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RESEARCH ARTICLE

Effects of Cottonseed Meal on Hematological, Biochemical and Behavioral Alterations in Male Japanese Quail (*Coturnix japonica*)

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ABSTRACT

The present study was carried out to find toxico-pathological effects of cottonseed meal (CSM) in male Japanese quail (C*oturnix japonica*). Male birds (n=48) were equally divided into four groups (A to D). Three isonitric and isocaloric experimental feeds were formulated by replacing soybean meal with three levels of CSM i.e., 13, 27 and 41%. The respective feed was offered to the birds *ad libitum* for the duration of experiment (42 days). Clinical signs, behavioral alterations, feed consumption, body weight, absolute and relative organ weight, hematological and biochemical parameters along with gross and histopathological lesions were studied. In group B and C, birds were temporarily depressed but later on became active. In group D, birds remained dull and depressed and 66.7% mortality was recorded. Body weight, absolute and relative organ weight was non-significantly different in treatment groups compared with control. Feed intake at week 1 was significantly low in group D while during remaining experiment; it differed non-significantly in all the treatment groups compared with control. Testicular volume at day 21 was significantly ($P<0.05$) low in group D. Significantly low serum total proteins and albumin in groups B and C and hematocrit values in all the groups and hemoglobin concentration in group D were recorded at day 42 of experiment. It was concluded that CSM 13% level did not have any deleterious effect on the feed conversion and body weight but the reproductive performance of the male Japanese quail was affected.

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INTRODUCTION

Cotton is a leading crop of Pakistan agricultural industry (Iqbal *et al*., 2010). It not only provides fiber for domestic textile industry but also accounts for about 60% of the vegetable oil and 90% of the high protein meal. Cottonseed meal (CSM), a by-product left after extraction of oil from cottonseeds contains 40-46% crude protein and about 2110 Kcal/Kg metabolizable energy (Mahmood *et al*., 2011). It is cheaper and a good source of protein and abundantly available in Pakistan. CSM in form of cakes is frequently used to feed dairy animals as a protein source but its incorporation in poultry feed is limited due to presence of toxic substances including gossypol, and cyclopropenoid fatty acids. It is also low in lysine and has high fiber contents. It is present in two forms, free and bound. Free gossypol is chemically reactive and toxic whereas bound gossypol is covalently bound to amino acids mainly lysine and is non-toxic. Free gossypol contents of cottonseed and CSM varies from 0.22-2.6% and 0.03-0.29%, respectively (Mujahid *et al.,* 2000). Experimental gossypol toxicity by dietary administration of gossypol containing glands resulted in depressed weight gain, increased feed intake, decreased feed efficiency, and increased mortality in chickens (Smith, 1970; Ilyas *et al*., 2007). Henry *et al.* (2001) documented that oral administration of free gossypol in broilers resulted in enlarged gall bladder, billiary hyperplasia and perivascular mild lymphoid aggregation. Gossypol has also been reported as an anti-fertility agent resulting in arrest of spermatogenesis in different species like rats (Gafvels *et al.,* 1984), hamster (Sarivasta *et al*., 1989) and cock (Mohan *et al*., 1989). However, the pathomorphological changes induced in testes by ingestion of CSM have not been reported in the accessible literature. Keeping in view the paucity of literature about

effects of CSM upon male reproductive system, the present project has been designed to study the pathological effects of feeding different levels of CSM upon the testes, thyroid and adrenals of male Japanese quail (*Coturnix japonica*).

MATERIALS AND METHODS

Experimental design and bird's management

A total of 48 male Japanese quail (*Coturnix japonica*) at 40 days of age apparently free from any clinical aliment were procured from a local farm. After acclimatization of three days, birds were divided into four equal groups, i.e., A to D. Birds were kept in individual wire cages and kept on basal feed had 21% total protein. Three experimental feeds were prepared by replacing soybean meal with CSM at varying level, i.e., 0, 13, 27 and 41% for groups A, B, C and D, respectively. Quail in all the groups were offered respective feed and water *ad libitum*. The duration of the experiment was 42 days. Five birds from each group were killed humanely on day 21 while remaining birds were killed at day 42 of the experiment.

Parameters studied

Clinical signs and behavioral alterations included alertness of birds, response on stimulus, mating behavior (mounting on the pen mates) and foaminess in the faces in all the groups were observed twice a day. Feed intake and mortality of birds from all the groups was recorded daily and body weight weekly.

Blood of each bird was collected with and without anticoagulant for hematological and serum biochemical studies, respectively. Hematological parameters including hematocrit, hemoglobin (Benjamin, 1978) and erythrocytic and leukocytic counts (Natt and Herrick, 1952) were determined. Biochemical parameters including serum total protein and albumin were determined following the methods described by Oser (1976) and Varley *et al*. (1980), respectively. Testes volume was determined by volume displacement technique.

Statistical Analysis

The data thus obtained were analyzed statistically by Analysis of Variance test using a computer software package (MSTAT-C). Different group means were compared by Duncan's multiple range test. The level of the significance was $P \leq 0.05$.

RESULTS

Clinical signs and behavioral alterations

Clinical sings were dose dependent. Mounting and foaminess in droppings was consistent feature in all treatment groups (Table 1). Crowing decreased in dose related manner and minimum was in 41% CSM group.

Bodyweight, feed intake and organ weight

Non-significant difference was observed in body weight of treatment groups compared with control group throughout the experiment (Table 2). Feed intake of the birds in group D (41% CSM) was significantly ($P<0.05$) lower than that of control group A through out the experiment (Table 3). All the remaining groups were non-significantly different from control group. Absolute weight and relative weight of testes, thyroid and adrenals obtained at days 21 and 42 were non-significantly different from control group (Table 3).

Testes Volume

At day 21, the volume of the testes of group D was significantly lower compared with control group A (Table 4). However, volume of testes of groups B and C was non-significantly different from group A. At day 42, there was non-significant difference in testes volume among all treatment groups compared with control group.

Table 1: Clinical signs and behavioral alterations in male Japanese quail (*Coturnix japonica*) fed various levels of cottonseed meal

Parameters	Groups			
	A	B	C	D
Depression	0	+1	+2	+4
Attraction towards feed	+4	+3	+2	+2
Attraction towards water	+4	+3	+3	+2
Mounting upon pen mates	+4	+4	+3	+3
Crowing	+4	+4	+4	+3
Foaminess in droppings	+4	+4	+4	+3

Group A served as control while groups B, C and D were kept on feed containing 13, 27 and 42% cottonseed meal, respectively.

Table 2: Body weight and feed intake of the male Japanese quail fed different levels of cottonseed meal

Parameters/ Groups	Periods (Weeks)					
	1	2	3	4	5	6
Body weight (g)						
A	146.80±20.0	146.00±19.9	154.00±3.6	140.00±21.8	144.00±16.0	154.20±18.4
B	151.00±12.4	143.40±20.0	152.60±13.6	146.20±13.8	159.00±11.7	148.4 ±13.3
C	144.80±17.5	147.00±14.4	156.40±10.6	150.00±9.9	151.40±3.6	151.60±12.4
D	134.40±26.9	137.40±23.6	131.80±27.8	144.00±26.8	156.00± 8.2	142.60±12.0
Feed intake (g/birds/ day)						
A	158.04±22.5a	135.80±19.4	147.06±21.9	149.23±21.2	130.73±18.6	161.57±20.7
B	128.42±18.3ab	135.40±19.4	146.27±20.8	163.87±23.4	154.98±22.1	138.87±19.7
C	124.96±17.8ab	119.63±17.9	134.57±19.4	163.63±23.6	153.62±22.2	136.24±19.5
D	119.95±17.1b	140.14±20.5	145.32±20.7	153.31±21.9	128.00±17.2	125.00±17.4

Group A served as control while groups B, C and D were kept on feed containing 13, 27 and 42 % cottonseed meal, respectively. Values (Mean±SD) of each parameter in each column followed by different letters differ significantly ($P \leq 0.05$).

Table 3: Absolute weight and relative organs weights of male Japanese quail fed different levels of CSM

Experimental	Absolute weight			Relative weight		
Day/Groups	Testes	Adrenal	Thyroid	Testes	Adrenal	Thyroid
Day 21						
A	4.59±1.08	0.013±0.01	0.061±0.05	3.05±0.66	0.011±0.01	0.041±0.05
B	3.38±0.77	0.012±0.001	0.010±0.01	2.18±0.46	0.006±0.001	0.006±0.001
C	3.89±0.45	0.039±0.04	0.007±0.001	2.67±0.26	0.025±0.02	0.003±0.001
D	2.18±0.89	0.015±0.01	0.041±0.03	1.87±0.69	0.014±0.001	0.039±0.03
Day 42						
A	4.53±0.34	0.049±0.02	0.021±0.03	3.17±0.18	0.031±0.02	0.014±0.02
B	4.62±0.42	0.03±0.001	0.004±0.001	3.18±0.49	0.029±0.02	0.005±0.001
C	4.39±0.40	0.068±0.02	0.038±0.01	2.77±0.13	0.038±0.01	0.005±0.001
D	4.34±0.79	0.121±0.17	0.09±0.10	2.77±0.55	0.112±0.18	0.031±0.04

Group A served as control while groups B, C and D were kept on feed containing 13, 27 and 42 % cottonseed meal, respectively. Values (Mean±SD) of each experimental day in each column followed by different letters differ significantly (P≤0.05).

Table 4: Testicular volume of male Japanese quail feed different levels of cottonseed meal (Mean±SD).

Experimental Days	A	B	C	D
21	4.500±1.12a	3.400±0.65a	3.600±0.42a	2.02±.86b
42	4.500±0.50	4.500±0.50	4.300±0.45	4.800±.04

Group A served as control while groups B, C and D were kept on feed containing 13, 27 and 42 % cottonseed meal, respectively. Values (Mean±SD) in each row followed by different letters differ significantly (P≤0.05).

Hematological parameters

Hematocrit levels at day 21 were non-significantly different in all the groups in comparison with group A. However, at day 42, the hematocrit value of group B and D were significantly lower from control while group C differed non-significantly (Table 5). Concentration of hemoglobin on day 21 was non-significantly different among all the groups. However, at day 42, hemoglobin concentration of group D was significantly lower as compared with control, while all the remaining groups differed non-significantly. Erythrocyte as well as leukocyte counts at days 21 and 42 in all the groups were found non-significantly different from control group.

All groups showed non-significant changes of serum total protein as compared with control group at day 21 (Table 6). On day 42, serum total proteins in group B and C were significantly lower than that of groups A and D. However, values in group A were non significant with Group D.

Serum albumin at day 21 remained non-significantly different in all the groups compared with control group A. On day 42, albumin in serum of group B and C was significantly lower than of group A. However, group A was non significant with Group D (Table 6).

Table 5: Hematological parameters in male Japanese quail fed different levels of cottonseed meal

Experimental Days/Groups	Hemato-Crit (%)	Hemo-Globin (g/dl)	Erythro-Cyte (10^6/µL)	Leukocyte (10^3/µL)
Day 21				
A	32.20±1.48	15.06±0.39	4.63±0.22	32.80±2.68
B	30.40±3.58	13.86±1.15	4.67±0.22	30.0±3.16
C	29.80±3.56	14.44±0.78	4.92±0.92	28.40±5.73
D	32.0±1.87	14.22±0.40	4.488±0.35	28.400±4.10
Day 42				
A	48.40±1.67a	16.03±0.45a	4.56±0.22	30.80±2.68
B	42.40±5.22b	14.89±1.97a	4.26±0.64	27.60±5.18
C	43.2±4.55ab	14.89±2.04a	4.55±0.54	27.20±4.82
D	39.0±3.54b	12.21±1.05b	4.49±0.15	30.80±5.40

Group A served as control while groups B, C and D were kept on feed containing 13, 27 and 42 % cottonseed meal, respectively. Values (Mean±SD) of each experimental day in each column followed by different letters differ significantly (P≤0.05).

Table 6: Serum albumin and total protein levels in male Japanese quail fed different levels of cottonseed meal

Groups	Day 21		Day 42	
	Albumin g/dl	Total protein g/dl	Albumin g/dl	Total protein g/dl
A	2.784±0.46	4.874±0.60	2.526±0.25a	4.710±0.35a
B	2.758±0.19	5.436±0.44	2.002±0.09c	4.280±0.11b
C	2.374±0.38	4.808±0.20	2.196±0.25bc	4.226±0.08b
D	2.742±0.38	4.864±0.29	2.402±0.25a	4.664±0.38a

Group A served as control while groups B, C and D were kept on feed containing 13, 27 and 42 % cottonseed meal, respectively. Values (Mean±SD) in each column followed by different letters differ significantly (P≤0.05).

DISCUSSION

Cottonseed meal (CSM) is incorporated in livestock and poultry feeds but its use is limited due to presence of a substance gossypol which is toxic. Apart from the injurious effects upon liver, kidneys and hemopoietic system, gossypol has also been reported as an anti-fertility agent resulting in arrested spermatogenesis in rats (Gafvels *et al*., 1984) and cocks (Mohan *et al*., 1989). Pathological effects of gossypol naturally present in the CSM upon the male reproductive system of avian species have not been extensively studied.

Crowing, cloacal gland secretion (foamy droppings) and mounting on the pen mates were behavioral index of the quail. In present study, the crowing frequency was linearly decreased with the increasing percentage of CSM, while the mounting and frequency of foaminess in dropping remained consistent. Crowning and foaminess in feces along with mounting are the indication of reaching puberty. Decreased mounting and a gradual decrease in semen and fertility index in orally administrated gossypol acetic acid in domestic cocks were also reported by Mohan *et al*. (1989).

In the present study Japanese quail receiving CSM at higher percentage exhibited non significant difference in feed consumption and body weight. Similar results were reported in broiler chicks (Watkins *et al*., 1993; Gamboa

et al., 2001). However feeding of CSM in higher level have been reported to have negative effect on bird performance with reference to weight gain and FCR (Henry *et al.,* 2001; Watkins *et al.,* 2002) which could be due to the presence of an anti-nutritive factor like free gossypol in CSM (Couch *et al.,* 1955; Heywang and Bird, 1954), low lysine level (Anderson and Warnick, 1966) and reduced protein digestibility. Absolute as well as relative weight of thyroid, adrenal and testes were non significantly different from that of the control group. While the volume of testes in quail fed 41% CSM was significantly lower than control which may be due to degenerative changes induced by gossypol in the testes or decrease production of intra-testicular spermatozoa. Similar results have been reported by Ilyas *et al*. (2007), where testes volume was decreased with increase in CSM dose. These findings coincided with the observations of Lin *et al*. (1988) who administered gossypol acetic acid intramuscularly at the rate of 25mg/kg body weight in Japanese quail.

Level of serum total proteins at day 21 was non-significantly different in all the groups compared with control group. While on day 42 in birds receiving 13 and 27 % CSM had significantly lower values as compared to control birds. Hypoproteinemia and hypoalbuminemia observed in CSM fed quail in the present study could be due to reduced synthesis of albumin in the degenerated hepatocytes.

A non significant difference in hematological parameters like hemoglobin, erythrocyte counts and hematocrit levels at higher CSM levels suggested no inhibitory effect of CSM upon the erythropoiesis. Similar findings in broiler chickens fed 20% CSM were observed by Henry *et al.* (2001) who found no difference in the erythrocyte and leukocyte count in the treated and control group. Variations in hematology and chemistry variables were also reported as decreasing in Holstein bulls receiving diets containing.

It could be concluded that high levels of CSM up to 13% did not have any deleterious effect on the feed conversion and body weight but the reproductive performance of the male Japanese quail was affected with the effect of probably gossypol on the spermatogenesis and production of more degenerative changes in the spermatogenesis.

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