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PATHOLOGICAL EFFECTS OF COTTONSEED MEAL WITH AND WITHOUT FERROUS SULPHATE IN MALE JAPANESE QUAILS (*COTURNIX JAPONICA*)

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ABSTRACT

This experiment was designed to study the toxic effects of cottonseed meal in Japanese quails. A total of 84 male Japanese quails (*Coturnix japonica*) of 40 days of age were randomly divided into seven equal groups (A to G). Group A was fed on control diet, while three isonitric and isocaloric experimental feeds prepared by replacing soybean meal with varying proportions of cottonseed meal (CSM) were offered to groups B and C (13% CSM), D and E (27% CSM) and F and G (41% CSM). Feeds of groups C, E and G were also supplemented with 0.065, 0.135 and 0.205% ferrous sulphate, respectively. Duration of experiment was 42 days. Frequency of mounting, crowing and presence of foamy droppings were lower in groups receiving high percentage of CSM. At week 2, body weights of groups D and F were significantly lower, while addition of ferrous sulphate in feed rendered this difference non-significant in groups C, E and G. Relative weights of liver and kidneys were significantly higher in group F compared to control group. A non significant difference was seen in feed intake among all the groups throughout the experiment. At 21 days, serum albumin was significantly lower in groups B, C and E compared to control. At 21 days, significantly lower serum total proteins was observed in groups B, C, D, E and G compared to control. Seminiferous tubules of all CSM fed groups had necrotic cells, characterized by dark and small pyknotic nuclei among round spermatids in some tubules. Liver of groups B, C, D and E had cytoplasmic vacoulation of hepatocytes and newly formed bile ducts. Supplementation of ferrous sulphate in experimental feeds partially ameliorated the effects of cottonseed meal on body weight, testes weight, feed intake and clinical signs.

Key words: Japanese quails, cottonseed meal, ferrous sulphate, pathological effects.

INTRODUCTION

Cottonseeds have long been recognized as a cheap source of vegetable proteins. Cottonseeds and their by-products (oil, cake and hulls) have been used extensively as protein supplement in dairy animals ration to increase fat and milk production. Cottonseeds contain chemically reactive and antinutritional factors including gossypol (Berardi and Goldblatt, 1969), cyclopropenoid fatty acids, malvalic acid and sterculic acid and are deficient in lysine (Altschul *et al*., 1958; Phelps, 1966). Cottonseed meal (CSM) is a byproduct left after extraction of oil from cottonseeds and contains 40-46% crude proteins and about 2110 Kcal/Kg metabolizable energy (ME). However, it has a limited utilization in poultry feed because of the presence of gossypol, low lysine and high fiber contents (Altschul *et al.,* 1958; Phelps, 1966).

Toxic effects of gossypol have extensively been studied in different species including rat, mice (Lin and Rikihisa, 1988)), cattle (Chenoweth *et al*., 1994) sheep, goat (Nagalakshmi *et al*., 2000) and birds (Henry *et al*., 2001; Nadeem, 2001). Several techniques have been used to alleviate the toxicity of free gossypol in poultry ration. These studies have shown that addition of ferrous sulphate to ration containing CSM reduces the adverse effects of gossypol in poultry (Barraza *et al*., 1991; Panigrahi and Morris, 1991; Watkins *et al*., 1993; Smith and Pesti, 1998). However, little information is available about pathological changes induced in different organs in the avian species. Therefore, the present study was designed to investigate the pathological effects of feeding different levels of CSM with and without ferrous sulphate in male Japanese quails (*Coturnix Japonica*).

MATERIALS AND METHODS

Experimental design

A total of 84 male Japanese quails (*Coturnix japonica*) of 40 days of age having average body weight of 150 g and apparently free from any clinical ailment were procured from a commercial quail farm. These birds were kept in metal-wire cages under naturally prevailing climatic conditions in an experimental poultry house. After acclimatized to the experimental environment for three days, they were divided randomly into seven equal groups (A-G) and were randomly allocated experimental feeds. Group A was offered basal feed, while three isonitric and isocaloric experimental feeds prepared by replacing soybean meal with varying proportions of cottonseed meal (CSM) were offered to groups B and C (13% CSM), D and E (27% CSM) and F and G (41% CSM). Feeds of groups C, E and G were also supplemented with 0.065, 0.135 and 0.205% ferrous sulphate, respectively. The duration of the experiment was 42 days.

Parameters studied

Birds in all groups were observed twice daily and subjectively evaluated for clinical signs. Behavioral parameters including alertness, response to stimulus, attraction to the feed, frequency of mounting upon pen mates, foamy material in the droppings and crowing were recorded. Feed intake of birds of all the groups was recorded on daily basis. The birds in each group were weighed weekly. Five birds from each group were slaughtered at day 21 and remaining birds were sacrificed at end of the experiment.

After slaughtering, internal organs were examined for the presence of gross lesions. Testes, liver, kidneys, spleen and heart were weighed and their relative weights as percent of body weight were computed. Testes volume was determined by water displacement technique.

Tissue samples of 3-5 mm thickness from testes, liver and kidneys were fixed in 10% neutral buffered formalin and processed for histopathological studies, using routine paraffin embedding method. Sections of 4-5 µm thick were cut and stained using hematoxylin and eosin and examined under light microscope. Blood was collected from all birds sacrificed in the study. Serum separated from the clotted blood was used for the determination of albumin by dye binding method (Varley *et al.,* 1980) and total proteins by Biurette method (Oser, 1976).

Statistical analysis

The results obtained were subjected to analysis of variance test using completely randomized design. Different group means were compared by Duncan's multiple range test using statistical software (M-STATC) at $p \leq 0.05$.

RESULTS

Clinical signs and mortality

No clinical signs and behavioral alterations were observed in birds of control group (A). They remained active throughout the experimental period. They rushed toward feed and water at the time of serving and frequently mounted upon their pen mates. Crowing and foaminess of droppings was maximum in this group. No bird died during the experiment.

Quails from different treatment groups showed variable clinical signs and behavioral alterations throughout the experimental period compared to control. Response to feed and water was normal. Birds of groups B and C did not show any clinical signs and depression throughout the experiment. Response towards feed was poor in groups D and F. Mounting upon pen mates, crowing and foaminess in the droppings was less frequent in these groups compared with control birds (group A). Most of the birds showed depression from weeks 2 to 6 of the experiment. Response towards feed was the lowest in birds of group F.

In group E, response towards feed was quick compared to groups D, F and G. Mounting upon pen mates, crowing behavior and frequency of foamy droppings did not show any change throughout the experimental period. Birds in group G showed a slower response towards feed in weeks 5 and 6 of the experiment compared to control group (A). Mounting and crowing were normal during the weeks 1-3 but it decreased in the remaining period of the experiment. Foamy droppings remained normal throughout the duration of the experiment. Four (33.3%) birds in group B, 1(8.3%) in group C, 2(16.6%) in group D, 3(25%) in group E, 8(66.6%) in group F and 3(25%) birds in group G died during the experiment.

Feed intake

Feed intake of different groups fed different levels of cotton seed meal with and without ferrous sulphate has been presented in Table 1. Difference among all the groups was non significant throughout the length of experiment.

Body weights, relative organ weights and testis volume

Body weights of all groups of Japanese quails at week 1 differed non significantly from one another (Table 2). In week 2, a significant decrease in body weights was observed in groups D and F compared to control group. In week 3, groups D, F and G showed significantly lower weights than control group. In week 4, group F showed significantly lower body weight compared with control group. In week 5, groups B, D, E, F and G had significantly lower body weights compared with control group. In week 6, only group E had significantly lower body weight than control group.

On days 21 and 42, the difference in relative weight of heart among all the experimental groups was non significant (Table 3), while relative weight of liver was significantly higher in group F compared to control and other treated groups. Relative weight of spleen in all groups showed a non significant difference from control on days 21, while on day 42, groups E, F and G had significantly higher relative weight compared to control. Relative weight of kidneys in group F was significantly higher on day 21 compared to control, while on day 42 differences among all the groups were non significant.

Birds of groups B and F showed significantly lower relative weight of testes compared to control group on day 21, while at day 42 all the groups were non significantly different from control. Volume of testes of Japanese quails of group F was significantly lower compared to control group at day 21 of experiment (Table 4), while at day 42 of experiment, differences among all groups were non significant.

Serum total proteins and albumin

Serum total proteins and albumin contents of different groups of quails are presented in Table 5. Serum total proteins on day 21 were significantly lower in groups B, C, D, E and G compared to control. On day 42, all groups differed non significantly from group A. On day 21, birds of groups B, C and E had significantly lower serum albumin values than group A. On day 42, serum albumin contents of groups C, E and G were significantly lower than control group.

Gross and histopathological lesions

No gross lesions were observed in any organ of any bird. Histopathologically, testes of birds in group A showed a normal histological picture with active spermatogenesis.

Seminiferous tubules of treated groups also showed active spermatogenesis. However, some seminiferous tubules of these groups (B-G) had necrotic cells characterized by pyknotic nuclei among the round spermatids. Seminiferous tubules of birds of groups D, E, F and G also contained necrotic cells with clumped chromatin material among spermatocytes.

Fatty change was observed in the hepatocytes of birds of group D and was more severe in groups E, F and G. Liver parenchyma in these groups exhibited infiltrating aggregates of cells with indistinct cytoplasm. These cells increased in number in the groups from B to G and were accumulated around blood vessels. In group D, biliary hyperplasia was present and was more prominent in groups E, F and G. In groups E, F and G, liver parenchyma had cells with large prominent nuclei and pinkish cytoplasm among the infiltrating cells. The macrophages were present in different areas and newly formed bile ducts in areas of cell infiltration were also seen.

Kidneys of the quails from the control group showed normal histological pattern. Kidneys from other groups showed some areas with tubular epithelial degeneration and necrosis characterized by granulation and vacuolation of cytoplasm and pyknotic nuclei. However, no extensive injury to the kidneys was seen.

Table 1: Feed intake (g/bird/day) of male Japanese quails fed different levels of cottonseed meal with and without $FeSO_4$ (mean ± SD)

Groups	Periods (weeks)					
	1	2	3	4	5	6
A	22.5 ± 2.07	19.4 ± 2.91	20.9 ± 2.51	21.2 ± 6.43	18.6 ± 1.10	20.7 ± 4.10
B	18.3 ± 1.58	19.4 ± 2.68	20.8 ± 1.78	23.4 ± 4.49	22.1 ± 2.72	19.7 ± 1.87
C	22.8 ±.0.67	21.2 ± 1.22	23.5 ± 4.37	20.1 ± 5.46	17.3 ± 1.51	17.4 ± 2.33
D	17.8 ± 1.25	17.9 ± 1.34	19.4 ± 3.05	23.6 ± 2.92	22.2 ± 4.39	19.0 ± 2.22
E	20.5 ± 4.20	20.8 ± 1.56	25.7 ± 4.93	18.3 ± 6.75	15.8 ± 2.76	18.1 ± 1.95
F	17.1 ± 1.95	20.5 ± 2.04	20.7 ± 2.46	21.9 ± 6.57	17.2 ± 4.03	17.4 ± 2.47
G	20.5 ± 2.65	19.4 ± 1.21	22.9 ± 4.05	18.2 ± 2.87	14.2 ± 3.30	16.8 ± 2.41

A, B, C, D, E, F and G represent the inclusion rate of cottonseed meal and ferrous sulphate as 0+0,13+0, 13+0.065, 27+0, 27+0.135, 41+0 and 41+0.205%, respectively.

Table 2: Body weight (g) of male Japanese quails fed different levels of cottonseed meal with and without $FeSo_4$ (mean ± SD)

Groups	Periods (weeks)					
	1	2	3	4	5	6
A	152.4 ± 4.7	157.2 ± 7.3a	156.0 ± 4.8a	148.6 ± 7.0ab	166.0 ± 9.0a	149.0 ± 5.9ab
B	148.6 ± 8.6	147.8 ± 7.5ab	147.0 ± 5.8abc	142.2 ± 7.3bc	148.6 ± 8.8bc	145.2 ± 11.2abc
C	147.0 ± 6.1	149.8 ± 3.4ab	153.4 ± 4.5ab	155.0 ± 3.5a	156.4 ± 4.6ab	154.6 ± 3.8a
D	147.6 ± 5.5	143.4 ± 3.5b	137.6 ± 7.4c	145.8 ± 7.4abc	139.8 ± 14.7cd	142.8 ± 5.9abc
E	147.4 ± 5.2	145.8 ± 8.2ab	156.0 ± 9.6a	141.0 ± 8.5bc	143.4 ±11.4cd	135.2 ± 12.8c
F	139.6 ± 3.6	138.6 ± 6.6b	125.8 ± 24.3d	134.0 ± 22.3c	135.2 ± 2.6d	137.0 ± 7.5bc
G	146.8 ± 5.8	147.4 ± 6.6ab	142.2 ± 10.0bc	153.2 ± 11.0ab	146.8 ± 9.3bcd	141.0 ± 5.4bc

Values in each column followed by different letters are significantly different from one another ($P \leq 0.05$).

Table 3: Relative weight (% of body weight) of different organs in male Japanese quails fed different levels of cottonseed meal with and without $FeSo_4$ (mean ± SD)

Groups	Heart	Liver	Spleen	Kidneys	Testis
21 days					
A	0.852 ± 0.12	2.132 ± 0.86cb	0.078 ± 0.05ab	0.688 ± 0.02bc	3.066 ± 0.66ab
B	0.760 ± 0.06	2.362 ± 0.43bcd	0.092 ± 0.05ab	0.666 ± 0.04bc	2.182 ± 0.46cd
C	0.886 ± 0.04	2.050 ± 0.13d	0.174 ± 0.01ab	0.844 ± 0.09ab	3.582 ± 0.63a
D	1.038 ± 0.46	2.186 ± 0.27bcd	0.062 ± 0.02b	0.566 ± 0.33c	2.682 ± 0.25bc
E	0.936 ± 0.02	2.814 ± 0.20bc	0.138 ± 0.04ab	0.806 ± 0.10ab	3.442 ± 0.58ab
F	0.994 ± 0.10	3.864 ± 0.98a	0.180 ± 0.18a	0.972 ± 0.16a	1.890 ± 0.70d
G	0.998 ± 0.13	2.904 ± 0.25b	0.104 ± 0.05ab	0.756 ± 0.10bc	3.142 ± 0.58ab
42 days					
A	0.938 ± 0.21	1.876 ± 0.30 b	0.080 ± 0.04bc	0.710 ± 0.11	3.202 ± 0.30abc
B	0.800 ± 0.05	2.336 ± 0.36b	0.094 ± 0.03abc	0.742 ±. 09	3.062 ± 0.23abc
C	0.748 ± 0.05	2.130 ± 0.19b	0.120 ± 0.05ab	0.848 ± 0.24	3.044 ± 0.32bc
D	0.812 ± 0.08	2.212 ± 0.19b	0.074 ± 0.02c	0.742 ± 0.02	2.970 ± 0.20bc
E	0.818 ± 0.06	2.334 ± 0.17b	0.130 ± 0.02a	0920 ± 0.08	3.734 ± 0.34a
F	0.894 ± 0.11	3.860 ± 0.45a	0.134 ± 0.04a	0.978 ± 0.23	2.776 ± 0.55c
G	0.832 ± 0.05	2.206 ± 0.14b	0.130 ± 0.06a	0.778 ± 0.08	3.460 ± 0.56abc

Values in each column followed by different letters are significantly different from one another ($P \leq 0.05$).

Table 4: Testes volume in Japanese quails fed different levels of cottonseed meal (mean ± SD)

Groups	Age (days)	
	21	42
A (control)	4.40 ± 0.89abc	4.70 ± 0.45
B (13% CSM)	3.40 ± 065cd	4.40 ± 0.55
C (13% CSM + $FeSo_4$)	5.20 ± 1.04a	4.60 ± 0.55
D (27% CSM)	3.50 ± 0.42 c	4.20 ± 0.27
E (27% CSM + $FeSo_4$)	4.60 ± 0.42ab	5.0 ± 0.50
F (41% CSM)	2.50 ± 1.12d	4.60 ± 0.89
G (41% CSM + $FeSo_4$)	4.40 ± 0.42abc	4.80 ± 0.45

Values in each column followed by different letters are significantly different from one another ($P \leq 0.05$).

DISCUSSION

In quails, crowing and mounting frequency was gradually decreased with the increase in dietary levels of CSM, while the frequency of foamy droppings remained unchanged. Crowning, foamy dropping and mounting are the indications of puberty in male Japanese quails (Adkins–Regan, 1999). Gossypol acetic acid given orally to domestic cock resulted in decreased mounting, gradual decrease in semen quality and fertility index (Mohan *et al*., 1989).

The present study suggested a non significant difference in feed intake of quails kept on different levels of CSM compared to birds of control group given no dietary CSM. The body weight of CSM fed groups also remained non significantly different from control birds. Similar results were reported in broiler chicks fed CSM containing ration formulated on digestible amino acid basis (Gamboa *et al*., 2001). However, feeding of 12 to 20 percent CSM has been reported to induce deleterious effect on weight gain and FCR in broiler chicks (Henry *et al.,* 2001; Nadeem, 2001). These adverse effects of CSM in broiler chicks might be due to the presence of an antinutritive factor like free gossypol, low lysine level and reduced protein digestibility (Yu *et al.,* 1996). A non significant difference in feed intake and body weight in CSM fed quail in the present study might be due to slow growth rate of Japanese quail compared with that of broilers, hence, the deleterious effects of CSM upon body weight might not be statistically elaborated.

Decreased mounting and crowing observed with 27% CSM feeding (group D) were ameliorated by concurrent feeding of ferrous sulphate and CSM (group E), however, no such ameliorative effects could be recorded in birds fed 41% CSM (group G). This observation suggests that the inhibitory effects of CSM/gossypol upon the sexual behavior of male quail could be partially prevented by ferrous sulfate. Degenerative changes in liver comprising fatty change, individual cell necrosis, cellular infiltration and areas of necrosis are suggestive of acute hepatitis. Cellular infiltration and presence of newly forming bile ducts suggest a persistent sub acute or chronic liver injury. Since gossypol is known to induce mitochondrial injury (Tanphaichitr *et al.*, 1988), damage to the liver tissue in the present study could be due to injured mitochondria of hepatocytes because of gossypol present in the CSM.

Table 5: Serum albumin and total proteins (g/dl) in male Japanese quails fed different levels of cottonseed meal (mean ± SD)

Groups	21 days		42 days	
	Total proteins	Albumin	Total proteins	Albumin
A	4.710 ± 0.35a	2.486 ± 0.18a	4.874 ± 0.60ab	2.784 ± 0.46a
B	4.260 ± 0.11b	2.002 ± 0.09d	5.436 ± 0.44a	2.758 ± 0.19a
C	4.254 ± 0.22b	2.140 ± 0.01cd	4.906 ± 0.91ab	2.126 ± 0.47b
D	4.224 ± 0.08b	2.276 ± 0.31abc	4.808 ± 0.20ab	2.374 ± 0.38ab
E	4.302 ± 0.04b	2.168 ± 0.03bcd	4.998 ± 0.62ab	1.860 ± 0.27b
F	4.522 ± 0.45ab	2.402 ± 0.25a	4.864 ± 0.29ab	2.742 ± 0.38a
G	4.196 ± 0.13b	2.394 ± 0.11ab	4.322 ± 0.18b	2.120 ± 0.44b

Values in each column followed by different letters are significantly different from one another ($P \leq 0.05$).

Grossly, no change was seen in testes of the birds fed different levels of CSM. However, testes weight of 13 and 41% CSM fed quails on day 21 was lower than those kept on basal feed. Decrease in testes weight of quail fed 41% CAM was also accompanied by decrease in volume of testes. Histopathologically, testes of CSM fed quail also exhibited necrotic and degenerative changes in the seminiferous tubules. These observations suggest degenerative and retrogressive effects of CSM/gossypol upon the testes in quails. Many authors have reported degenerative and necrotic effects in testes by administration of gossypol through different routes or oral feeding of CSM in different species including rats (Lin and Rikihisa, 1988; Kalla *et al.,* 1990), hamsters (Sarivasta *et al.,* 1989) and rams (Nagalakshmi *et al*., 2000). Morris *et al.* (1986) reported inhibition of LDH-X in testes of rats administered gossypol. Some authors have reported deleterious effects of gossypol acetic acid (25 mg/kg intramuscularly) upon testes of Japanese quails (Young *et* al., 1988). However, no report described the degenerative effects of CSM upon testes of Japanese quail. Administration of ferrous sulphate concurrently with 41% CSM resulted in an improvement in weight and volume of testes at day 21 of the experiment. However, the histological changes induced by CSM remained unaltered, suggesting a partial ameliorative effect of ferrous sulphate upon CSM induced alterations. Ferrous sulphate has been reported as a gossypol detoxifying agent and improved the feed efficiency and body weight gain in broiler birds kept on CSM based feeds (Barraza *et al*.1991; Panigrahi and Plumb, 1996; Watkins *et al*., 1993).

Present study suggested that the administration of CSM in the feed of quails at 27% and higher levels induced pathological changes in the liver and testes. Concurrent feeding of ferrous sulphate only partially ameliorated the toxic effects of cottonseed meal.

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