DIETARY SUPPLEMENTATION OF AMLA AND GRAPE SEED ON TESTOSTERONE CONCENTRATION AND SEMEN CHARACTERISTICS IN BROILER BREEDERS

K.T. Priya1, D.K. Elizabeth Manju2, A. Thangavel3, V. Leela4
Department of Veterinary Physiology
Madras Veterinary College
Chennai - 600 007.

ABSTRACT

The effect of dietary supplementation of amla (Emblica officinalis) and grape (Vites venifera) seed on plasma testosterone level and semen characteristics in broiler breeder stock were assessed in this study. This trial was conducted in twenty four Poultry Research Station B2 (PRS B2) broiler breeder cocks from 32 to 37 weeks of age. Experimental birds were randomly divided in to four groups viz; Control: Standard broiler ration, Treatment - I: Broiler ration + 1 per cent amla powder, Treatment - II: Broiler ration + 1 per cent grape seed powder and Treatment - III: Broiler ration + amla powder and grape seed powder each at 0.5 per cent level. Dietary supplementation with the combination of amla and grape seed powder indicated significantly higher testosterone concentration as well as significantly improved semen characteristics in the broiler breeder cocks.

Key words: Amla (Emblica officinalis), Grape (Vites venifera) seed - Testosterone - Semen characteristics - Broiler breeder stock.

INTRODUCTION

Modern intensive poultry industry demands more rapid growth in a confined housing environment leads to great susceptibility to stress in broiler breeds. Stress related changes in the semen characteristics contribute infertility problems in broilers breeders (Karaca et al., 2002). Production of Reactive oxygen species (ROS) in the reproductive tract is detrimental not only to the fluidity of the sperm plasma membrane, but also to the integrity of DNA in the sperm nucleus. Avian sperm cell membranes have high content of long chain polyunsaturated fatty acids. Lipid peroxidation of the long chain polyunsaturated fatty acids in the sperm cell membrane is the primary cause of infertility (Aitken et al., 1989; Cecil and Bakst, 1993). Amla (Emblica officinalis) is the potent source of antioxidants such as carotenoids, phenolic acids and flavonoids. Proanthocyanins, the biologically active constituents of grape (Vites venifera) seed had a potent antioxidant activity (Nakamura et al.,

1. Part of M.V.Sc., thesis submitted by the first and second authors to the Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600 051.
3. Professor and Head, 4. Professor, Department of Veterinary Physiology, Madras Veterinary College, Chennai - 600 007.
Transresveratrol of grape seed exhibited a productive effect against lipid peroxidation in the sperm cell membrane and DNA damage caused by ROS (Bhat et al., 2001; Roemer and Roemer, 2002 and Aziz et al., 2003).

Ascorbic acid supplementation stimulated testicular steroid dehydrogenase activity and increased plasma testosterone levels (Biswas et al., 1996). Oxidative stress showed decreased testosterone secretion by reducing the levels of enzymatic and non-enzymatic antioxidants in Leydig cells (Cao et al., 2004). Hence this feeding trial was undertaken with an objective to study the antioxidant effect of amla and grape seed on testosterone concentration and semen characteristics in broiler breeder cocks.

**MATERIALS AND METHODS**

This trial was conducted in twenty four PRS B2 broiler breeder cocks from 32 to 37 weeks of age. Experimental birds were randomly divided in to four groups viz; Control: Standard broiler ration, Treatment - I: Broiler ration + 1 per cent amla powder, Treatment - II: Broiler ration + 1 per cent grape seed powder and Treatment - III: Broiler ration + amla powder and grape seed powder each at 0.5 per cent level.

The birds were reared in deep litter system under standard managerial practice throughout the experimental period.

This experiment was approved by the Institutional Animal Ethical Committee (1774/DFBS/A/2006 dt. 02.08.2007).

Feeding trial was conducted for a period of six weeks. Experimental rations were fed to the respective treatment groups for first three weeks (treatment period). Subsequently the experimental birds were fed with normal breeder ration without any supplementation for the next three weeks (post - treatment period).

Weekly blood samples were collected from the wing vein during treatment and post treatment periods and centrifuged at 50 x G for 10 minutes for the separation of the plasma.

The levels of testosterone in the plasma were estimated by ELISA using ADALTIS EIAgen testosterone kits.

Semen samples were collected at weekly intervals by massage technique (Burrows and Quinn, 1937) during early hours of the day. The milky drops of the ejaculate were immediately aspirated by sterile tuberculin syringes and the semen volume of each bird was measured directly from the tuberculin syringes. The semen samples were then transferred to sterile test tubes kept in a water bath at 18-20°C. Sperm concentration was estimated according to the procedure of Allen and Champion, (1955). Sperm motility was assessed as per the method of Parker et al. (1942). Sperm livability was estimated according to the method of Tienhoven and Steel, (1957).

Statistical analysis was done by randomized block design as per Snecdor and Cochran, (1989).

**RESULTS AND DISCUSSION**

The effects of amla and grape seed supplementation on plasma testosterone concentrations (ng/ ml Mean+ SE) in broiler cocks are presented in the Table.

A significant variation was recorded during the treatment and post treatment periods of amla and grape seed supplementations. The birds supplemented with the combination of amla and grape seed each at 0.5 per cent level showed the highest testosterone concentration followed by treatment I and treatment II. In all the treated birds, testosterone concentrations were found to increase during the second and third week of treatment period which significantly declined during post treatment period.
The result of the present study may be due to the stimulatory effect of ascorbic acid content of amla on 3β HSD activity. Steroidogenesis appeared to be ascorbic acid dependent particularly at hydroxylation steps (Tsuji et al., 1989; Goralczyk et al., 1992; Luck et al., 1995) and synergistic antioxidant activities of amla and grape seed. Biswas et al. (1996) also reported that ascorbic acid supplementation stimulated testicular steroid dehydrogenase activity and increased plasma testosterone levels.

It is reported that ascorbic acid activated the release of LH from the anterior pituitary gland by means of nitric oxide. LH causes the release of testosterone from the Leydig cells (Karanath et al., 2001).

Synergistic antioxidative activities of amla and grape seed may also be responsible for the significant increase in the testosterone concentrations in the birds supplemented with amla and grape seed each at 0.5 per cent level. This is in agreement with the reports of Cao et al. (2004) who indicated that oxidative stress reduced the levels of key enzymatic and non-enzymatic antioxidants in Leydig cells, resulted in decline in testosterone secretion. Emila Juan et al. (2005) also stated that the natural antioxidant of grape, transresveratrol had increased the serum concentration of testosterone in rats which might have shifted the levels of testosterone in this study also.

The mean semen volume of 0.86 ± 0.03 ml at 1st week of post treatment was comparatively higher than the reports of Ramamurthy (1983) in IC² Strain in White Cornish breed of chicken, Kamar et al. (1984) in Fayoumi, Plymouth Rock and Rhode Island Red breeds of fowls, Stephens (1986) in White Leghorn cocks, Petrovska el al. (1987) in Rhode Island Red cocks and Bah et al. (2001) in local breeder cocks. Significant improvement in the semen volume could be due to ascorbic acid induced testosterone synthesis and therefore accessory glands secretion.

Treatment III revealed significantly higher (P < 0.01) sperm concentration (3091 ± 97.54 x 10⁶/ml) in the 1st week of post treatment period in comparison with treatment I and II. Sperm concentration observed in the present study was higher in comparison with the observations of Lillie et al. (1974) in White Cornish cocks and Bah et al. (2001) in local breeder cocks, and lower than those reported by Lake and Stewart, (1978) and Ramamurthy (1983).

Significant improvement in the sperm count in the birds supplemented with amla and grape seed powder (Treatment III) suggested synergistic effect of ascorbic acid and trans resveratrol (Akmal et al., 2006) (Emilia Juan et al., 2005).

Sperm motility indicated significantly higher (P < 0.01) percentage (90 ± 0.00%) during first week of post treatment period. The mean sperm motility of the present study indicated higher percentages than the observations of Ramamurthy (1983) in White Cornish cocks and Bah et al. (2001) in local breeder cocks. High content of ascorbic acid in amla might have a beneficial effect on sperm motility (Monsi and Onitchi, 1990; Eskenazi et al., 2005 and Akmal et al., 2006). Significant improvement in sperm motility in treatment III could also be due synergistic effect of amla and grape seed on facilitated glucose transport by GLUT -5 gene expression in sperm during oxidative stress (Ajit Vaze, 2007).

Significantly higher (P < 0.01) percentage of sperm livability (90 ± 0.02%) was recorded during first week of post treatment period in the birds supplemented with amla and grape seed powder each at 0.5 per cent level when compared to control. Ramamurthy (1983) in White Cornish, Kamar et al. (1984) in Fayoumi, Plymouth Rock and Rhode Island Red breeds, Stephens (1986) in White Leghorn and Bah et al. (2001) in local breeder cocks have reported lower percentage of live sperms than the observation of the present study.
All the three treatments revealed significantly lower (P < 0.01) percentage of sperm abnormality (4 ± 0.02%) in comparison with the control birds during 1st weeks of post treatment period. Significant improvement in the sperm viability and decrease in sperm abnormality might be due to the synergistic actions of the antioxidants of amla and grape seed which reduced the oxidative damage and maintained the membrane integrity of cell membrane (Luck et al., 1995). The reduced sperm abnormality might also be due to the ability of antioxidants to resist the oxidative DNA damage and genetic alterations in the spermatozoa (Luck et al., 1995; Bagachi et al., 1997).

**CONCLUSION**

Synergistic antioxidative activities of amla and grape seed significantly increased plasma testosterone concentration and also significantly improved semen characteristics in broiler breeder cocks.

**REFERENCE**


### Table

**Dietary supplementation of amla and grape seed powder on testosterone level and semen characteristics in broiler breeder cocks**

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma testosterone concentrations (ng/ml) Mean ± SE</th>
<th>Semen volume (ml) Mean ± SE</th>
<th>Semen concentration x 10^6/ml Mean ± SE</th>
<th>Semen motility (%) Mean ± SE</th>
<th>Live sperm (%) Mean ± SE</th>
<th>Sperm abnormality (%) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st week 2nd week 3rd week 1st week 2nd week 3rd week</td>
<td>1st week 2nd week 3rd week 1st week 2nd week 3rd week</td>
<td>1st week 2nd week 3rd week 1st week 2nd week 3rd week</td>
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<td>1st week 2nd week 3rd week 1st week 2nd week 3rd week</td>
</tr>
<tr>
<td>Control</td>
<td>10.59 ± 1.20 11.86 ± 0.65 11.96 ± 0.38 11.89 ± 0.18 11.48 ± 0.32 11.89 ± 0.67</td>
<td>0.21 ± 0.02 0.23 ± 0.02 0.28 ± 0.02 0.26 ± 0.01 0.26 ± 0.02</td>
<td>1843 ± 78.03 1855 ± 85.74 1861 ± 85.29 1880 ± 79.62 1858 ± 85.74</td>
<td>58 ± 0.03</td>
<td>65 ± 0.01</td>
<td>14 ± 0.01</td>
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<tr>
<td>Treatment - I</td>
<td>13.04 ± 0.50 15.72 ± 0.68 15.90 ± 0.49 15.67 ± 0.47 14.40 ± 0.47 12.32 ± 0.12</td>
<td>0.33 ± 0.01 0.43 ± 0.01 0.55 ± 0.01 0.63 ± 0.03 0.60 ± 0.04 0.56 ± 0.04</td>
<td>2320 ± 130.66 2478 ± 129.75 2835 ± 118.41 2840 ± 132.02 2853 ± 88.47</td>
<td>68 ± 0.02</td>
<td>66 ± 0.01</td>
<td>10 ± 0.02</td>
</tr>
<tr>
<td>Treatment - II</td>
<td>12.89 ± 0.98 13.63 ± 0.74 13.47 ± 0.44 12.7 ± 0.35 11.90 ± 0.28 11.84 ± 0.19</td>
<td>0.30 ± 0.02 0.38 ± 0.02 0.50 ± 0.02 0.55 ± 0.03 0.48 ± 0.04 0.43 ± 0.04</td>
<td>2178 ± 99.81 2198 ± 102.53 2218 ± 99.81 2241 ± 98.90 2260 ± 96.63</td>
<td>63 ± 0.05</td>
<td>71 ± 0.01</td>
<td>7 ± 0.01</td>
</tr>
<tr>
<td>Treatment - III</td>
<td>14.73 ± 0.72 17.55 ± 0.30 16.75 ± 0.63 16.59 ± 0.61 15.36 ± 0.61</td>
<td>0.38 ± 0.01 0.55 ± 0.01 0.75 ± 0.04 0.86 ± 0.03 0.81 ± 0.02 0.75 ± 0.03</td>
<td>2641 ± 123.17 2723 ± 96.63 2925 ± 93.00 3091 ± 97.54 2991 ± 94.59</td>
<td>60 ± 0.03</td>
<td>74 ± 0.02</td>
<td>10 ± 0.03</td>
</tr>
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**Note:** Means sharing a common letter within a column are not significantly different (P>0.05).