production, and its proportion in the complete feed formula of meat ducks usually ranges between 6 and 12%. In recent years, the level of gossypol from CSM in duck diets has generated considerable interest in food safety and bird welfare. The risk of gossypol toxicity is the primary concern of poultry industry, and this has negatively affected the level of CSM that can be incorporated into poultry feeds. Gossypol from cottonseeds has antisteroidogenic and antireproductive activities in male rats (El-Sharaky et al., 1993). Gossypol and its metabolites, such as gossypolone, can induce inhibition of glucose uptake, which may be related to the generation of lipid peroxides and consequently membrane damage (Kanwar et al., 1990; Fornes et al., 1993). The cottonseed toxin, gossypol, is not easily cleared from animal tissues, leading to gossypol accumulation (Sharma et al., 1966); if contaminated meat is eaten, it may indirectly be damaging to human health (Semon, 2012). Abou-Donia and Dieckert (1975) observed that 1 d after the pigs were fed a single oral dose of 6.7 mg [14C] gossypol/kg (3.7 μCi), the liver had 32.9% of the administered dose, which was decreased to 10.7% at d 10 and subsequently to 1.2% at d 20. When gossypol was withdrawn in cow diets after they were fed a gossypol-containing diet for 84 d, plasma gossypol concentrations only achieved 30 and 10% of the maximal concentrations at 14 d and 28 d after gossypol withdrawal, respectively (Mena et al., 2004). Therefore, gossypol clearing from animal tissue is affected by the gossypol concentration of diet, animal

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type or species, and the length of gossypol intake and withdrawal.

Gossypol in CSM and consequently diets exists in free and bound forms. The 2 forms together are designated as total gossypol (**TG**). Free gossypol (**FG**) is toxic to animals, whereas the bound form is considered nontoxic. However, it has been reported that during the digestion process, bound gossypol may be released as FG and can be absorbed by the digestive tract (Blackwelder et al., 1998; Noftsger et al., 2000; Mena et al., 2001). Mena et al. (2004) suggested that when determining guidelines for the safe feeding of gossypol containing products to dairy cows, the amounts of FG as well as the source of TG should be considered. Numerous researchers have reported that high levels of gossypol in broilers depressed weight gain (Phelps, 1966; Waldroup, 1981) and feed efficiency (Couch et al., 1955). However, several authors have shown that chick performance is not depressed when dietary level of FG is lower than 250 mg/kg of feed (Heywang and Bird, 1955; Hermes et al., 1983). Other investigators have shown that CSM can replace up to 75% soybean meal (**SBM**) without any adverse effects on performance, hematological values, and carcass quality of the broilers (Adeyemo and Longe, 2007). Furthermore, several factors influence the tolerance of gossypol for animals. These factors include the source of CSM, dose or concentration of CSM, the processing technique the CSM has been subjected to, the level of nutrients in the diet, age of birds, animal type or species, and dietary iron and lysine (Nagalakshmi et al., 2007). The 2 most important factors considered when evaluating CSM for poultry nutrition are FG levels and protein quality. Fernandez et al. (1994) reported that heat damage to CSM proteins linearly decreased digestibility of all amino acids, except methionine, arginine, and histidine. Consequently, all experimental diets should be considered to formulate on a digestible amino acid basis when the toxicity of gossypol is studied. By far, most gossypol and CSM studies were conducted with broilers, laying hens, and other animals such as fish and dairy cows, but information regarding ducks is limited. To our knowledge, there are few studies that have evaluated the effects of FG and TG on the performance and health of ducks. Therefore, the objective of this research was to determine the effects of gossypol from CSM on the performance, blood characteristics, and hepatic histopathological effects of Cherry Valley ducks fed with graded levels of CSM and to examine the recovery performance of these ducks after 2 wk of CSM withdrawal.

MATERIALS AND METHODS

Birds and Diets

A total of 900 1-d-old mixed sex Cherry Valley ducks (450 males and 450 females) were used for this study. Ducks were randomly assigned to 5 dietary treatments with 12 pens (6 females and 6 males) per treatment and 15 ducks/pen, according to a completely randomized design. Five isonitrogenous and isocaloric experimental diets were formulated. The experimental diets were formulated to produce diets in which 0% (diet 1, control diet, a corn-soybean basal diet, without TG and FG), 25% (diet 2, with 465 mg of TG/kg and 35.2 mg of FG/kg), 50% (diet 3, with 929 mg of TG/kg and 77.2 mg of FG/kg), 75% (diet 4, with 1,393 mg of TG/kg and 103.1 mg of FG/kg), and 100% (diet 5, with 1,857 mg of TG/kg and 152.9 mg of FG/kg) of protein from SBM were replaced with that from CSM. The TG and FG concentration of all diets were the analyzed values. The composition and calculated nutrient and energy levels of the basal diet (diet 1) and diet 5 are presented in Table 1. Diets 2, 3, and 4 were generated by blending diets 1 and 5 at a ratio of 3:1, 1:1, and 1:3, respectively. The 100% CSM diet was supplemented with L-lysine HCl, DL-methionine, L-threonine, and L-tryptophan, and it contained the same level of these amino acids as the control diet on a digestible basis. The ratio of digestible lysine to digestible methionine, threonine, and tryptophan in all diets was 44, 67, and 23 (Chen and Zhang, 2004). Animal and vegetable blend oil was added to keep lipid and energy contents in all treatments similar. There were 2 dietary phases including starter phase (1 to 3 wk) and grower phase (4 to 5 wk). In the grower phase, all ducks were fed a corn-soybean meal-based diet, which was formulated to meet or exceed nutrient requirements of ducks according to NRC (1994; Table 1), to examine the recovery performance of meat ducks after the withdrawal of CSM-containing diets. All ducks were reared in cages (2.2 × 1.2 × 0.9 m) in a temperature- and humidity-controlled room with a 24 h constant light schedule and had free access to water and feed throughout the experimental period. Feed was supplied in pellet form and the diameter of the pellets was 2 mm in the starter and 3 mm in the grower diet. The Institutional Animal Care and Use Committee of Sichuan Agricultural University approved all procedures used in the study.

Sampling Procedure and Analytical Methods

All test diets were assessed for TG and FG content by HPLC. Free gossypol was immersed and extracted through micropore filter and then analyzed by HPLC. The conditions of HPLC were as follows: the column of chromatogram sepax sapphire C_{18} 5 μm 120 A (250 mm × 4.6 mm), mobile phase acetonitrile/water (1.2% H_3PO_4) = 80/15 (vol/vol), UV-254 nm detector, column temperature 25°C, flow rate 1 mL/min. All test diets were assessed for TG content by HPLC as described by Hron et al. (1990).

At the end of each week during the starter phase, after 12 h feed withdrawal, ducks were weighed and

Table 1. Composition and nutrition level of the basal and experimental diets[1]

Item	Diet 1 (corn-soybean meal diet)	Diet 5 (100% SBM in basal diet replaced by 100% CSM)	Finisher basal diet (%)
Ingredient, %			
Corn	62.32	59.39	71.45
Blend oil[2]	1.09	3.23	1.53
Soybean meal (43% CP)	33.11	0.00	23.27
Cottonseed meal (43% CP)	0.00	33.11	0.00
L-Lysine·HCl	0.09	0.63	0.13
DL-Methionine	0.22	0.26	0.17
Calcium carbonate	0.48	0.72	0.75
Dicalcium phosphate	1.58	1.27	1.03
L-Threonine	0.00	0.24	0.00
L-Tryptophan	0.07	0.11	0.07
Bentonite	0.00	0.00	0.57
Salt	0.35	0.35	0.35
Choline chloride	0.15	0.15	0.15
Vitamin premix[3]	0.03	0.03	0.03
Mineral premix[4]	0.50	0.50	0.50
Antioxidant	0.01	0.01	0.01
Total	100.00	100.00	100.00
Calculate value			
ME, kcal/kg	2,900	2,900	3,000
CP, %	19.5	19.5	16.0
Calcium, %	0.65	0.65	0.6
Available phosphorus, %	0.40	0.40	0.30
Digestible lysine, %	1.01	1.01	0.812
Digestible methionine, %	0.48	0.48	0.39
Digestible threonine, %	0.66	0.66	0.54
Digestible tryptophan, %	0.27	0.27	0.22
Analyzed value, mg/kg of diet			
Total gossypol	0	1,857	—
Free gossypol	0	152.9	—

[1]CSM = cottonseed meal; SBM = soybean meal. Diets 2, 3, and 4 were made by blending diets 1 and 5 at the ration of 3:1, 1:1, and 1:3 to produce diets containing 8.3, 16.6, and 24.8% CSM, respectively.

[2]Blend oil: animal and vegetable blend oil.

[3]Provided per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 1,500 IU; vitamin E, 7.5 IU; thiamine, 0.6 mg; riboflavin, 4.8 mg; pyridoxine, 1.5 mg; vitamin B_{12}, 0.009 mg; calcium pantothenate, 7.5 mg; folate, 0.15 mg; niacin, 20 mg.

[4]Provided per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$), 8 mg; Fe ($FeSO_4 \cdot 7H_2O$), 80 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 90 mg; Mn ($MnSO_4 \cdot H_2O$), 70 mg; Se ($NaSeO_3$), 0.3 mg; I (KI), 0.4 mg.

feed consumption was recorded weekly by pen. While in the grower period, ducks were weighed and feed consumption was recorded by pen at the end of the experiment. Average daily gain, ADFI, and feed-to-gain ratio were calculated. The ducks that were unable to reach the feeders and drinkers due to leg problems were culled. Mortality was recorded daily. One duck with weight closest to the pen average was selected and bled through a jugular vein. The blood samples from the jugular vein were immediately placed on ice, transported to the laboratory within 3 h of collection, and centrifuged at 2,000 × *g* for 15 min in a refrigerated centrifuge at about 10°C. Serum was collected and stored at −20°C until analyzed for the biochemical parameters. Serum alanine aminotransferase (**ALT**) and aspartate aminotransferase (**AST**) activity, total protein (**TP**), globulin, and albumin contents are the important index to evaluate liver function, which were analyzed using Bio-chemistry Analyzer (Yellow Springs Instrument Co. Inc., Yellow Springs, OH). For example, ALT and AST are usually increased in serum when hepatocytes have suffered cellular damage (Turk and Casteel, 1997).

Following this, the 12 selected birds per treatment were euthanized by cervical dislocation, and the left lateral lobe of the liver was dissected and fixed in 10% neutral formalin. The fixed tissues were trimmed and embedded in paraffin. Thin sections (5 µm) were sliced and mounted on a slide, and stained with hematoxylin and eosin for histopathological examination by a pathologist. According to severity of liver steatosis, severity of lesions was scored subjectively as follows: 0 = normal histological structure; 1 = hepatic steatosis was recognizable in a particle of liver cells; 2 = diffuse fatty degeneration was observed in hepatocytes. A pathologist was blinded to treatment when evaluating slides.

Statistical Analysis

The ANOVA, linear and quadratic effects of dietary gossypol concentrations on performance and blood parameters were assessed by GLM procedure of SAS software (SAS Institute Inc., 2006). When dietary treatment was significant ($P < 0.05$), means were compared using LSD procedure of SAS software (SAS Institute Inc., 2006). In the present study, broken-line regression analysis (Robbins et al., 2006) was used to estimate the maximum level for no effects of dietary FG and TG intake by using the nonlinear regression (NLIN) proce-

dure of SAS (SAS Institute Inc., 2006). The broken-line model was $Y = L − U(X − R)$, where $Y = ADG$, $X =$ average daily dietary FG or TG intake (mg/d per bird), $R =$ breakpoint, $L =$ the response at $X = R$, and $U =$ the slope of the curve. In this model, $Y = L$, when $X < R$.

RESULTS

Growth Performance

Mortality and cull rate of ducks showed no significant differences among all treatments. During the whole experimental period, the mortality and cull rate of ducks in diets 1, 2, 3, 4, and 5 were 1.12, 0.56, 0, 1.68, and 2.82%, respectively.

The growth performance of ducks fed graded levels of dietary CSM is reported in Table 2. Increasing dietary level of CSM linearly ($P < 0.05$) and quadratically ($P < 0.05$) reduced BW at d 7 and 14. Increasing levels of CSM resulted a linear and quadratic decrease ($P < 0.05$) in ADG and ADFI during wk 1 and 2. Ducks fed diet 5 had the poorest growth performance ($P < 0.05$) compared with ducks fed diets 1, 2 3, and 4, whereas growth performance did not differ between diets 1, 2, 3, and 4. During d 14 to 21, increasing level of CSM did not affect ($P > 0.05$) growth performance. During the dietary CSM withdrawal phase, ADFI and feed-to-gain ratio increased ($P < 0.05$) linearly and quadratically (Table 2).

Using a one-slope broken-line analysis, the breakpoint (**BP**) for ADG of FG intake on d 1 to 7 occurred at 2.637 mg/d and TG intake occurred at 32.234 mg/d (equation of FG: $Y = 25.1435–2.2747 × Z1$, $P < 0.01$, Figure 1; equation of TG: $Y = 25.1435–0.1619 × Z1$, $P < 0.01$, Figure 2). The corresponding values for d 7 to 14 were 9.618 mg/d (FG) and 92.17 mg/d (TG). Equation of FG: $Y = 59.4807–3.9242 × Z1$, ($P < 0.01$, Figure 3); equation of TG: $Y = 59.4331–0.045 × Z1$ ($P < 0.01$, Figure 4).

Blood Profiles

Blood parameters are presented in Table 3. On d 7, increasing level of CSM linearly increased ($P < 0.05$) serum ALT. On d 14, feeding diets containing graded levels of CSM linearly and quadratically increased ($P < 0.05$) the activity of AST with the diet containing the highest level of CSM resulting in the highest AST activity compared with ducks on diets 1, 2, and 4. Albumin showed a quadratic response (decrease, $P < 0.05$) to increasing dietary gossypol level only on d 21. The levels of TP, globulin, and albumin-to-globulin ratio did not significantly differ among treatments.

Hepatic Histopathology

The score of hepatic histopathology is presented in Table 4, and the corresponding pictures from which hepatic scores were based are shown in Figure 5. The

Table 2. Effect of feeding graded levels of gossypol on growth performance of meat ducks from 1 to 35 d of age

Item	Diet 1[1]	Diet 2[1]	Diet 3[1]	Diet 4[1]	Diet 5[1]	SEM	ANOVA	Linear	Quadratic
BW, g/duck									
d 1	58.3	58.3	58.2	58.2	58.2	0.1	0.91	0.59	0.30
d 7	233[a]	236[a]	236[a]	229[a]	203[b]	3.0	<0.01	<0.01	<0.01
d 14	653[a]	648[a]	658[a]	649[a]	591[b]	9.2	<0.01	<0.01	<0.01
d 21	1,120	1,212	1,213	1,193	1,172	16.3	0.38	0.15	0.13
d 35	2,301	2,255	2,237	2,270	2,217	30.7	0.38	0.12	0.28
ADG, g/d per duck									
Wk 1	25.0[a]	25.4[a]	25.5[a]	24.5[a]	20.7[b]	0.2	<0.01	<0.01	<0.01
Wk 2	59.5[a]	58.9[a]	60.3[a]	60.0[a]	55.4[b]	0.5	<0.01	<0.05	<0.01
Wk 3	77.6	80.5	79.2	77.6	83.0	0.8	0.25	0.25	0.36
Wk 4–5	78.1	74.5	73.1	76.9	74.7	0.7	0.14	0.30	0.21
Wk 1–5	64.1	62.8	62.3	63.2	61.7	0.9	0.12	0.28	0.38
ADFI, g/d per duck									
Wk 1	32.0[a]	32.5[a]	32.6[a]	31.4[a]	26.7[b]	0.5	<0.01	<0.01	<0.01
Wk 2	89.3[a]	90.1[a]	91.4[a]	90.6[a]	82.6[b]	1.4	<0.01	<0.01	<0.01
Wk 3	142	145	146	148	147	2.4	0.53	0.10	0.21
Wk 4–5	188[ab]	185[a]	184[a]	190[ab]	194[b]	2.1	<0.01	<0.05	<0.01
Wk 1–5	125	125	125	128	127	0.6	0.10	0.26	0.40
Feed/gain, g/g									
Wk 1	1.28	1.28	1.28	1.28	1.29	0.01	0.88	0.51	0.57
Wk 2	1.49	1.53	1.52	1.51	1.49	0.02	0.37	0.77	0.22
Wk 3	1.94	1.92	1.93	2.05	1.89	0.05	0.15	0.83	0.67
Wk 4–5	2.40[a]	2.50[ab]	2.51[ab]	2.50[ab]	2.61[b]	0.06	0.13	<0.05	<0.05
Wk 1–5	2.09[a]	2.13[ab]	2.13[ab]	2.15[ab]	2.18[b]	0.02	<0.01	<0.05	<0.08

[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$).

[1]Diet 1 = control group, a basal diet, without free gossypol (FG) and total gossypol (TG); diet 2, contained 465 mg of TG/kg and 35.2 mg of FG/kg; diet 3, contained 929 mg of TG/kg and 77.2 mg of FG/kg; diet 4 contained 1,393 mg of TG/kg and 103.1 mg of FG/kg; diet 5 contained 1,857 mg of TG/kg and 152.9 mg of FG/kg.
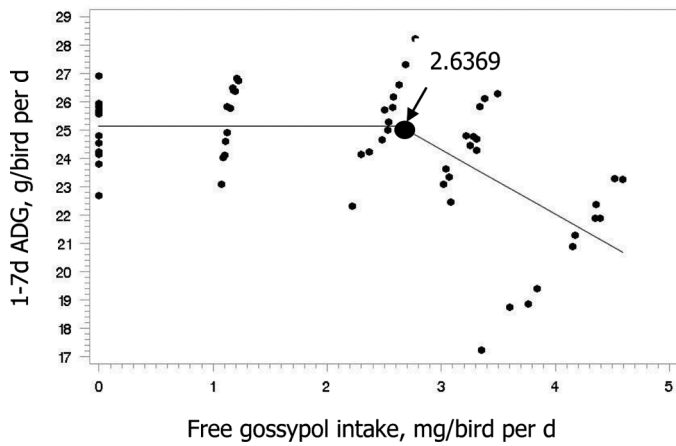
Figure 1. A broken-line analysis of ADG on ducks at 1 to 7 d of age (n = 60) with increasing daily free gossypol intake. A breakpoint (BP) of 1 to 7 d ADG occurred at 2.6369 mg/bird per d of free gossypol intake, showing that the decreasing 1 to 7 d ADG was obtained when free gossypol intake was 2.6369 mg/d ($P < 0.01$, $R^2 = 0.706$). One-slope broken-line model; equation: $Y = 25.1435 - 2.2747Z1$; BP = 2.6369 mg/d (if daily free gossypol intake is <BP, then $Z1 = 0$; if daily free gossypol intake is >BP, then $Z1$ = daily free gossypol intake − BP).
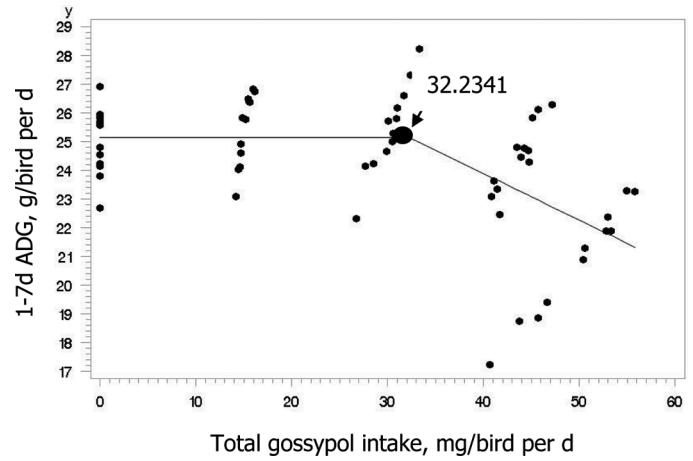

Figure 2. A broken-line analysis of ADG on ducks at 1 to 7 d of age (n = 60) with increasing daily total gossypol intake. A breakpoint (BP) of 1 to 7 d ADG occurred at 32.2341 mg/bird per d of total gossypol intake, showing that the decreasing 1 to 7 d ADG was obtained when total gossypol intake was 32.2341 mg/d ($P < 0.01$, $R^2 = 0.563$). One-slope broken-line model; equation: $Y = 25.1435 - 0.1619Z1$; BP = 32.2341 mg/d (if daily total gossypol intake is <BP, then $Z1 = 0$; if daily free gossypol intake is >BP, then $Z1$ = daily total gossypol intake − BP).

liver cells of birds on diet 1 had small amounts of lipid droplets in the cytoplasm of hepatocytes and all the liver samples had normal histological structure at the end of each week; thus, the total score was 0. The total score increased as the dietary gossypol increased and the length of CSM intake increased. The total scores

for diets 4 and 5 on d 7, 14, and 21 were higher than for birds on the other treatments. On d 21, the score 1 in diet 2 increased. This increase in score is an indication that the dietary gossypol concentration and the length of gossypol intake have an additive toxicity effect on the liver of meat ducks.
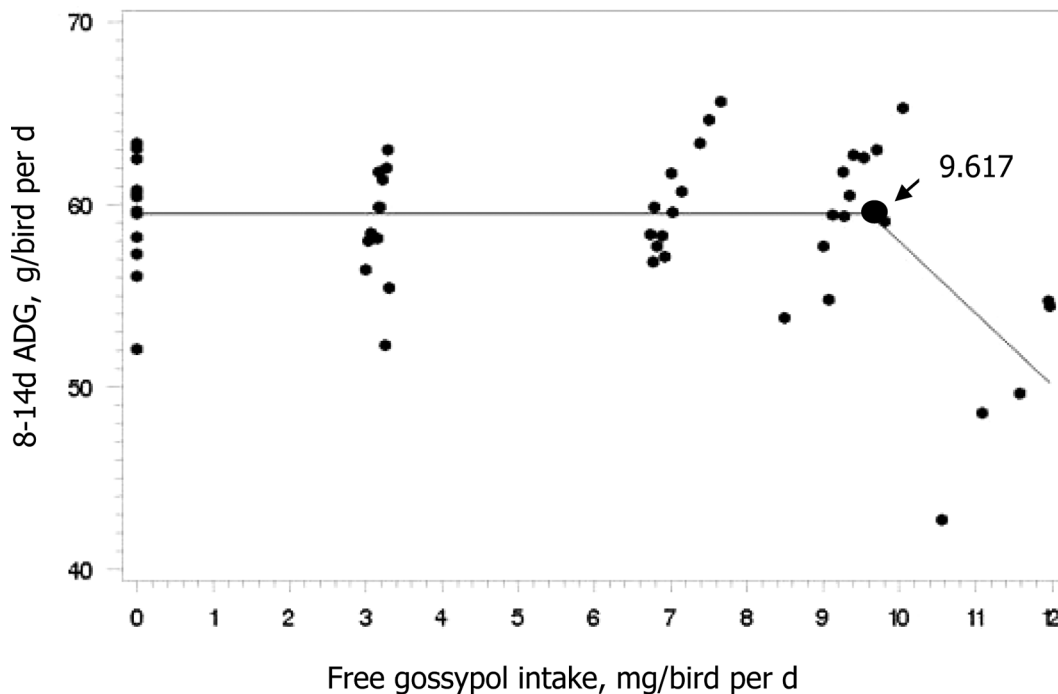

Figure 3. A broken-line analysis of ADG on ducks at 8 to 14 d of age (n = 60) with increasing daily free gossypol intake. A breakpoint (BP) of 8 to 14 d ADG occurred at 9.6175 mg/bird per d of free gossypol intake, showing that the decreasing 8 to 14 d ADG was obtained when free gossypol intake was 9.6175 mg/d ($P < 0.01$, $R^2 = 0.741$). One-slope broken-line model; equation: $Y = 59.4807 - 3.9242Z1$; BP = 9.6175 mg/d (if daily free gossypol intake is <BP, then $Z1 = 0$; if daily free gossypol intake is >BP, then $Z1$ = daily free gossypol intake − BP).
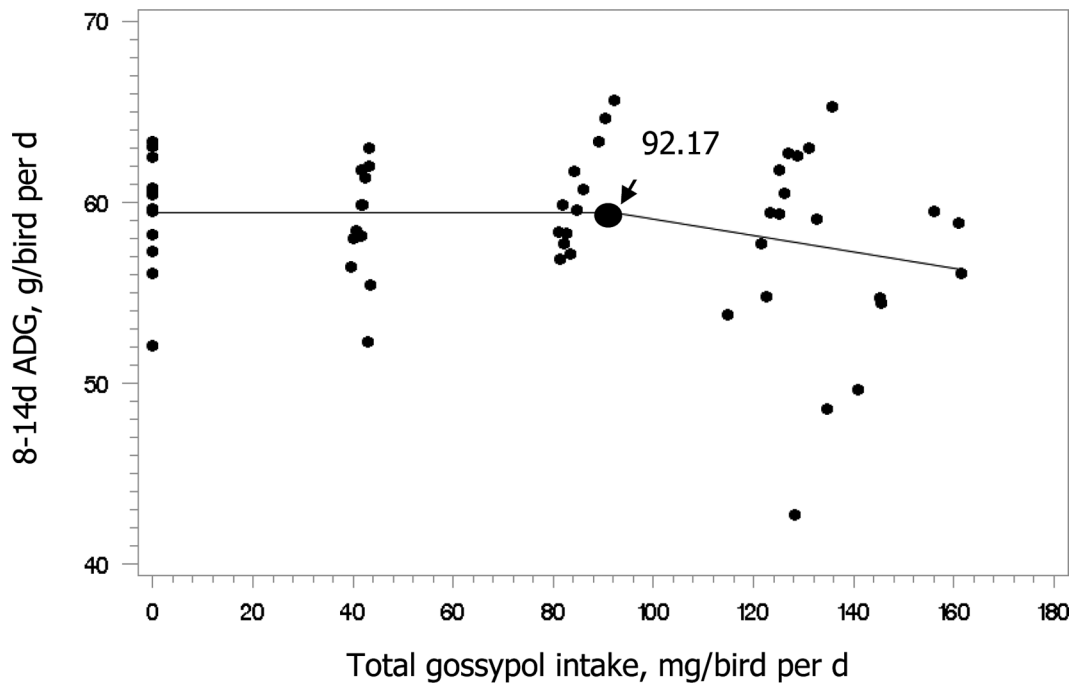
Figure 4. A broken-line analysis of ADG on ducks at 8 to 14 d of age (n = 60) with increasing daily total gossypol intake. A breakpoint (BP) of 7 to 14 d ADG occurred at 92.17 mg/bird per d of total gossypol intake, showing that the decreasing 7 to 14 d ADG was obtained when total gossypol intake was 92.17 mg/d ($P < 0.01$, $R^2 = 0.932$). One-slope broken-line model; equation: $Y = 59.4331 – 0.045Z1$; BP = 92.17 mg/d (if daily total gossypol intake is <BP, then $Z1 = 0$; if daily free gossypol intake is >BP, then $Z1$ = daily total gossypol intake – BP).

Table 3. Effect of feeding graded levels of gossypol on duck serum profiles

Item	Diet 1[1]	Diet 2[1]	Diet 3[1]	Diet 4[1]	Diet 5[1]	SEM	*P*-value ANOVA	Linear	Quadratic
Alanine aminotransferase, U/L									
d 7	50.3[a]	57.3[ab]	56.3[ab]	63.8[b]	60.8[ab]	4.0	0.17	<0.05	0.07
d 14	49.4	47.6	51.1	45.6	53.9	3.5	0.52	0.53	0.57
d 21	42.6	37.8	44.3	49.8	50.6	4.6	0.26	0.06	0.14
d 35	34.8	34.6	35.4	33.9	32.3	2.4	0.92	0.46	0.64
Aspartate aminotransferase, U/L									
d 7	39.7	40.7	47.5	47.1	42.8	4.4	0.18	0.09	<0.05
d 14	40.3[a]	40.6[a]	43.8[ab]	39.3[a]	53.1[b]	3.5	<0.05	<0.04	<0.04
d 21	39.2	46.0	40.8	47.3	46.3	4.5	0.61	0.27	0.53
d 35	57.0[ab]	64.8[a]	57.8[ab]	65.9[a]	53.6[b]	3.3	<0.05	0.61	0.14
Total protein, g/L									
d 7	49.1	51.3	50.6	51.0	50.6	1.8	0.93	0.62	0.75
d 14	46.4	48.1	49.3	47.4	47.2	1.3	0.62	0.64	0.47
d 21	56.9	53.8	54.9	49.0	54.1	4.1	0.74	0.42	0.60
d 35	53.7	54.0	54.1	52.7	52.9	2.2	0.99	0.66	0.89
Albumin, g/L									
d 7	14.3	13.9	12.9	14.0	13.8	1.2	0.94	0.83	0.83
d 14	20.0	21.5	21.8	21.6	22.0	1.5	0.75	0.24	0.43
d 21	23.5[a]	19.5[ab]	16.2[b]	20.0[ab]	20.9[ab]	1.6	<0.04	0.38	<0.02
d 35	15.9	17.7	15.9	16.9	17.3	1.8	0.90	0.74	0.94
Globulin, g/L									
d 7	34.8	37.4	37.7	37.0	36.8	2.0	0.86	0.56	0.57
d 14	26.5	27.5	27.5	25.8	25.2	2.1	0.92	0.53	0.67
d 21	33.3	34.4	41.7	36.9	33.2	3.2	0.32	0.83	0.21
d 35	37.9	36.3	38.6	35.9	35.6	1.9	0.77	0.42	0.68
Albumin to globulin ratio									
d 7	0.41	0.39	0.35	0.40	0.40	0.05	0.91	0.86	0.75
d 14	0.84	0.86	0.90	0.91	1.00	0.12	0.91	0.34	0.62
d 21	0.77	0.73	0.64	0.77	0.80	0.15	0.95	0.85	0.80
d 35	0.44	0.53	0.41	0.49	0.51	0.07	0.74	0.65	0.89

[a,b]Means in the same column with different superscripts are significantly different ($P < 0.05$).

[1]Diet 1 = control group, a basal diet, without free gossypol (FG) and total gossypol (TG); diet 2 contained 465 mg of TG/kg and 35.2 mg of FG/kg; diet 3 contained 929 mg of TG/kg and 77.2 mg of FG/kg; diet 4 contained 1,393 mg of TG/kg and 103.1 mg of FG/kg; diet 5 contained 1,857 mg of TG/kg and 152.9 mg of FG/kg.

Table 4. Effect of feeding graded levels of gossypol on hepatic histopathology in ducks

Item[1]	Score 0[2]	Score 1[2]	Score 2[2]	Total score[2]
d 7				
Diet 1	12	0	0	0
Diet 2	10	2	0	0.17
Diet 3	8	3	1	0.42
Diet 4	5	6	1	0.67
Diet 5	5	2	5	1
d 14				
Diet 1	12	0	0	0
Diet 2	9	3	0	0.25
Diet 3	8	4	0	0.33
Diet 4	4	6	2	0.83
Diet 5	3	2	7	1.33
d 21				
Diet 1	12	0	0	0
Diet 2	5	6	1	0.67
Diet 3	7	2	3	0.67
Diet 4	6	2	4	0.83
Diet 5	2	1	9	1.58

[1]Diet 1 = control group, a basal diet, without free gossypol (FG) and total gossypol (TG); diet 2 contained 465 mg of TG/kg and 35.2 mg of FG/kg; diet 3 contained 929 mg of TG/kg and 77.2 mg of FG/kg; diet 4 contained 1,393 mg of TG/kg and 103.1 mg of FG/kg; diet 5 contained 1,857 mg of TG/kg and 152.9 mg of FG/kg.

[2]Score 0 = normal histological structure; score 1 = hepatic steatosis was recognizable in a particle of liver cells; score 2 = diffuse fatty degeneration was observed in hepatocytes. Total score = (score 0 × the duck number + score 1 × the duck number + score 2 × the duck number)/total duck number; for example, at 7 d of age, total score of diet 5 = (5 × 0 + 2 × 1 + 5 × 2)/12 = 1.
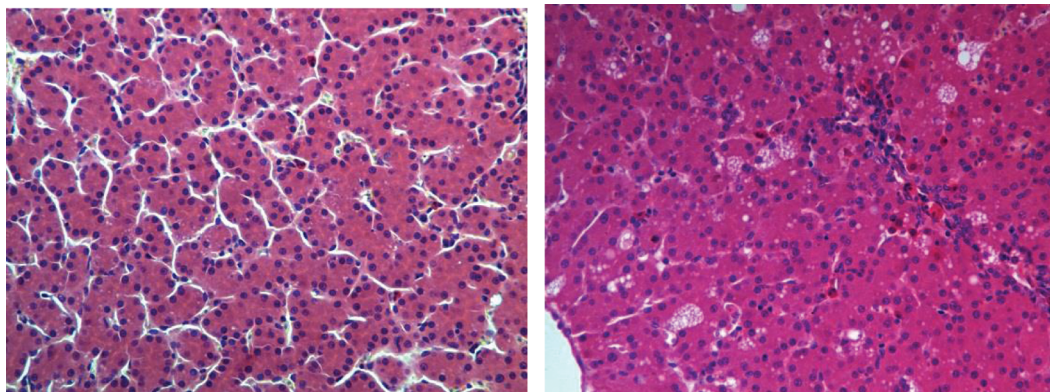
400A, Normal, Score = 0

400B, Score = 1
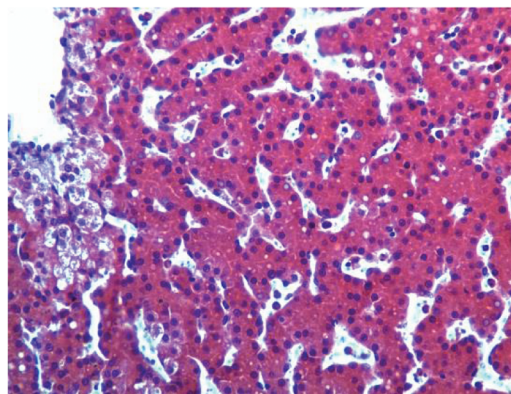
400C, Score = 2

Figure 5. Morphology of duck livers from control and cottonseed meal groups from d 21. 400A: control, normal histological structure (score 0), 400×; 400B: hepatic steatosis was recognizable in a particle of liver cells (score 1), 400×; 400C: diffuse fatty degeneration was observed in hepatocytes (score 2), 400×. Color version available in the online PDF.

DISCUSSION

The current study was designed to evaluate the effects of feeding graded levels of gossypol from CSM on performance and blood biochemical profiles of ducks. Visible signs of gossypol toxicity including dyspnea, anorexia, weakness, and sudden death were not observed in any of the ducks during the experimental period. Mortality and cull rate of ducks did not differ significantly among treatments. In the current study, there was no difference on BW, ADG, and ADFI when dietary FG and TG was under 103 and 1,393 mg/kg, respectively (diet 4) in the first 2 wk, which is in agreement with what Waldroup (1981) reported that dietary FG of up to 100 mg/kg was acceptable to broilers. Similar results were also reported in broilers by Sterling et al. (2002) who reported that at slightly higher dietary protein levels (26%), CSM could replace SBM in broiler grower diets to achieve similar performance. Similarly, Watkins et al. (1993) reported that rations formulated with 30% low gossypol CSM (41% CP) and fed to 21-d-old broilers had no adverse effect on BW gain. However, diet 5 (FG: 152 mg/kg and TG: 1,857 mg/kg) significantly depressed BW, ADG, and ADFI in the first 2 wk of this study. This confirms what has been reported earlier that reduction in BW gain and feed intake are common signs of gossypol toxicosis (Henry et al., 2001; Blevins et al., 2010). Thus, the reduction in ADFI and ADG was a consequence of a high level of gossypol in diet 5.

This report is the first to show the threshold level of gossypol at which ADG is affected. The threshold of daily TG and FG intake based on ADG on d 0 to 7 and 7 to 14 was 32.2 and 2.64 mg/d, and 92.12 and 9.62 mg/d, respectively. If feed intake is 30 g/duck per day, the dietary TG and FG should be lower than 1,047.5 and 87.8 mg/kg, respectively, during d 0 to 7. During d 7 to 14, assuming feed intake is 90 g/duck per day, the dietary TG and FG should be lower than 1,023.6 and 106.9 mg/kg, respectively. However, when dietary TG and FG were 1,857.4 and 152.3 mg/kg, there was no significant effect on growth performance from d 14 to 21. These results indicated that the tolerance of ducks to gossypol is influenced by the age of the bird. Compared with broiler chicks, meat ducks have higher sensitivity to dietary gossypol because several feeding trials have shown that chick performance is not significantly affected when the dietary level of FG is close to 250 mg/kg of feed (Heywang and Kemmerer, 1966; Hermes et al., 1983).

In the current study, during the last 2 wk (d 21–35) for recovery, growth performance of ducks were not different among the 5 treatments, but diet 5 had the highest ADFI. The possible reason was a compensatory growth when the CSM diets were replaced with gossypol-free diets. These results indicated that 14 d of withdrawal from dietary gossypol may be sufficient for blood and tissue gossypol concentrations to return to an almost undetectable level. Mena et al. (2004) re-

ported that after cows receiving the highest TG (1,894 mg/kg) and FG (960 mg/kg) diet for 84 d were fed a gossypol-free diet for 28 d, plasma gossypol concentrations declined sharply in a quadratic manner and were only 10% of the maximal concentrations at 28 d post-treatment (declined from >4.0 to <0.4 µg/mL). Therefore, when determining guidelines for the safe feeding of gossypol containing products to meat ducks to ensure the safety of food and human health, the elimination length of dietary gossypol should be considered.

Aspartate aminotransferase and ALT are the important enzymes to evaluate liver function as their plasma levels reflect the health status of the liver. These increases are usually associated with leakage from the cytoplasm from injured cells or as a result of increased synthesis of gamma glutamyltransferase. In the current study, dietary gossypol concentration linearly increased serum ALT activity on d 7 with diet 4 having the highest value. Serum AST levels in birds on diet 5 was significantly higher than the other groups by d 14. The increase in serum AST and ALT levels with increasing CSM inclusion in the diets is an indication that gossypol from CSM had negatively affected liver functions. This observation is similar to what Blevins et al. (2010) reported. Blevins et al. (2010) reported that the level of gamma glutamyltransferase was elevated in chickens fed diets containing 1,000 mg/kg of gossypol at d 14. The content of TP included albumin and globulin in serum, reflecting synthetic function of the liver. In this experiment, we did not find any significant effect of gossypol on TP, albumin, and globulin. However, the albumin level in the control group was higher than for birds on diet 3 although this difference disappeared 15 d after feed withdrawal. These results are in line with what Mena et al. (2004) reported that dairy diets with up to 960 mg/kg of FG and 1,894 mg/kg of TG had only minor effects on the integrity and functions of hepatic cells as indicated by changes in enzyme activity in the serum.

The liver may be one of the first tissues affected by the ingested dietary gossypol because the liver had the highest concentration of TG (71.4 to 313.6 µg/g of DM) followed by the kidney (9.2 to 36.3 µg/g of DM), plasma (3.0 to 14.6 µg/mL), and muscle (2.1 to 9.8 µg/g of DM) when diets containing 53.8 to 61.3% of TG in CSM were fed to broiler chickens (Gamboa et al., 2001). In the current study, hepatic steatosis became serious with increasing substitution of CSM with birds on diets 2 and 3 having minor damage to their livers. This is indicative of lipid storage and thus liver malfunction (Plaa and Charbonneau, 2008). Lipidosis is reversible and there were no histological indicators of permanent liver damage (Blevins et al., 2010). Thus, it can be hypothesized that the health of the liver would be improved in ducks on diets 2 and 3 if they were switched to a gossypol-free diet. However, if these birds were exposed to gossypol for a long period of time, the degree of damage to the liver could have been worse. Ducks fed diets 4 and 5 had severe lesions on their liv-

ers. These degenerative changes in their livers included fatty degeneration, individual cell necrosis, and cellular infiltration. Cellular infiltration as a result of chronic liver injury could be due to injured mitochondria of hepatocytes because of the presence of gossypol in the diets (Tanphaichitr et al., 1988). There is a similar report showing that chicks fed 400 mg of gossypol/kg had mild perivascular lymphoid aggregate formations and biliary hyperplasia in the liver (Henry et al., 2001). Smith (1957) found that dietary gossypol resulted in the destruction of the parenchyma and hepatocytes in the liver of pigs. Furthermore, many authors have reported degenerative and necrotic effects in testes by administration of gossypol through different routes including oral feeding of CSM in different species including rats (Lin and Rikihisa, 1987; Kalla et al., 1990), hamsters (Srivastava et al., 1989), and rams (Nagalakshmi et al., 2000). Severe cases of perivascular lymphoid aggregate formation, biliary hyperplasia, and hepatic cholestasis were observed in chicks fed 800 and 1,600 mg/kg of pure gossypol in feed (Henry et al., 2001). Henry et al. (2001) also showed that histopathologic changes in liver due to gossypol also occur at levels lower than the levels that affect BW. The same results were observed in diets 4 and 5. Impairments of hepatic functions and metabolism induced by high dietary gossypol concentration could mirror the reduced performance and changes of blood profiles in ducks.

In conclusion, dietary gossypol concentration affected duck growth performance and liver function. From a broken-line analysis, ADG was negatively affected when the ingestion of dietary TG and FG were larger than 32.2 mg of TG/d and 2.6 mg of FG/d from d 1 to 7 and 92.1 mg of TG/d and 9.6 mg of FG/d from d 7 to 14. With increasing dietary TG and FG and with the length of gossypol intake, the degree of liver damage increased. Based on our findings, the dietary TG and FG concentration should be lower than 929 and 77 mg/kg of diet, respectively, during the first 3 wk of age and that a 14 d withdrawal of feed contaminated with gossypol should be considered.

ACKNOWLEDGMENTS

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