Antifertility Effect of *Neem* (*Azadrichta indica*) Seed Kernel Meal in Chickens

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ABSTRACT : The reproductive performance of forty two male broilers divided into three similar groups and fed on isocaloric and isonitrogneous diets containing 0, 10 or 20% water washed neem seed kernel meal (WWNSKM) was investigated from 20 to 32-wks of age. Results on semen characteristics revealed that feeding of WWNSKM led to significant ($p < 0.05$) reduction in semen volume, sperm concentration associated with increased incidences of morphological abnormalities in the spermatozoa when compared to that of the control birds. A drastic reduction in the fertilizing ability of spermatozoa was observed, the adverse effects being more at higher inclusion level of the cake. Hatchability of eggs also declined in the WWNSKM fed groups. Histological examination of testes revealed a higher number of degenerating cells and poor spermatogenesis alongwith multinucleated giant cells in the seminiferous tubules of the testes of birds receiving the high dose of WWNSKM in diet. It may be concluded that the feeding of WWNSKM by incorporating in isocaloric and isonitrogneous diets to cockerels is associated with adverse effect on their fertility.
(**Key words**: *Neem* Seed, Chicken, Semen, Fertilizing Ability, Histological Examination)

INTRODUCTION

The versatile medicinal properties of *neem* (*Azadrichta indica*, family meliaceae) tree have been well documented in Indian and Unani system of medicine since antiquity. Recently, the anti-HIV effect of *neem* extract has been investigated (Upadhyay et al., 1993). An enzyme which inhibits division of the AIDS virus-infected cells has also been isolated from *neem* (Anonymous, 1994). Considering the enormous benefits of *neem* in controlling cancer, pregnancy and sexually transmitted diseases, it is possible that the modern medical problems may just find solutions in such plant products (Anonymous, 1995).

During the last decade, however, attention is focussed on the involvement on *neem* formulations in regulation of fertility (Lal et al., 1986; Riar et al., 1990). Ingestion of neem constituents have been reported to affect spermatogenesis adversely in rats (Vijjan and Parihar, 1983) and pigs (Agarwal and Sastry, 1985). *Neem* oil has been found to possess strong spermicidal property against the rhesus monkey and human spermatozoa (Riar et al., 1990; Riar et al., 1993). Intrauterine administration of a purified extract from *neem* seeds was found to cause long-term blockage of fertility in redents and monkeys (Garg et al., 1993).

No literature pertaining to the effects of *neem* formulations on fertility in avian species appears to be available. Further the chicken is considered as an experimental model for such a study because bird is a short living creature and the fertility experiments are conducted with relative ease as compared to other livestock. In view of above, the present studied were, therefore, carried out to examine the effect of feeding water washed *neem* seed kernel meal (WWNSKM) on the reproductive performance of male chickens. This study was conducted with water washed *neem* seed kernel meal since, the raw *neem* seed kernel meal had been found unpalatable and rich of toxic principles (Gupta et al., 1975; Sadagopan et al., 1982) and that the washed meal was relatively superior in its feeding values (Nath et al., 1983).

MATERIALS AND METHODS

Forty-two, 12 week old synthetic male broiler chicken from the same hatch and breed were divided equally into three groups. The birds were housed in individual battery cages. The first group was fed a standard breeder ration for the next 20 weeks and served as the control. The

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second and third groups of birds were simultaneously fed diets containing 10 (low dose) or 20% (high dose) WWNSKM (w/w) for groundnut cake in the control rations. All the three diets so prepared were isocaloric and isonitrogneous (table 1). Water washed *neem* seed kernel meal was prepared as per the procedure followed by Rajagopal and Nath (1983). The experiment was continued until the response of WWNSKM was stabilized (32 week).

Table 1. Composition of the experimental diets (%)

Ingredient	Dietary group		
	Control group (0% WWNSKM)	Low dose group (10% WWNSKM)	High dose group (20% WWNSKM)
Maize	40	40	40
Broken rice	20	18	16
Rice polish	11	13	15
Groundnut cake	20	10	0
Fish meal	5	5	5
WWNSKM	0	10	20
Dical. Phosphate	1	1	1
Salt	0.2	0.2	0.2
Other supplements*	2.8	2.8	2.8

* included mineral mixture, trace elements, Vitamin mixture and coecidiostat and supplied as per requirements.

Following 21st week of age, the semen samples were collected by the massage method (Burrows and Ouinn, 1937). During the study, semen volume was measured by a pipette and sperm concentration was determined with a Neubauer haemocytometer (Lake, 1960). The number of abnormal spermatozoa were counted according to Lake and Stewart (1978). For histological studies, the testes were fixed in bouin's fluid and 5-6 micron thick paraffin sections were stained with hematoxylin and eosin.

For the artificial insemination, semen was diluted (1:4) with BPSE dilutor (Sexton, 1977) within 30 minutes of collection. Subsequently, the fertilizing ability of spermatozoa had been tested on 54 laying hens divided in 3 equal groups. On 25th week of treatment, 18 hens in each groups were inseminated separately with semen (60×10^6 spermatozoa) collected from the cocks of control, low and high dose groups. Under the similar conditions, again the fertility trials were carried out at the termination of the experiment (32 week). The fertility was examined by incubating the eggs layed by hens 2 to 10 days after intravaginal insemination with diluted semen. The eggs were assessed by candling on 5 days of incubation to determine fertility.

Questionable eggs thought to be infertile were broken open and examined macroscopically for the conformation of embryonic development. Fertile eggs were incubated to determine hatchability.

The data were analysed with one way classification following Completely Randomized Design (Snedecor and Cochran, 1980).

RESULTS

Data on semen characteristics revealed that feeding of WWNSKM led to a significant ($p < 0.05$) reduction in the semen volume and sperm concentration associated with increased ($p < 0.05$) number of morphological abnormalities in the spermatozoa in comparison to control group (table 2, 3 and 4). However, during the last three weeks of the study, no further alteration in percent morphological abnormalities was observed in low dose group whereas high dose group spermatozoa differed significantly ($p < 0.05$) in this respect from other groups (table 3). Results indicated that feeding of WWNSKM delayed the sexual maturity in cocks leading to the production of poor quality semen donated by less number of treated cocks in comparison to those of the control group. The number of semen producing cocks in each group increased by 25th week of age, and thereafter the semen was produced by a fixed number of cocks (except 28th and 29th week of age in high dose group)

Table 2. Effect of WWNSKM on semen volume (ML) of cocks (mean ± standard error of mean) with number of semen producing cocks shown in parenthesis

Week	Control group (0% WWNSKM)	Low dose group (10% WWNSKM)	High dose group (20% WWNSKM)
21	0.29 ± 0.03 (5)	0.20 (2)	0.07 (1)
22	0.35 ± 0.03^a (6)	0.28 ± 0.03^b (5)	0.10 ± 0.02^c (3)
23	0.37 ± 0.03^a (6)	0.30 ± 0.02^b (5)	0.10 ± 0.02^c (4)
24	0.35 ± 0.02^a (9)	0.30 ± 0.02^b (7)	0.13 ± 0.01^c (4)
25	0.37 ± 0.02^a (11)	0.33 ± 0.02^b (9)	0.18 ± 0.02^c (7)
26	0.38 ± 0.03^a (11)	0.34 ± 0.02^b (9)	0.18 ± 0.02^c (7)
27	0.40 ± 0.02^a (11)	0.35 ± 0.02^b (9)	0.19 ± 0.02^c (7)
28	0.41 ± 0.04^a (11)	0.35 ± 0.02^b (9)	0.22 ± 0.02^c (8)
29	0.43 ± 0.03^a (11)	0.34 ± 0.02^b (9)	0.22 ± 0.03^c (8)
30	0.44 ± 0.05^a (11)	0.32 ± 0.02^b (9)	0.25 ± 0.04^c (7)
31	0.43 ± 0.03^a (11)	0.31 ± 0.01^b (9)	0.23 ± 0.04^c (7)
32	0.44 ± 0.04^a (11)	0.32 ± 0.02^b (9)	0.25 ± 0.04^c (7)

Means bearing different superscripts (a,b,c) within the rows differed significantly ($p < 0.05$).

Table 3. Effect of WWNSKM on sperm cocentration ($\times 10^9$/ml) of cocks (mean ± standard error of mean) with number of semen producing cocks shown in parenthesis

Week	Control group (0% WWNSKM)	Low dose group (10% WWNSKM)	High dose group (20% WWNSKM)
21	2.94±0.19 (5)	1.02 (2)	0.82 (1)
22	3.11±0.23^a (6)	2.74±0.40^b (5)	2.15±0.20^c (3)
23	3.10±0.28^a (6)	2.54±0.35^b (5)	2.25±0.48^b (4)
24	3.73±0.34^a (9)	2.92±0.33^b (7)	2.34±0.32^c (4)
25	3.88±0.26^a (11)	3.45±0.37^b (9)	2.59±0.23^c (7)
26	4.09±0.21^a (11)	3.40±0.34^b (9)	2.73±0.37^c (7)
27	4.58±0.16^a (11)	3.44±0.44^b (9)	2.56±0.28^c (7)
28	4.49±0.20^a (11)	3.79±0.49^b (9)	2.77±0.34^c (8)
29	4.52±0.20^a (11)	3.82±0.38^b (9)	2.69±0.36^c (8)
30	4.63±0.22^a (11)	3.48±0.37^b (9)	2.78±0.39^c (7)
31	4.66±0.30^a (11)	3.52±0.32^b (9)	2.74±0.36^c (7)
32	4.56±0.23^a (11)	3.56±0.34^b (9)	2.88±0.43^c (7)

Values bearing different superscripts (a,b,c) within rows differed significantly ($p < 0.05$).

Table 4. Effect of WWNSKM on percent abnormal spermatozoa of cocks (mean ± standard error of mean) with number of semen donating cocks shown in parenthesis

Week	Control group (0% WWNSKM)	Low dose group (10% WWNSKM)	High dose group (20% WWNSKM)
21	14.80±2.31 (5)	22.00 (2)	29.00 (1)
22	13.83±1.75^a (6)	19.40±1.94^b (5)	28.33±3.15^c (3)
23	12.16±2.25^a (6)	18.00±2.00^b (5)	22.00±3.48^c (4)
24	10.00±1.16^a (9)	16.28±1.90^b (7)	19.00±2.16^b (4)
25	10.49±1.11^a (11)	13.00±1.50^a (9)	18.00±2.49^b (7)
26	9.36±0.80^a (11)	14.22±1.79^b (9)	17.28±1.82^b (7)
27	8.72±0.94^a (11)	12.88±1.37^b (9)	16.42±2.52^c (7)
28	7.90±0.73^a (11)	11.66±1.51^b (9)	15.00±1.65^c (8)
29	7.63±0.86^a (11)	10.55±1.77^b (9)	14.87±2.30^c (8)
30	7.18±0.85^a (11)	9.88±1.61^a (9)	13.71±2.98^b (7)
31	7.54±0.85^a (11)	9.11±2.00^a (9)	13.56±1.87^b (7)
32	7.45±1.00^a (11)	9.77±1.78^a (9)	13.00±2.07^b (7)

Values in same row bearing different superscripts (a,b,c) are different ($p < 0.05$).

irrespective of the treatment. At this stage, out of 14 birds, semen was not given by 3, 5 and 7 cocks from control, low dose and the high dose groups, respectively. No noticeable reduction in body weight and testicular weight was observed throughout the experimental period among the control and treated groups. The mean body weights of cocks fed 0, 10 or 20% WWNSKM at 21st and 32rd wks of age were 3.18±0.02, 3.12±0.04, 3.16±0.03 and 3.55±0.04, 3.49±0.03, 3.41±0.04 kg, respectively. The mean testes weights measured at the end of the experiment (after 32-wk) by sacrificing the cocks were 28.30±1.13, 28.07±1.09 and 26.88±0.90 g, respectively.

The histological picture indicated a remarkable differences in testis of cocks among the control and treated groups (figure 1, 2, 3). In the treated groups spermatogonia, primary spermatocytes and supporting cells revealed mild vacuolar degeneration. These changes were more marked in high dose treated group. In addition, the seminiferous epithelium was detached in some tubules at several places. Spermatids were few and had formed multinucleated giant cells in the seminiferous tubules. The spermatogenesis process was markedly reduced with less number of spermatozoa and other series of cells (figure 3).

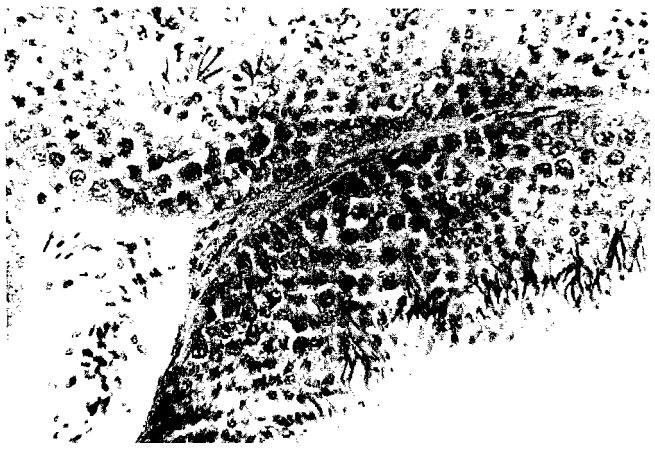

Figure 1. Control testis (32 weeks) showing normal architectural details of seminiferous tubules H/E (400 ×).

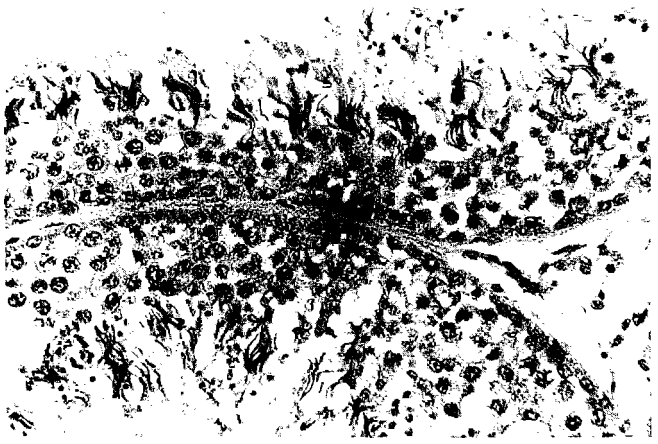

Figure 2. WWNSKM (low dose) treated testis (32 weeks) showing mild vacuolar degeneration of seminiferous tubules H/E (400 ×).

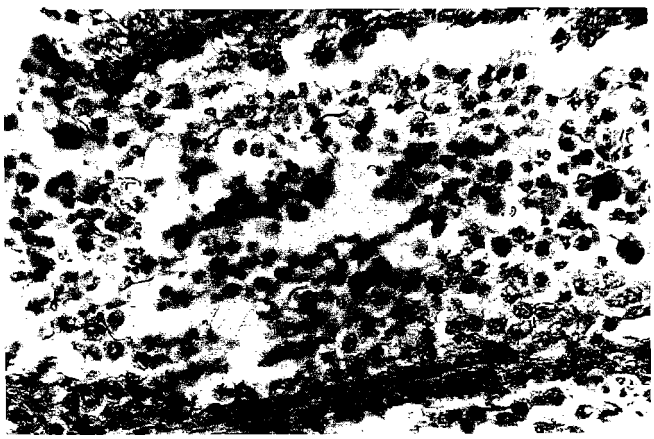

Figure 3. WWNSKM (high dose) treated testis (32 weeks) showing the detached seminiferous epithelium and degenerated spermatogenic series of cells in lumen and formation of giant cells H/E (400 ×).

Data on the fertilizing ability of spermatozoa and hatchability determined at 25th and 32nd (termination of experiment) week of age have been summarized in table 5. Fertility decreased by 9.56 and 18.14% at 25th week of age and 7.93 and 13.14% at 32nd week of age in groups with low and high dose WWNSKM, respectively in comparison to the untreated group. Accordingly, the hatchability also decreased in the treated groups (table 5).

Table 5. Percent fertility and hatchability of eggs laid by hens inseminated with semen from cocks fed with or without WWNSKM diets

	Week	Control group (n = 11)	Low dose group (n = 9)	High dose group (n = 7)
Fertility	25	79.58	70.02 (9.56)	61.44 (18.14)
	32	82.37	74.44 (7.93)	68.23 (14.14)
Hatchability	25	76.60	57.22 (19.38)	55.16 (21.44)
	32	78.77	63.77 (15.00)	59.86 (18.91)

Figures in parenthesis indicate percent decline in fertility and hatchability rates over those of control.

DISCUSSION

Results of this study indicated that semen characteristics and fertilizing ability of spermatozoa from cocks receiving WWNSKM were adversely affected. It had been reported that the para-aortic lymphnode cells from the *neem* treated animals show significantly higher lymphocyte proliferative response to mitogens. It appeared that the local immune cell population became activated following exposure to the extract and produced factors that possibly disturb the spermatogenic process (Upadhyay et al., 1993). The poor spermatogenesis in the present study appeared to be due to this reason thus resulted in the reduction in semen volume and also the sperm concentration linked with more number of morphologically abnormal spermatozoa. The results of the present study showed that WWNSKM caused a significant ($p < 0.05$) reduction in sperm concentration (table 3) and which are in good agreement with the earlier observations in rats administered with neem oil (Sampathraj et al., 1993).

Plasma testosterone concentration had been reported to correlate positively with body weights (Glimore, 1969; Gemmell et al., 1986) and testicular weight (Sharp, 1975; Sharp et al., 1977). However, in our study, no significant differences were found in body and testicular weights among the control and treated groups. These findings suggested that the plasma testosterone level might had been unaffected during the course of WWNSKM feeding to the avian species. Similar observations had been made by other workers (National Coordinating Group, 1978; Chang et al., 1980) in the mammalian species fed gossypol containing diet and found no change in body weight, testicular weight and weight of accessory sex organs and suggested that gossypol did not interfere in the androgen synthesis. Hence, the marked degenerative changes in seminiferous tubules of WWNSKM fed birds (figure 3) as observed in the present investigation may confirm the earlier report (Upadhyay et al., 1993) where in the rate of spermatogenesis process was arrested by a factor present in the *neem* seed extract and not by the involvement of the plasma testosterone. Therefore, this factor may be responsible for the poor fertility observed in hens inseminated with semen obtained from WWNSKM treated cocks (table 5). Besides the drop in fertility may be associated with an increased number of morphological abnormalities and more number of degenerating cells as evident by histological studies. A significant reduction in fertility due to morphological abnormalities in the spermatozoa in chicken fed with gossypol acetic acid had been demonstrated earlier (Mohan et al., 1989).

It may be concluded that feeding of WWNSKM even at 10% level in isocaloric and isonitrogneous diets was

found associated with adverse effects on the fertility of male broiler chickens.

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