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# Lysine Induced Modulation of Toxico-Pathological Effects of Cottonseed Meal in Broiler Breeder Males

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**Abstract.-** Broiler breeder males fed diets containing cottonseed meal (CSM) from 10-30% and lysine (2%) to explore the toxic effects on male reproductive performance. A significant ($P \leq 0.05$) decrease in body weight, comb area and vent size was observed in 20 and 30% CSM fed groups. A decrease in serum testosterone, semen volume and sperm counts occurred with the increase in dietary level of CSM. Testes volume, absolute and relative weights were significantly ($P \leq 0.05$) lower in all the treated groups. Testes of males kept on ration containing up to 20% CSM fed groups did not show any pathological changes. Histologically, testes of 30% CSM fed birds exhibited increased intertubular connective tissue proliferation. In most of the tubules, round spermatids had necrotic nuclei. In some birds, seminiferous tubules had smaller diameter than those of birds of control group. These tubules had 1-2 layers of cells having vesicular nuclei with a nucleolus and fine chromatin. In the present study, all levels of CSM incorporated in diets of broiler breeder males had adverse effects on the reproductive performance. Lysine supplementation in the ration only partially alleviated the deleterious effects of CSM.

**Keywords**: Cottonseed meal, lysine, broiler breeder cocks, semen characteristics, testicular pathology.

## INTRODUCTION

Cottonseed meal (CSM), a by-product obtained after oil extraction from cottonseeds, contains about 222.0 to 560.2 g.Kg$^{-1}$ total proteins and 7.4 to 11.99 MJ.Kg$^{-1}$ metabolizable energy (Nagalakshmi *et al*., 2007). Incorporation of CSM in poultry feed is limited due to presence of gossypol which is a biologically active terpenoid aldehyde mainly present within the puncta or ‘glands’ of cotton seeds. Free gossypol is toxic and chemically reactive. When free gossypol covalently binds to amino acids mainly lysine, it becomes non-toxic and is known as bound gossypol. The availability of lysine in CSM is less because the free gossypol binds with lysine in meal during processing resulting in bound gossypol (Ryan *et al*., 1986). Feeding of pigment glands in chicken led to depressed weight gain, decreased feed efficiency, and increased mortality (Smith, 1970). Gossypol, as an anti-fertility agent has previously been reported in different species including rat (Singh and Rath, 1990), rabbit (Saksena *et al*., 1981) and domestic fowl (Kalla *et al*., 1990). Most of the accessible literature described adverse effects of feeding cottonseeds and CSM on feed efficiency and body weights of broiler chickens. CSM based feeds are frequently supplemented with lysine to bind and inactivate the gossypol (Watkins *et al*., 1993; Begum *et al*., 2010). However, accessible literature provides scant information about toxico-pathologic effects of CSM on chicken’s male reproductive system and its amelioration by lysine. Therefore, this manuscript deals with toxico-pathological effects in broiler breeder males by inclusion of different levels of CSM and modification of these effects by dietary supplementation of lysine.

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## MATERIALS AND METHODS

### *Experimental birds and feeds*

A total of 80 broiler breeder males (Starbro), aged 40 weeks, having similar body weight and apparently free from any clinical ailments, were procured from a local farm. All the birds kept in wire cages at ambient temperature (23 to 26ºC). Daily light/dark cycle was 16/8 hours. Fresh water was available *ad libitum*. After a week of acclimatization, birds randomly divided into 8 equal groups.

CSM had 47.15% total protein, 3.62% fiber and 4.27% ash. The free gossypol content of CSM was 0.075% as determined by A.O.C.S Official Method Ba 7-58 (Anonymous, 1987). A corn, soybean meal having 15% total proteins contents used as basal feed (Khan *et al*., 2010). Four isonitric and isocaloric experimental feeds were prepared by incorporating CSM (obtained from Al-Noor Oil Extraction Plant, Multan, Pakistan) at 0, 10, 20 and 30 % level in the basal feed by replacing soybean meal. Another set of four experimental feeds by adding 2% Lysine along with CSM was prepared. These feeds were designated as A (basal feed, 0 % CSM), AL (0 % CSM + lysine), B (10 % CSM), BL (10 % CSM + lysine), C (20 % CSM), CL (20 % CSM + lysine), D (30 % CSM) and DL (30 % CSM + lysine). Randomly assigned experimental feeds to broiler breeder males continued for a period of 10 weeks.

*Parameters studied*

Birds in each group weekly examined for body weight, comb area and vent length. The comb area of each bird was measured using a planimeter (Takeda, Japan), while the vent length was measured using a vernier caliper. Semen from each rooster collected through abdominal massage (Burrows and Quinn, 1937). Semen volume (ml) and sperm counts of each sample were determined by using hemocytometer (Brillard and McDaniel, 1985).

At the end of the experiment, from each bird blood/serum sample collected and stored at –20°C. Serum testosterone concentration was determined by using ELISA kit (Cat # EIA-1559, DRG® Instruments, GmbH, Germany) according to instructions of the manufacturer.

After humane killing of the birds at the end of experiment, testes removed, weighed and examined for gross lesions. Relative weight of testes as percent of body weight was calculated. Fluid displacement technique (Khan *et al*., 2008) used to determine testes volume. Tissue samples of testes fixed in 10% neutral buffered formalin and processed for histopathological studies following routine paraffin embedding method (Zahoor-ul-Hassan *et al*., 2010).

*Statistical analysis*

Data thus collected for body weight, comb area, vent size, testes volume, serum testosterone and absolute and relative organ weights analyzed by analysis of variance test using completely randomized design using statistical software (M-STAT). Means of different group compared by Duncan's multiple range test. The significance level was $P \leq 0.05$.

## RESULTS

*Free gossypol intake*

The free gossypol contents of feed containing 10, 20 and 30% CSM in this experiment were 75, 150 and 225 mg/kg, respectively (Table I). The average amounts of free gossypol ingested by birds given 10, 20 and 30% CSM based feed were 2.19, 4.56 and 7.17 mg/kg body weight/day, respectively. Birds in groups B and BL ingested the lowest amount of free gossypol while the highest in groups D and DL.

*Physical parameters*

Body weights (Table II) were significantly lower for birds of groups C, D and DL compared to the control (group A) throughout the experimental period. Body weights for birds in groups D and DL were not significantly different from each other but were significantly ($P \leq 0.05$) lower than all other groups on day 28–49. On day 70, the lowest body weight observed in the group D was significantly different from all other groups except group DL.

Comb area (Table III) among all groups was not significantly different on days 7 and 14. On days 21, 42 and 56, all the groups had significantly ($P \leq 0.05$) decreased comb size than control (group A). On day 63, all the groups except AL had significantly lower values than group A. However, on day 70 all the groups except AL and B had significantly lower values than group A.

Vent size (Table III) of birds of all groups on days 7 – 28 differed not significantly from each other. On day 35, group D had significantly ($P \leq 0.05$) lower value compared to group A. On days 42- 49, groups D and DL had significantly lower values than all other groups. On day 56 lowest values was present in birds of group CL followed by groups D and DL, which were significantly lower

**Table I.-** **Free gossypol [mg/bird/day (mg/kg body weight/day)] ingested by mature broiler breeder males fed different levels of dietary CSM with and without lysine.**

| Days | Daily ration (mg)/ bird/ day | Groups* A | AL | B | BL | C | CL | D | DL |
|---|---|---|---|---|---|---|---|---|---|
| 7 | 140 | 0.00 | 0.00 | 10.5 (2.09) | 10.5 (2.10) | 21.0 (4.27) | 21.0 (4.25) | 31.5 (6.43) | 31.5 (6.20) |
| 14 | 145 | 0.00 | 0.00 | 10.87(2.15) | 10.8 (2.15) | 21.6 (4.36) | 21.6 (4.34) | 32.61 (6.99) | 32.61 (6.84) |
| 21 | 150 | 0.00 | 0.00 | 11.25(2.23) | 11.25 (2.23) | 22.5 (4.49) | 22.5 (4.47) | 33.75 (7.19) | 33.75 (7.15) |
| 28 | 155 | 0.00 | 0.00 | 11.62(2.24) | 11.62 (2.28) | 23.2 (4.64) | 23.2 (4.57) | 34.86 (7.56) | 34.86 (7.52) |
| 35 | 160 | 0.00 | 0.00 | 12.0 (2.30) | 12.0 (2.33) | 24.0 (4.82) | 24.0 (4.70) | 36.0 (7.75) | 36.0 (7.95) |
| 42 | 162 | 0.00 | 0.00 | 12.15(2.32) | 12.15 (2.34) | 24.3 (4.87) | 24.3 (4.72) | 36.45(7.95) | 36.45 (8.03) |
| 49 | 164 | 0.00 | 0.00 | 12.3(2.34) | 12.3 (2.37) | 24.6(4.90) | 24.6 (4.76) | 36.9(8.02) | 36.9 (7.98) |
| 56 | 165 | 0.00 | 0.00 | 12.3(2.31) | 12.3 (2.30) | 24.6 (4.88) | 24.6 (4.73) | 36.9 (7.97) | 36.9 (7.89) |
| 63 | 165 | 0.00 | 0.00 | 12.3 (2.29) | 12.3 (2.32) | 24.6 (4.84) | 24.6 (4.71) | 36.92 (7.93) | 36.9 (7.80) |
| 70 | 165 | 0.00 | 0.00 | 12.37(2.28) | 12.37 (2.32) | 24.74 (4.84) | 24.74 (4.70) | 37.12 (7.85) | 37.12 (7.71) |

*Birds in different groups were given feeds containing cottonseed meal (CSM) and lysine as follows: A (basal feed, control) = 0 % CSM, AL = 0 % CSM + lysine, B = 10 % CSM, BL = 10 % CSM + lysine, C= 20 % CSM, CL= 20 % CSM + lysine, D = 30 % CSM, DL = 30 % CSM + lysine.

**Table II.-** **Body weight (g) of adult broiler breeder males fed different dietary levels of cottonseed meal with and without lysine**

| Days | Groups* A | AL | B | BL | C | CL | D | DL |
|---|---|---|---|---|---|---|---|---|
| 7 | 5040±99.3 | 5000±134.1 | 5020±78.2 | 4985±124.7 | 4910±84.9 | 4930±148.1 | 4895±124.3 | 5080±48.9 |
| 14 | 5105±111.6a | 5075±105.7a | 5055±108.1a | 5015±150.1ab | 4950±45.3ab | 4972±184.6ab | 4665±117.1b | 4765±101.6ab |
| 21 | 5163±82.9a | 5125±82.6a | 5110±53.1a | 5031±156.1ab | 5005±41.1ab | 5025±128.9ab | 4690±107.7b | 4715±103.2b |
| 28 | 5230±74.6a | 5170±111.6a | 5180±62.8a | 5090±100.4a | 5000±113.7a | 5071±162.2a | 4610±102.4b | 4630±42.2b |
| 35 | 5279±107.1a | 5210±185.8a | 5197±95.3a | 5140±156.6a | 4977±117.2a | 5103±136.1a | 4640±69.4b | 4525±109.3b |
| 42 | 5311±89.6a | 5251±144.7a | 5230±95.5a | 5172±94.9a | 4985±105.9a | 5147±127.8a | 4580±111.6b | 4535±90.6b |
| 49 | 5363±127.5a | 5295±115.0a | 5250±43.2a | 5185±133.5a | 5015±127.4a | 5167±140.4a | 4597±82.5b | 4622±125.0b |
| 56 | 5418±113.5a | 5337±80.2a | 5310±87.4ab | 5327±134.3ab | 5032±116.4b | 5192±95.3ab | 4625±84.7c | 4675±106.7c |
| 63 | 5450±114.7a | 5425±130.2a | 5370±103.3ab | 5281±104.3ab | 5073±97.5b | 5218±62.2ab | 4653±120.4c | 4730±106.5c |
| 70 | 5480±170.3a | 5472±84.8a | 5415±147.5ab | 5321±147.5ab | 5109±186.1bc | 5253±138.0ab | 4725±87.6 | 4810±60.4cd |

Values (Means±SE) in each row followed by different letters are significantly different (P≤ 0.05). *Birds in different groups were given feeds containing cottonseed meal (CSM) and lysine as follows: A (basal feed, control) = 0 % CSM, AL = 0 % CSM + lysine, B = 10 % CSM, BL = 10 % CSM + lysine, C= 20 % CSM, CL= 20 % CSM + lysine, D = 30 % CSM, DL = 30 % CSM + lysine.

from all other groups. From day 63- 70 groups D and DL were not significantly different from each other but significantly ($P \leq 0.05$) lower from all other groups.

*Semen volume and sperm counts*

Semen volume (Table IV) showed a not significant difference between all groups on day 7. On day 14, groups AL, B, and BL had not significant difference, while other groups had significantly ($P \leq 0.05$) lower values than group A. On day 21, groups AL, B, and CL differed not significantly from group A. On day 28, groups AL and B showed not significant difference while other groups had significantly lower values than group A. On day 35, semen volume of groups AL, B, BL and CL were not significantly different from A group. On day 42, groups D and DL had significantly lower values than A. On day 49, values in all groups except AL and B were significantly ($P \leq 0.05$) lower from group A. On day 56, groups CL, D, and DL yielded significantly lower volume of semen than that of group A. On day 63, groups BL, CL, D, and DL had significantly lower values from that of group A. On day 70, groups CL and D had significantly lower values as compared to group A.

Sperm counts (Table IV) showed not significantly different among all experimental groups on day 7. On day 14, significantly ($P \leq 0.05$) lower values were observed in groups C, D, and DL

**Table III.- Comb and vent area of the male broiler breeders fed different dietary levels of cottonseed meal with and without lysine**

| Days | Groups* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | AL | B | BL | C | CL | D | DL |
| **Comb area ($mm^2$)** | | | | | | | | |
| 7 | 2521±100.6 | 2345±62.1 | 2321±56.6 | 2325±50.6 | 2363±70.3 | 2314±74.2 | 2340±70.0 | 2325±97.0 |
| 14 | 2553±25.6 | 2351±73.2 | 2341±86.2 | 2340±38.5 | 2398±80.7 | 2335±49.2 | 2365±71.3 | 2356±79.6 |
| 21 | 2596±21.1a | 2309±119.6b | 2271±39.5b | 2369±41.7b | 2420±65.0b | 2368±55.5b | 2375±92.4b | 2379±125.2b |
| 28 | 2553±14.0ab | 2389±105.6b | 2342±71.6b | 2389±55.1b | 2640±221.6a | 2390±62.3b | 2396±47.3b | 2390±210.1b |
| 35 | 2597±46.9a | 2397±92.8ab | 2373±77.2b | 2409±55.9ab | 2435±68.9ab | 2420±81.7ab | 2435±75.1ab | 2415±170.4ab |
| 42 | 2556±56.7a | 2441±73.3b | 2402±76.3b | 2405±75.6b | 2453±71.3b | 2466±74.0b | 2421±72.8b | 2421±86.7b |
| 49 | 2660±28.1 | 2502±83.6 | 2468±50.4 | 2454±82.8 | 2489±76.7 | 2496±65.6 | 2469±44.6 | 2449±136.5 |
| 56 | 2736±50.4a | 2518±34.6b | 2535±46.1b | 2489±43.7b | 2508±39.3b | 2521±51.06b | 2464±53.3b | 2449±203.1b |
| 63 | 2801±85.4a | 2638±23.5ab | 2575±31.0bc | 2407±80.0f | 2303±86.5ef | 2539±62.9bcd | 2358±66.5def | 2419±116.9cde |
| 70 | 2830±111.1a | 2677±44.0ab | 2653±29.8ab | 2554±46.2bc | 2556±69.5bc | 2566±36.70bc | 2290±55.9d | 2409±102.5cd |
| **Vent size (cm)** | | | | | | | | |
| 7 | 2.62±0.05 | 2.63±0.13 | 2.65±0.13 | 2.60±0.06 | 2.55±0.11 | 2.62±0.09 | 2.65±0.07 | 2.68±0.09 |
| 14 | 2.65±0.05 | 2.79±0.11 | 2.74±0.06 | 2.68±0.07 | 2.66±0.07 | 2.67±0.09 | 2.65±0.09 | 2.63±0.10 |
| 21 | 2.72±0.10 | 2.80±0.14 | 2.75±0.12 | 2.63±0.06 | 2.77±0.07 | 2.67±0.10 | 2.81±0.06 | 2.60±0.08 |
| 28 | 2.72±0.09ab | 2.83±0.11a | 2.74±0.07ab | 2.71±0.16ab | 2.77±0.07ab | 2.72±0.08ab | 2.50±0.14b | 2.64±0.07ab |
| 35 | 2.82±0.07a | 2.83±0.65a | 2.76±0.08a | 2.76±0.06a | 2.74±0.07a | 2.71±0.07a | 2.41±0.13b | 2.65±0.06ab |
| 42 | 2.80±0.13a | 2.84±0.10a | 2.80±0.08a | 2.77±0.07a | 2.79±0.07a | 2.71±0.07a | 2.44±0.10b | 2.65±0.07b |
| 49 | 2.84±0.10a | 2.80±0.08a | 2.80±0.08a | 2.71±0.06a | 2.80±0.08a | 2.71±0.10a | 2.23±0.06b | 2.37±0.08b |
| 56 | 2.83±0.07a | 2.84±0.12a | 2.84±0.10a | 2.76±0.06a | 2.80±0.11a | 2.73±0.07c | 2.20±0.06b | 2.34±0.09b |
| 63 | 2.85±0.12a | 2.85±0.10a | 2.80±0.08a | 2.81±0.11a | 2.82±0.10a | 2.81±0.08a | 2.14±0.04b | 2.31±0.07b |
| 70 | 2.86±0.09a | 2.85±0.05a | 2.84±0.07a | 2.81±0.10a | 2.82±0.07a | 2.81±0.06a | 2.10±0.03b | 2.25±0.06b |

Values (Means±SE) in each row followed by different letters are significantly different ($P \leq 0.05$). *Birds in different groups were given feeds containing cottonseed meal (CSM) and lysine as follows: A (basal feed, control) = 0 % CSM, AL = 0 % CSM + lysine, B = 10 % CSM, BL = 10 % CSM + lysine, C= 20 % CSM, CL= 20 % CSM + lysine, D = 30 % CSM, DL = 30 % CSM + lysine.

as compared to group A. On day 21, groups CL, D and DL had significantly lower values from group A. On days 28-63, all the groups except AL had significantly lower values from group A. On day 70, groups AL, B, and BL were not significantly different while all other groups had significantly lower values from group A.

Serum testosterone level in all the groups except AL was significantly ($P \leq 0.05$) lower than group A. Birds of group D had lowest level that was followed by group DL that had significantly higher level than group D (Table V).

*Testicular weight, volume and testosterone*

Absolute weights of testes were significantly lower in all groups except AL and B compared to group A. While the relative weights and volume of testes were significantly ($P < 0.05$) lower in all the groups except AL, B and BL compared to group A (Table V). Serum testosterone level in all the groups except AL was significantly ($P < 0.05$) lower than group A.

Birds of group DL followed the lowest level of testosterone in birds of group D. However, lysine adverse effects observed upon all these parameters in males fed diet containing 0 or 10% CSM (Table V).

*Gross and histopathological observations*

Absolute weight of testes was significantly lower in all groups as compared to control (group A) whereas weight of testes of groups AL and B did not show significant difference. Relative weight and testes volume showed significantly ($P \leq 0.05$) lower values in all the groups as compared to control except in groups AL, B and BL (Table V).

Grossly, there was smaller size of testes in CSM fed groups as compared to those of group A (Fig. 1A). Testes of the group A had seminiferous tubules lined by all cells of spermatogenesis including spermatogonia, spermatocytes, spermatids and spermatozoa. Immature spermatozoa in bunches

**Table IV.- Semen characteristics of the male broiler breeders fed different dietary levels of cottonseed meal with and without lysine**

| Days | Groups* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | AL | B | BL | C | CL | D | DL |
| **Semen volume (ml)** | | | | | | | | |
| 7 | 0.32±0.01 | 0.35±0.01 | 0.36±0.02 | 0.36±0.02 | 0.32±0.01 | 0.33±0.03 | 0.35±0.01 | 0.32±0.02 |
| 14 | 0.36±0.02ab | 0.30±0.01bc | 0.31±0.01bc | 0.38±0.02a | 0.21±0.03d | 0.25±0.01cd | 0.29±0.02c | 0.29±0.01c |
| 21 | 0.37±0.02a | 0.38±0.02a | 0.32±0.01abc | 0.29±0.02bcd | 0.24±0.01d | 0.34±0.02ab | 0.30±0.01bcd | 0.26±0.01cd |
| 28 | 0.36±0.02a | 0.32±0.02ab | 0.32±0.02ab | 0.29±0.02bc | 0.27±0.01bcd | 0.25±0.02cd | 0.22±0.02d | 0.27±0.01bcd |
| 35 | 0.35±0.02ab | 0.30±0.007bcd | 0.29±0.02bcd | 0.38±0.05a | 0.23±0.01d | 0.32±0.02abc | 0.27±0.03cd | 0.28±0.01cd |
| 42 | 0.29±0.02a | 0.28±0.01a | 0.30±0.02a | 0.27±0.01ab | 0.24±0.02ab | 0.26±0.01ab | 0.20±0.01 b | 0.20±0.01b |
| 49 | 0.36±0.01a | 0.32±0.08a | 0.32±0.01a | 0.24±0.006b | 0.25±0.01b | 0.24±0.01b | 0.18±0.01c | 0.18±0.01c |
| 56 | 0.28±0.01ab | 0.26±0.02b | 0.33±0.01a | 0.25±0.02b | 0.28±0.009ab | 0.18±0.02c | 0.18±0.01c | 0.18±0.009c |
| 63 | 0.30±0.01a | 0.29±0.01a | 0.28±0.01a | 0.20±0.01b | 0.28±0.01a | 0.17±0.02b | 0.16±0.01b | 0.19±0.009b |
| 70 | 0.26±0.02ab | 0.33±0.01a | 0.27±0.009ab | 0.26±0.01b | 0.24±0.009b | 0.12±0.09c | 0.13±0.004c | 0.21±0.01b |
| **Sperm Count ($x10^6$)** | | | | | | | | |
| 7 | 2.23±0.11ab | 2.19±0.17ab | 1.96±0.13b | 1.95±0.12b | 1.99±0.12b | 2.08±0.15ab | 2.23±0.14ab | 2.43±0.08a |
| 14 | 2.31±0.15a | 2.26±0.16ab | 2.00±0.11abc | 1.98±0.10abc | 1.84±0.08c | 1.84±0.11c | 1.80±0.05c | 1.91±0.21bc |
| 21 | 2.27±0.12ab | 2.30±0.09a | 1.90±0.05bc | 2.04±0.08abc | 1.66±0.09cd | 1.92±0.08abc | 1.43±0.12d | 1.37±0.14d |
| 28 | 2.44±0.14a | 2.12±0.14ab | 2.04±0.22b | 1.49±0.13c | 1.62±0.11c | 1.76±0.15bc | 1.53±0.21c | 1.76±0.13bc |
| 35 | 2.37±0.09a | 2.40±0.07a | 1.41±0.10c | 1.64±0.12bc | 1.74±0.10bc | 1.85±0.14b | 1.82±0.19b | 1.69±0.08bc |
| 42 | 2.37±0.12a | 2.26±0.11a | 1.62±0.10bc | 1.76±0.12b | 1.32±0.11cd | 1.54±0.09bc | 1.56±0.07bc | 1.08±0.20d |
| 49 | 2.41±0.17a | 2.18±0.15ab | 1.96±0.09b | 1.92±0.12b | 1.34±0.12c | 1.40±0.12c | 1.21±0.09c | 1.27±0.18c |
| 56 | 2.49±0.12a | 2.26±0.16ab | 1.97±0.13b | 1.89±0.14b | 1.27±0.12c | 1.29±0.14c | 1.13±0.10c | 1.21±0.14c |
| 63 | 2.27±0.14a | 2.31±0.13a | 1.85±0.03b | 1.81±0.03b | 1.18±0.13c | 0.71±0.14d | 0.88±0.12cd | 0.79±0.12d |
| 70 | 2.19±0.08a | 2.28±0.11a | 2.10±0.09a | 2.14±0.12a | 1.05±0.10c | 0.65±0.05c | 0.63±0.11c | 0.55±0.04c |

Values (Means±SE) in each row followed by different letters are significantly different (P≤ 0.05). *Birds in different groups were given feeds containing cottonseed meal (CSM) and lysine as follows: A (basal feed, control) = 0 % CSM, AL = 0 % CSM + lysine, B = 10 % CSM, BL = 10 % CSM + lysine, C= 20 % CSM, CL= 20 % CSM + lysine, D = 30 % CSM, DL = 30 % CSM + lysine.

**Table V.- Absolute and relative testes weight and volume of adult male broiler breeders fed different dietary levels of cottonseed meal with and without lysine**

| *Groups | Absolute weight of testes (g) | Relative weight of testes (% of body weight) | Testes volume (ml) | Testosterone (ng/ml) |
|---|---|---|---|---|
| A | 41.6±5.55a | 0.81±0.14a | 33.2±1.64a | 1.452±0.03a |
| AL | 37.2±2.89ab | 0.73±0.05ab | 31.9±1.36ab | 1.272±0.07ab |
| B | 36.1±3.66ab | 0.73±0.07ab | 29.8±1.41abc | 1.159±0.10bc |
| BL | 29.0±2.86bc | 0.54±0.07abc | 24.3±1.79abc | 1.131±0.08bc |
| C | 26.7±4.19bc | 0.48±0.06bc | 20.7±0.78cd | 1.006±0.08c |
| CL | 28.7±3.62bc | 0.36±0.09c | 22.1±0.57bcd | 1.063±0.13bc |
| D | 17.6±4.46c | 0.41±0.05c | 12.9±0.73d | 0.363±0.03e |
| DL | 25.6±4.43bc | 0.56±0.09ab | 19.5±1.94cd | 0.753±0.04d |

Values (Means±SE) in each column followed by different letters are significantly different (P≤ 0.05). * Birds in different groups were given feeds containing cottonseed meal (CSM) and lysine as follows: A (basal feed, control) = 0 % CSM, AL = 0 % CSM + lysine, B = 10 % CSM, BL = 10 % CSM + lysine, C= 20 % CSM, CL= 20 % CSM + lysine, D = 30 % CSM, DL = 30 % CSM + lysine.

facing toward the periphery of tubules was present. Lumen of some tubules contained spermatozoa while in some lumens a mixture of spermatocytes, spermatids spermatozoa was present.

Testes of birds fed 10 and 20% CSM with and without lysine (groups B, BL, C and CL) showed similar histological picture to that of control birds (group A). Histologically, two birds in-group D had smaller diameter of seminiferous tubules than those of group A and lined with 1-2 layer of cells having vesicular nuclei with a nucleolus and fine chromatin. In other three males, seminiferous

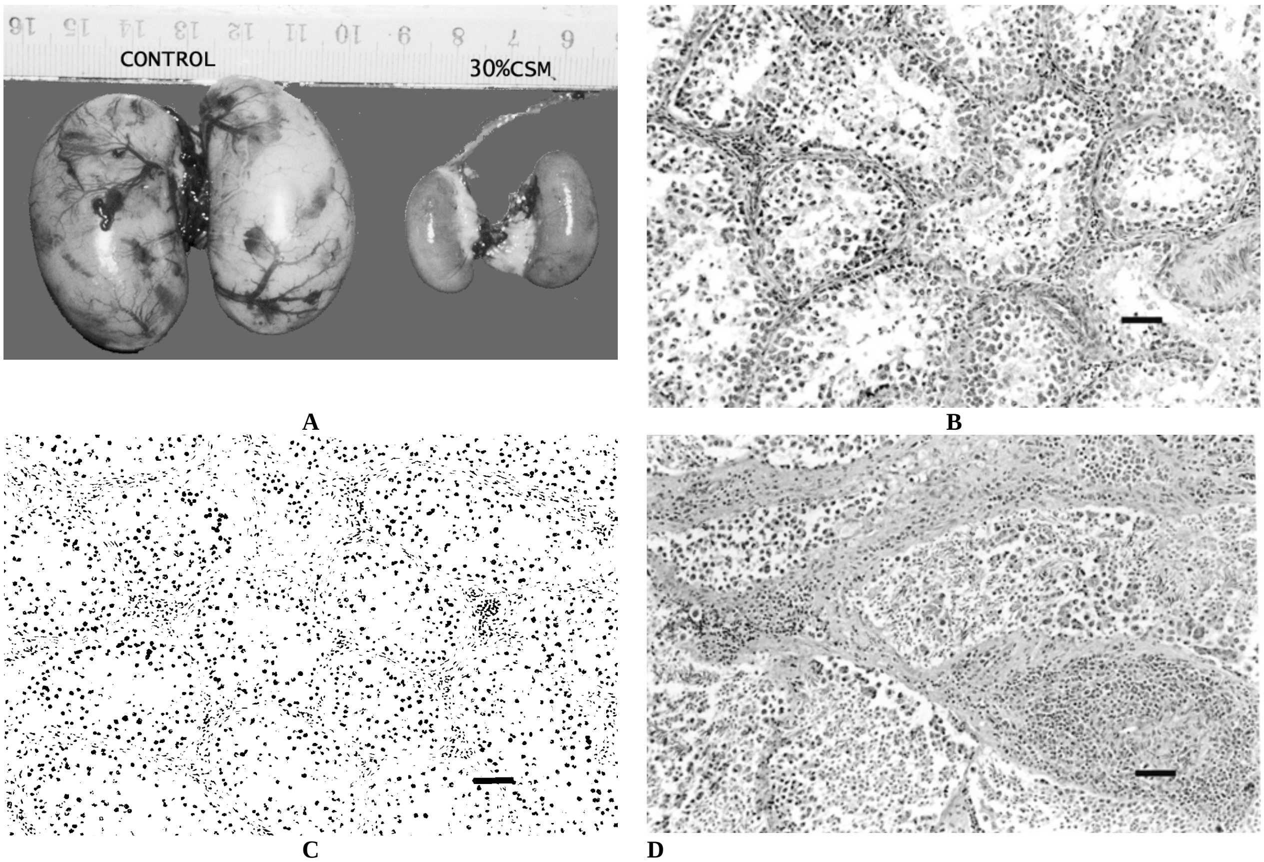


Fig. 1. Gross and histological structure of testes of broiler breeder of male *Gallus domesticus*; A, Testes of group A (control) in comparison with those of group D (30%). A marked decrease in size of testes of group D is evident; B, fed 30 % CSM (group D) showing absence of spermatozoa and condensed nuclei of round spermatids in seminiferous tubules; C, fed 30% CSM based diet showing increased connective tissue in intertubular spaces along with absence of spermatids and young spermatozoa in germinal epithelium; D, fed 30% CSM plus 2% lysine showing distortion of tubular structure and collapsed tubules being replaced by connective tissue and infiltrating cells.

Stain: Haematoxylin and Eosin, Magnification bar in B, C, D = 50 µm.

tubules lined with a mixture of normal and necrotic spermatids and their lumen contained spermatocytes, necrotic spermatids and spermatozoa (Fig. 1B). Increased connective tissue proliferation in the intertubular spaces along with absence of spermatids and young spermatozoa in germinal epithelium were present (Fig. 1C). Remaining five birds of this group showed the presence of all cells of spermatogenesis. Seminiferous tubules of these males, however, contained some spermatids with condensed nuclei.

Testes of birds of group DL receiving 30 % CSM along with addition of 2 % lysine in their diet showed a histological picture similar to that of group D, however, smaller diameter of seminiferous tubules with one or two layers of cells was present in four birds. There was distortion of tubular structure and replacement of collapsed tubules by connective tissue and infiltrating cells (Fig. 1D).

## DISCUSSION

Significantly lower body weights in the present study recorded in birds fed 20 and 30% cottonseed meal ingesting free gossypol 24.74 and 37.12 mg/kg b wt/day, respectively. Based on the gossypol intake, significantly lower body weights recorded in birds ingesting free gossypol ≥ 4.56

mg/kg body weight/day. Similar decrease in weight was observed in broilers fed 20% extruded cottonseed meal (0.044% free gossypol) up to 21 day, however, addition of 2% lysine the body weight gains of chicks were not significantly different from those fed the control diets (Henry *et al*., 2001a). This amelioration effect was not present in birds fed 30% CSM based feed with lysine in present study. Gamboa *et al.* (2001) fed diets containing 28% CSM (free gossypol 92 to 504 µg/g) with the same concentrations of digestible lysine and methionine did not show significant differences in body weights, feed conversions or mortality of birds up to 21 day. Similarly, addition of L-lysine-Hcl in diet containing 23% CSM (free gossypol 224 mg/kg of meal) counteracted the untoward effects of cottonseed on broiler performance (Sterling *et al*., 2002). Watkins *et al*. (1993) reported significantly depressed body weights, feed consumption in birds receiving 30% CSM (0.13 % free gossypol) in their diets however, with the addition of different series of energy levels the body weights, feed consumption and mortality were comparable to the performance of birds receiving the 0% cottonseed meal diets. A longer duration of feeding of gossypol containing rations in the present study (10 weeks) as compared with (3-7 weeks) reported by other authors could be the cause of decreased body weight at lower gossypol levels.

In chicken wide variations in the tolerance levels of dietary gossypol has been reported. In broiler chicks free gossypol at a dietary level of 0.06 % or above in the form as isolated pigment glands resulted in progressive increase in mortality, decreased weight gain rate and feed efficiency, although, addition of 1% lysine improved the growth rate and feed efficiency (Khadiga *et al*., 2009). Significant decreased body weight and feed intake by feeding purified gossypol at 800 and 1600mg/kg of feed in broilers has been reported (Henry *et al*., 2001b).

Comb area and vent size was significantly decreased in males fed 30% CSM feed (7.17 mg/kg body wt/day free gossypol). No report regarding the effects of CSM feeding to male chickens on the sexual behavior and development of secondary sex characters was available in the published literature. However, intramuscular injection of purified gossypol and gossypol acetic acid at 25 mg/kg in male quails showed testicular atrophy and drastic reduction in size of androgen- dependent cloacal gland (Lin *et al*., 1988). Subcutaneous injection of 25.0 mg free gossypol/kg b wt in rats decreased the weight of different secondary sex organs (Gafvels *et al*., 1984).

A decrease in semen volume and sperm counts following feeding of cottonseed flour in rats has also been reported (Herrera *et al*., 1983). Randel *et al*. (1992) reviewed the effect of gossypol and cottonseed products on reproductive parameters and described sperm immobility and depressed sperm counts in males of non-ruminant species.

Testosterone level in male birds has a profound effect on the development of comb and other secondary sex characters Sturkie (1976). The present study showed a decrease in testosterone level of birds given 20% or higher level of CSM feeds. Thus the decrease in comb and vent size in birds given 30% CSM might be related to decrease in the serum testosterone concentrations. Gossypol administration has shown to decrease the testosterone levels in rats (Gafvels *et al*., 1984), rabbits (Saksena *et al*., 1981) and in human (Onyenekwe *et al*., 2003).

Birds fed 20 and 30% CSM (daily free gossypol ingestion 4.56- 7.17 mg/kg b wt) showed a significant decrease in absolute and relative weight and volume of testes along with reduction in semen volume and sperm counts. These changes have direct relation with pathomorphological alterations in testes and low serum testosterone concentration. These results are comparable to the similar changes in domestic fowl induced by administration of purified gossypol (Mohan *et al*., 1989; Kalla *et al*., 1990).

In present study, untoward effect of lysine on testes weight, volume and testosterone concentration at 0 or 10% CSM noted. In the accessible literature, there is no report available on the toxic effects of lysine on testicular parameters in broiler breeder males. In general, lysine supplementation to CSM containing diets is effective in contracting the ill effects of free gossypol in chickens (Nagalakshami *et al*., 2007), however, lysine has also been reported to be toxic Edmonds and Baker (1987). Lysine at 4.61% induced reduction in weight gain (-25%), gain: feed ratio (-14%) and feed intake (-14%) in the treated chicks as compared to control (Carew *et al*., 1998). Contrary to the above findings, addition of lysine 1.1 and 1.2% in feeds resulted in significant body weight gains of broiler chicks (Saima *et al*., 2010).

Histopathological alterations in testes of male chickens given feed containing 30% CSM included increased inter-tubular connective tissue proliferation n, presence of necrotic spermatids in the tubular epithelial layer and absence of spermatozoa. These findings could not be compared as in accessible literature such reports were not present. However, absence of spermatozoa, necrosis of round spermatids, as observed in the present study have been reported with administration of free gossypol in Japanese quails Rikihisa and Lin (1988) and rats (Singh and Rath, 1990; Kalla *et al*., 1990). None of these authors had administered gossypol for more than 24 days whereas in the present study gossypol containing diets fed for ten weeks. Hence, a longer duration of the exposure to dietary gossypol might have been a cause of more extensive necrotic changes of germinal epithelium and increased intertubular connective tissue.

In most cereal based broiler rations, maintenance of adequate lysine level is necessary particularly when cottonseed meal is included in the ration, because the latter is deficient in lysine. The observations of the present study suggested that unlike a significant improvement in the body weight gain reported in the literature (Ilyas *et al*., 2007), dietary addition of lysine in CSM based feeds could only partially ameliorated the toxicological effects of CSM on testes size, weight and volume, semen volume, sperm count and serum testosterone level. However, CSM induced histopathological alterations in testes exhibited little amelioration by dietary supplementation of lysine.

Based on the present study, conclusions could be that all levels of CSM incorporated in diets of broiler breeder males had adverse effects on the reproductive performance. Lysine supplementation in the ration only partially alleviated the deleterious effects of CSM on weight gain, comb area, vent length, semen volume and sperm count, however, toxic effects of lysine upon testes weight, volume and testosterone concentration was observed when administrated in feeds having 0 or 10% CSM.

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## REFERENCES

ANONYMOUS, 1987. *Determination of free gossypol. Official method Ba7-58. Official and tentative methods of analysis*. 3rd Ed., American Oil Chemists Society, Chicago, USA.

BEGUM, I., AZIM, A., AKHTER, S., ANJUM, M.I. AND AFZAL, M., 2010. Mineral dynamics of blood and milk in dairy buffaloes fed on calcium and phosphorus supplementation. *Pak. Vet. J.*, **30**: 105-109.

BRILLARD, J.P. AND McDANIEL, G.R., 1985. The reliability and efficiency of various methods for estimating spermatozoa concentration. *Poult. Sci*., **64**: 155-158.

BURROWS, W.N. AND QUINN, J.P., 1937. A method of obtaining spermatozoa from domestic fowl. *Poult. Sci.,* **14:** 251-254.

CAREW, L.B., EVARTS, K.G. AND ALSTER, F.A., 1998. Growth, feed intake, and plasma thyroid hormone levels in chicks fed dietary excesses of essential amino acids. *Poult. Sci.,* **77:** 295-298.

EDMONDS, M.S. AND BAKER, D.H., 1987. Comparative effects of individual amino acid excesses when added to a corn soybean meal diet: Effects on growth and dietary choice in the chick. *J. Anim. Sci.,* **65:** 699-705.

GAFVELS, M., WANG, J., BERGH, A., DAMBER, J.E. AND SELSTAN, G., 1984. Toxic effects of the antifertility agent gossypol in male rats. *Toxicology,* **32:** 325-333.

GAMBOA, D.A., CALHOUN, M.C., KUHLMANN, S.W., HAQ, A.U. AND BAILEY, C.A., 2001. Use of expander cottonseed meal in broiler diets formulated on a digestible amino acid basis. *Poult. Sci.*, **80:** 789-794.

HENRY, M.H., PESTI, G.M., BAKALLI, R., LEE, J., TOLEDO, R.T., EITENMILLER, R.R. AND PHILLIPS, R.D., 2001a. The performance of broiler chicks fed diets containing extruded cottonseed meal supplemented with lysine. *Poult. Sci.*, **80:** 762-728.

HENRY, M.H., PESTI, G.M. AND BROWN, T.P., 2001b. Pathology and histopatholgy of gossypol toxicity in broiler chicks. *Avian Dis.,* **45:** 598-604.

HERRERA, J.I., MONTALVO, G., GONZALEZ-GARZA, M.T., SOTELO, A. AND BERMÚDEZ, J.A., 1983. Cottonseed flour effects on androgen testicular content and serum levels in rats. *Arch. Androl*., **11:** 161-165.

ILYAS, M., SALEEMI, M.K., MAHMOOD, F. AND KHAN, M.Z., 2007. Pathological effects of feeding cottonseed meal with and without lysine in male Japanese quails (*Coturnix japonica). Pak. Vet. J.*, **27:** 55-62.

KHADIGA, A., ATI, A., MOHAMMED, S., SAAD1, A. M. AND MOHAMED H. E., 2009. Response of broiler chicks to dietary monosodium glutamate. *Pak. Vet. J*., **29:** 165-168.

KALLA, N.R., CHADHA, V.K. AND FOO, J., 1990. Effect of gossypol on domestic fowl, gallus domesticus. *Acta Eur. Fertil*., **21:** 21-24.

KHAN, A., ULLAH, M. AND KHAN, M.Z., 2008. Pathological effects of sodium hypochlorite administration through drinking water in male Japanese quails (*Coturnix japonica*). *Hum. exp. Toxicol*., **27:** 773-780.

KHAN, R.U., DURRANI, F.R., CHAND, N. AND ANWAR, H., 2010. Influence of feed supplementation with *Cannabis sativa* on quality of broilers carcass. *Pak. Vet. J.,* **30:** 34-38.

LIN, Y.C., DIETRICK, T., RIKIHISA, Y. AND BEANE, W.L., 1988. Antifertility effect of gossypol in male Japanese quail. *Life Sci.,* **42:** 2283-2292.

MOHAN, J., PANDA, J.N., SINGH, U.S. AND MOUDGAL, R.P., 1989. Studies on antifertility effects of gossypol acetic acid in domestic cocks. *J. Reprod. Fert.,* **85:**73-78.

NAGALAKSHMI, D., RAO, S.V.R., PANDA, A.K. AND SASTRY, V.R.B., 2007. Cottonseed meal in poultry diets. A review. *J. Poult. Sci*., **44:** 119-134.

ONYENEKWE, C.C., MELUDU, S.C. AND NNAMMAH, N.K., 2003. Sex hormones and biochemical profiles of male gossypol users in South Western Nigeria. *Nigerian J. Phys. Sci.,* **18:** 52-58.

RANDEL, R.D., CHASE, C.C. AND WYSE, S.J., 1992. Effects of gossypol and cottonseed products on reproduction of mammals. *J. Anim. Sci*., **70:** 1628-1638.

RIKIHISA, Y. AND LIN, Y.C., 1988. Ultrastructure of the testis and the epididymis of the Japanese quail (*Coturnix coturnix*) administered gossypol. *Poult. Sci.,* **67:** 961-972.

RYAN, J.R., KRATZER, F.H., GRACE, C.R. AND VOHRA, P., 1986. Glandless cottonseed meal for laying and breeding hens and broiler chicks. *Poult. Sci.,* **65:** 949-955.

SAIMA, KHAN, M.Z.U., JABBAR, M.A., MEHMUD, A., ABBAS, M.M. AND MAHMOOD, A., 2010. Effect of lysine supplementation in low protein diets on the performance of growing broilers. *Pak. Vet. J.,* **30:** 17-20.

SAKSENA, S.K., SALMONSEN, R., LAU, I.F. AND CHANG, M.C., 1981. Gossypol: Its toxicological and endocrinological effects in male rabbits. *Contraception*, **24:** 203-214.

SINGH, S.K. AND RATH, S.K., 1990. Histologic changes in the mouse testis after treatment with gossypol tetra-acetic acid. *Arch. Histol. Cytol*., **53:** 393-396.

SMITH, K.J., 1970. Practical significance of gossypol in feed formulation. *JAOCS,* **47:** 448-450.

STERLING, K.G., COSTA, E.F., HENRY, M.H., PESTI, G.M. AND BAKALLI, R.I., 2002. Responses of broiler chickens to cottonseed- and soybean meal-based diets at several protein levels. *Poult. Sci*., **81:** 217-226.

STURKIE, P.D., 1976. *Avian physiology*. 3rd Ed. Springer Verlag, New York, USA., p: 342-344.

WATKINS, S.E., SKINNER, J.T., ADAMS, M.H. AND WALDROUP, P.W., 1993. An evaluation of low-gossypol cottonseed meal in diets for broiler chickens. 1. Effect of cottonseed meal level and lysine supplementation. *J. appl. Poult. Res*., **2:** 221-226.

ZAHOOR-UL-HASSAN, KHAN, M.Z., KHAN, A. AND JAVED, I., 2010. Pathological responses of White Leghorn breeder hens kept on ochratoxin A contaminated feed. *Pak. Vet. J.,* **30:** 118-123.

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