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

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ABSTRACT

A total of 84 male Japanese quails (*Coturnix japonica*) of 40 days of age were randomly divided into seven groups (A to G) with 12 birds in each group. A corn and soybean meal based feed having 25% proteins was offered to group A (control) as a basal feed. 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 2% lysine. Duration of experiment was 42 days. Frequency of mounting, crowing and presence of foamy droppings were lower in groups having high percentage of CSM. Body weights of CSM fed groups were significantly lower from that of control group till week 5 and dietary supplementation of lysine rendered this difference non significant. Differences in body weights at week 6, however, were non significant between control and treated groups. At week 3, testicular weight of birds in groups B and F were significantly lower than that of control, while lysine supplementation rendered this difference non significant. Seminiferous tubules of all CSM fed groups had necrotic cells characterized by dark and small pyknotic nuclei of round spermatids in some tubules. Liver of groups D, E, F and G had cytoplasmic vacoulation of hepatocytes and newly formed bile ducts. Supplementation of lysine in experimental CSM feeds partially ameliorated the effects of CSM on body weight, testes weight and clinical signs.

Key words: Japanese quails, cottonseed meal, lysine, body weight, feed intake.

INTRODUCTION

Cottonseed meal (CSM) is a by-product left after extraction of oil from the cottonseeds. It contains 40-46% crude protein, is abundantly available in Pakistan and is a cheap source of protein. However, its incorporation in poultry feed is limited due to presence of a toxic substance gossypol, low level of lysine and high fiber content. In the CSM, gossypol is present in free and bound forms. Free gossypol is biologically active and toxic. However, during processing, it binds itself to protein molecules present in the meal and becomes biologically inactive which is non toxic.

Toxic effects of free gossypol in chickens include depressed weight gain, decreased feed efficiency and increased mortality (Smith and Pesti, 1998). It also has adverse effects on the epididymides, testes and the developing germ cells in men (Frick and Danner, 1985). Antispermatogenic effects of gossypol have also been reported in rats (Gafvels *et al.,* 1984) and hamster (Sarivasta *et al*., 1989).

Supplemental lysine is incorporated into the poultry feeds to bind gossypol and eliminate its toxic effects but information in the accessible literature is scanty about the effect of dietary supplementation of lysine upon the antifertility effect of gossypol in CSM. Therefore, the present project was carried out to study the effects of naturally occurring gossypol by feeding different levels of CSM to male Japanese quails (*Coturnix japonica*). Lysine in the feed was added to observe its ameliorating effects upon the CSM-induced disorders in male reproductive system of the Japanese quails.

MATERIAS AND METHODS

Experimental birds and feeds

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. Basal feed used in this experiment was prepared by corn and soybean meal having 25% total proteins. Isocaloric and isonitrogenic experimental feeds were prepared from basal feed by replacing soybean meal with CSM at a rate of 0, 13, 27 and 41% of the feed by weight. All the birds were offered feed and water *ad libitum*.

Experimental design

The experimental birds were divided randomly into seven groups (A-G) with 12 birds in each group. These groups were acclimatized to the experimental environment for three days. Afterwards, these groups were randomly allocated to experimental feeds as shown in Table 1. The duration of the experiment was 42 days. Five birds from each group were slaughtered at day 21 and 42 of experiment.

Table 1: Experimental design

Groups	CSM (%)	Lysine (%)
A (control)	0.0	0.0
B	13.0	0.0
C	13.0	2.0
D	27.0	0.0
E	27.0	2.0
F	41.0	0.0
G	41.0	2.0

Parameters studied

Birds in all groups were observed twice daily and subjectively evaluated for clinical signs. Behavioural parameters including the alertness of the birds, response to stimulus and attraction to the feed, frequency of mounting upon the 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 till the end of experiment. After slaughtering, internal organs were examined for the presence of gross lesions. Testes, liver, kidney spleen and heart were weighed and their relative weight as percent of body weight was computed. 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 thickness were cut and stained using hematoxylin and eosin and examined under light microscope.

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-STAT). The level of the significance was 0.05 or lower ($p \leq 0.05$).

RESULTS

Clinical signs and behavioural alterations

There were no clinical signs and behavioral alterations observed in the control group A. All the birds remained healthy and active throughout the experimental period. They showed response and attraction towards feed at the time of feeding. They were frequently mounting upon their pen mates particularly in the morning. Quails were crowing frequently. Frequency of foamy droppings remained almost the same throughout the experimental period.

Quails of different treatment groups showed a range of clinical signs and behavioural alterations throughout the experimental period. Birds of the groups B and D did not show any clinical signs and deviation in behaviour from those of group A. In group D, some birds showed slight depression during weeks 2 and 3 of the experiment. Attraction to the feed decreased during weeks 1, 2 and 6 of the experiment. Frequency of mounting and crowing and presence of foamy droppings was lesser than that of control group. Birds of the group E did not show any clinical signs and deviation in behaviour from those of group A. Most of the birds in group F showed depression from week 2 to 6 and their response towards feed was lesser compared to groups A, B, C, D and E. Frequency of mounting, crowing and presence of foamy droppings was also lower than all other groups from week 2 to the end of the experiment.

Birds of group G showed a lesser response towards feed during weeks 5 and 6 of the experiment compared to groups A, C and E. Mounting and crowing were normal during weeks 1-3 but it decreased afterwards. Foamy droppings remained normal throughout the duration of the experiment.

Body weight and feed intake

In week 1, a non significant difference was observed in body weights of all groups (Table 2). In week 2, a significant reduction in body weight was observed in group F compared with A. In week 3, birds of groups D and F had significantly lower weights than all other groups. In week 4, weights were significantly lower in group F, while these were significantly higher in group G compared with group A. In week 5, groups B, D and F had significantly lower weights than that of group A. In week 6, all groups were non significantly different from group A. A non significant difference was observed in feed intake between all groups during each week of the experiment till the last week (Table 3).

Relative organ weights

Relative organ weights of quails in different groups are presented in Table 4. Heart showed a non significant difference between all groups on weeks 3 and 6 of experiment. Liver had significantly higher weight in group F than that of other groups on week 3 of the experiment. On week 6, liver weight of all groups differed non significantly from one another. Spleen

Table 2: Body weights (g) of the male Japanese quails fed different levels of cottonseed meal with and without lysine (mean ± SD)

Groups	Periods (weeks)					
	1	2	3	4	5	6
A	152.4 ± 4.7	157.2 ± 7.3ab	156.0 ± 4.8 a	148.6 ± 7.0bc	166.0 ± 9.0a	149.0 ± 5.9ab
B	148.6 ± 8.6	147.8 ± 7.5abc	147.0 ± 5.8abc	142.2 ± 7.3bc	148.6 ± 8.8bc	145.2 ± 11.2ab
C	144.4 ± 3.8	157.8 ± 7.9a	152.8 ± 9.5a	141.8 ± 20.5bcd	159.2 ±16.9ab	147.0 ± 17.3ab
D	147.6 ± 8.7	143.4 ± 3.6bc	137.6 ± 7.4bc	145.8 ± 7.5bcd	139.8 ± 14.7cd	142.8 ± 5.9 b
E	152.0 ±3.6	153.6 ± 6.6ab	157.8 ± 24.3a	153.0 ± 22.3b	155.2 ± 12.6ab	157.0 ± 7.5a
F	139.6 ± 3.6	138.4 ± 6.6c	125.6 ±7.4c	134.0 ± 6.7d	135.2 ± 7.0d	137.8 ± 5.9b
G	152.2 ± 7.7	149.0 ± 4.7abc	158.2 ± 9.1a	167.0 ± 11.0a	162.2 ± 7.4a	147.6 ± 5.2ab

A, B, C, D, E, F and G represent the inclusion rate of cottonseed meal and lysine as 0+0%, 13+0%, 13+2%, 27+0%, 27+2%, 41+0% and 41+2%, respectively.
Values in each column followed by different letters are significantly different ($P \leq 0.05$).

Table 3: Feed intake (g/bird/day) of male Japanese quails fed different levels of cottonseed meal with and without lysine (mean ± SD)

Groups	Periods (weeks)					
	1	2	3	4	5	6
A	22.5 ± 2.0	19.4 ± 2.9	20.9 ± 2.5	21.2 ± 6.4	18.6 ± 1.1	20.1 ± 4.1
B	18.3 ± 1.6	19.4 ± 2.7	20.8 ± 1.8	23.4 ± 4.5	22.1 ± 2.7	19.7 ± 1.8
C	23.5 ± 2.6	21.0 ± 5.2	17.6 ± 2.11	18.7 ± 4.0	17.7 ± 2.7	17.1 ± 2.3
D	17.8 ± 1.2	17.9 ± 1.3	19.4 ± 3.0	23.6 ± 2.9	22.2 ± 4.3	19.0 ± 2.2
E	25.3 ± 5.03	19.1 ± 3.0	20.8 ± 2.6	20.4 ± 2.7	19.3 ± 4.2	21.1 ± 2.2
F	17.1 ± 1.9	20.6 ± 2.0	20.7 ± 2.4	21.9 ± 6.5	17.23 ± 4.0	17.8 ± 2.4
G	22.7 ± 4.9	21.5 ± 5.0	21.3 ± 4.5	19.3 ± 5.5	18.0 ± 2.6	19.9 ± 1.7

A, B, C, D, E, F and G represent the inclusion rate of cottonseed meal and lysine as 0+0%, 13+0%, 13+2%, 27+0%, 27+2%, 41+0% and 41+2%, respectively.

Table 4: Relative weight (% of body weight) of different organs in male Japanese quails fed different levels of cottonseed meal with and without lysine (mean ± SD)

Groups	Heart	Liver	Spleen	Kidney	Testes
Week 3					
A	0.852 ± 0.12	2.132 ± 0.86b	0.078 ± 0.05ab	0.688 ± 0.02b	3.066 ± 0.66ab
B	0.760 ± 0.06	2.362 ± 0.43b	0.092 ± 0.05ab	0.666 ± 0.04b	2.186 ± 0.46cd
C	1.038 ± 0.46	2.12 ± 0.17b	0.055 ± 0.02b	0.696 ± 0.13b	2.964 ± 0.63bc
D	0.839 ± 0.12	2.186 ± 0.27b	0.062 ± 0.02b	0.566 ± 0.33b	2.682 ± 0.25bc
E	0.854 ± 0.13	2.47 ± 0.46b	0.128 ± 0.09ab	0.794 ± 0.2ab	3.678 ± 0.44a
F	0.994 ± 0.10	3.864 ± 0.98a	0.180 ± 0.18a	0.972 ± 0.16a	1.890 ± 0.70d
G	0.821 ± 0.39	2.26 ± 0.37b	0.083 ± 0.03ab	0.762 ± 0.10ab	2.986 ± 0.58ab
Week 6					
A	0.938 ± 0.21	1.876 ± 0.30	0.080 ± 0.04b	0.710 ± 0.11b	3.202 ± 0.30
B	0.800 ± 0.05	2.336 ± 0.36	0.094 ± 0.03b	0.742 ± 0.09b	3.062 ± 0.23
C	0.799 ± 0.07	2.084 ± 0.16	0.093 ± 0.02 b	0.775 ± 0.13b	3.338 ± 0.29
D	0.812 ± 0.08	2.212 ± 0.19	0.074 ± 0.02b	0.742 ± .02b	2.970 ± 0.20
E	0.965 ± 0.11	2.342 ± 0.72	0.093 ± 0.02 b	0.863 ± 0.06ab	3.092 ± 0.83
F	0.894 ± 0.11	3.860 ± 0.45	0.134 ± 0.04 a	0.978 ± 0.23a	2.776 ± 0.25
G	0.839 ± 0.08	2.120 ± 0.16	0.130 ± 0.06 a	0.782 ± 0.09b	3.346 ± 0.27

A, B, C, D, E, F and G represent the inclusion rate of cottonseed meal and lysine as 0+0%, 13+0%, 13+2%, 27+0%, 27+2%, 41+0% and 41+2%, respectively.
Values in each column for week 3 and week 6 followed by different letters are significantly different ($P \leq 0.05$).

weight of all groups differed non significantly in week 3 from group A. On week 6, groups F and G had significantly higher relative weight of spleen than all other groups. A significantly higher weight of kidneys was recorded in groups E and F on week 3 compared with group A. In week 6, group F had significantly higher weight than groups A, B, C, D and G. Testes weights on week 3 was significantly lower in groups B and F, whereas all other groups were non significantly different from group A. On week 6 testes weight of all the groups differed non significantly from one another.

Testes volume

Volume of testes of Japanese quails in different groups (Table 5) on week 3 was significantly higher in group E compared with all other groups. Groups B and F had significantly lower volumes from that of groups A, C and G. On week 6, difference between all the groups was non significant.

Pathology

No appreciable gross lesions were observed in different visceral organs of quails from different groups. Histopathologically, testes of birds in group A exhibited a normal histological picture with active spermatogenesis. Basement membrane of the tubules was lined by spermatogonia, followed by large spermatocytes, round and elongated spermatids. Bundles of immature spermatozoa were present in between rounded spermatids facing toward the basement membrane (Fig. 1). In some cases, spermatozoa were present in the lumen of seminiferous tubules.

Seminiferous tubules of quails from all other groups showed active spermatogenesis. However, some seminiferous tubules of these groups (B-G) had necrotic cells characterized by dark and small pyknotic nuclei of the round spermatids (Fig. 2). Moreover, groups D, E, F and G also contained necrotic cells with clumped chromatin material among spermatocytes.

Hepatocytes in group A had a normal histological picture. Fatty change was present in the hepatocytes of group D and it was more severe in groups E, F and G. Liver parenchyma in these groups had infiltrating aggregates of cells with indistinct cytoplasm (Fig. 3). These cells increased in population in the groups from B to G and were accumulating around blood vessels. In group D, newly formed bile ducts were present in the parenchyma (Fig. 4). These were more prominent in groups E, F and G. In these groups, cells with large prominent nuclei and pinkish cytoplasm were also present among the infiltrating cells.

Kidneys of the quails from the control group (A) showed intact epithelium of tubules. Glomeruli did not show any dilatation or accumulation of material in the Bowman's spaces. Kidneys from other groups also exhibited normal parenchyma along with 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 could be seen in the histological study.

DISCUSSION

In quails, crowing and mounting frequency gradually decreased with the increasing percentage of dietary CSM, while the frequency of foamy droppings remained unchanged. Crowing, foamy droppings and mounting are the indications of puberty in male Japanese quails (Adkins-Regan, 1999). Concurrent administration of lysine with 27% CSM ameliorated the decreased frequency of mounting and crowing observed in quails fed CSM alone. However, no such ameliorative effects of administration of lysine were observed in birds fed 41% CSM. This suggested that lysine partly prevented the adverse effect of CSM/gossypol upon the sexual behaviour of male quails.

The present study at the end of experiment suggested a non significant difference in feed

Table 5: Testes volume (ml) of male Japanese quails fed different levels of cottonseed meal with and without lysine (mean ± SD)

Period (weeks)	Groups						
	A	B	C	D	E	F	G
3	4.40 ± 0.89b	3.40 ± 0.35c	4.00 ± 0.94b	3.60± 0.42bc	5.70 ± 0.76a	2.50 ± 1.12c	4.40 ± 0.82b
6	4.70 ± 0.45	4.40 ± 0.55	4.80 ± 0.76	4.20 ± 0.27	4.10 ± 0.82	4.60 ± 0.89	4.70 ± 0.45

A, B, C, D, E, F and G represent the inclusion rate of cottonseed meal and lysine as 0+0%, 13+0%, 13+2%, 27+0%, 27+2%, 41+0% and 41+2%, respectively.
Values in a row with different letters differ significantly ($P<0.05$).

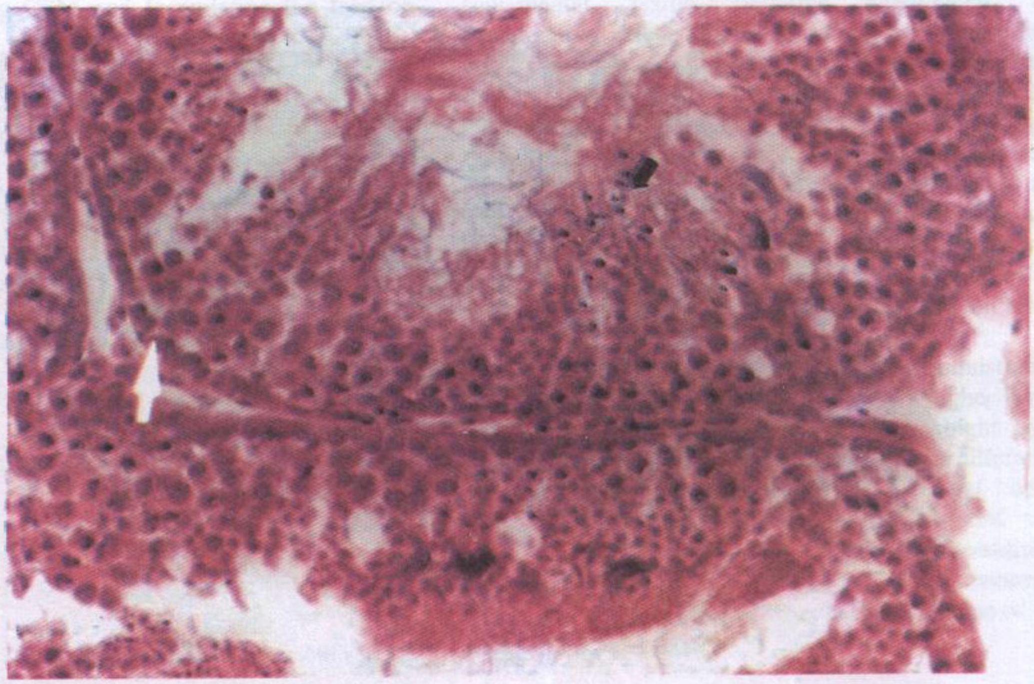

Fig. 1: Photomicrograph of testes of quail from group D showing pyknotic nuclei in the spermatids (arrow, 400X)

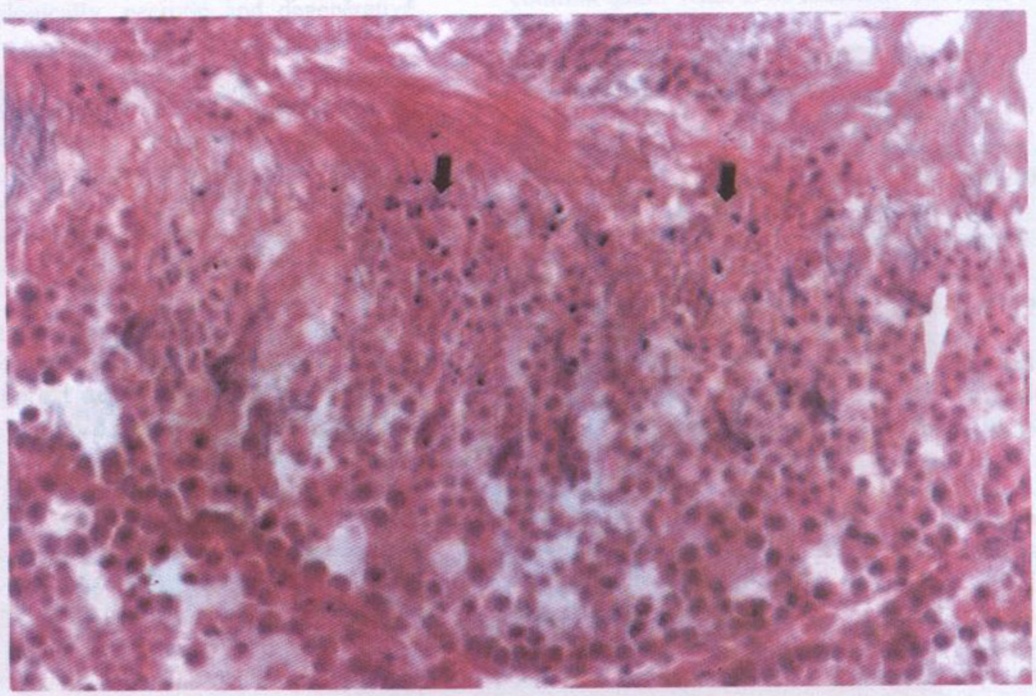

Fig. 2: Photomicrograph of testes of quail from group F showing dark stained clumped chromatin in the spermatocytes (arrow, 400X)

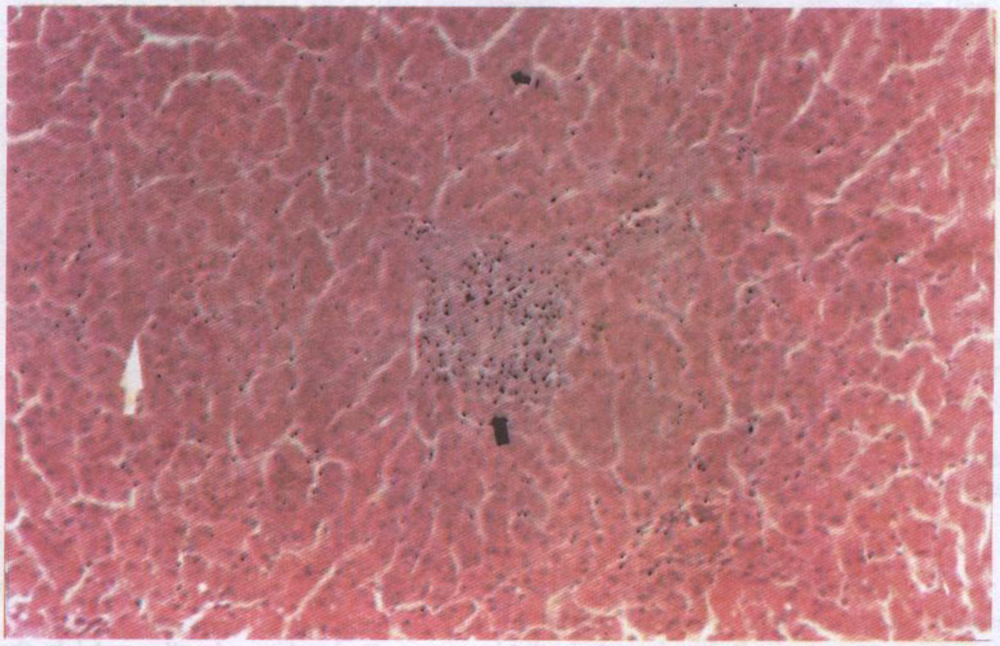

Fig. 3: Photomicrograph of liver of quail from group F showing cellular infiltration (arrow) at portal triad, fatty change, vacuolar degeneration in hepatocytes (200X)

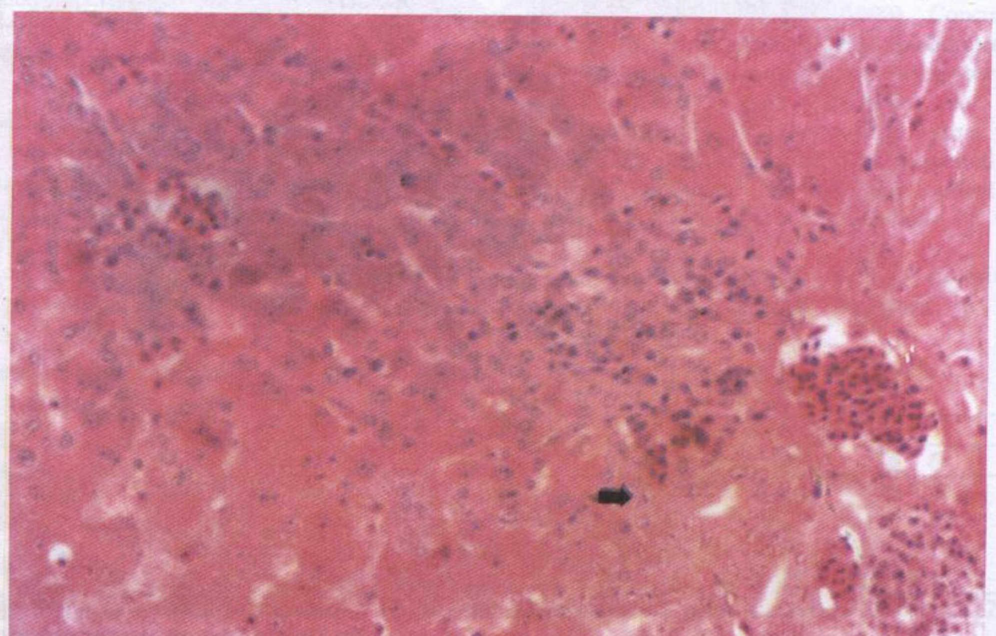

Fig. 4: Photomicrograph of liver of quail from group F showing cellular infiltration (arrow) at portal triad, fatty change, vacuolar degeneration in hepatocytes (400X)

consumption and body weights of quails kept on different levels of CSM than birds of group A given no dietary CSM. Similar results were reported in broiler chicks by feeding CSM and formulating the feed on digestible amino acid basis (Gamboa *et al*., 2001b). However, feeding of higher levels of CSM has been reported to induce deleterious effects on weight gain and FCR in broiler chicks (Henry *et al.,* 2001a; Nadeem, 2001). These adverse effects of CSM might be due to the presence of an antinutritive factor like free gossypol, low lysine level and reduced protein digestibility (Yu *et al.,* 1996). Non significant difference in feed consumption and body weight between control and CSM fed Japanese quail in the present study could be explained on the basis that quails being a slow growing bird had a poor feed efficiency compared with that reported for broiler chicks. With these reasons the inhibitory effect of CSM on body weight could not be appreciated.

Changes in liver e.g. fatty change, cellular infiltration and presence of newly formed bile ducts were suggestive of a moderate inflammatory reaction. There is no report of hepatic injury due to CSM or gossypol. This compound is known to induce mitochondrial injury (Tanphaichitr *et al.,* 1988) and damage to the liver tissues in the present study could be due to injured mitochondria of hepatocytes.

A significant reduction in testes volume was observed in quails offered feed containing 13 and 41% CSM. Histopathologically, necrotic and degenerative changes present in spermatids and spermatocyte layers in the seminiferous tubules suggested degenerative and retrogressive effects of CSM upon the testes in quails. Many authors have reported degenerative and necrotic effects in testes by administration of gossypol through different routes in rats (White *et al.,* 1988), mice (Kalla *et al.,* 1990), hamsters (Sarivasta *et al.,* 1989) and bovines (Brocas *et al*., 1997). In adult male chicken (cock), administration of gossypol acetic acid (40 mg/ kg b wt. daily) through intramuscular route resulted in degenerative effects in testes (Mohan *et al*., 1989). Some authors reported deleterious effects of gossypol acetic acid upon testes of Japanese quails (Lin *et al*., 1988). Degenerative effects in testes have also been produced by feeding CSM to lambs (Nagalakshmi *et al*., 2000) and male teddy goats (Zahid *et al.,* 2002). These changes had been considered due to presence of free gossypol. In view of the pathological effects reported due to free gossypol and CSM in different species, it could be postulated that in the present study pathological changes observed in the testes of quails could also be due to free gossypol contents of CSM present in the feed.

Administration of lysine concurrently with CSM resulted in a significant improvement in weight and volume of testes; this improvement indicated that suplemental lysine partially ameliorated the degenerative effects in testes of quails offered feed containing CSM. However, no amelioration occurred in the histopathological alterations induced by dietary CSM. Lysine has been reported as a gossypol detoxifying agent and its dietary incorporation has been reported to improve the feed efficiency and body weight gain in broiler birds kept on CSM based feeds (Watkins *et al*., 1993; Gamboa *et al*., 2001a; Henry *et al*., 2001b). No report described the detoxifying effect of lysine upon gossypol induced toxic effects on testes in any species.

The present study suggested that the administration of CSM in the feed of quails at 27% and higher levels might have deleterious effect on body weight gain. Liver showed degenerative and chronic proliferative changes following administration of 27% and higher levels of CSM in the feed. Feeding of CSM also decreased the testes weight and volume and resulted in microscopic degenerative lesions in testes. Concurrent feeding of lysine resulted in partial amelioration of the toxic effects of cottonseed meal.

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