EFFECT OF DIETARY SUPPLEMENTATION OF AMLA AND GRAPE SEED ON SEMEN CHARACTERSTICS OF BROILER BREEDER COCKS

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ABSTRACT

*This study was conducted to assess the effect of dietary supplementation of amla (*Emblica officinalis*) and grape (*Vites venifera*) seed on semen characterstics of broiler breeder cocks. Twenty four Poultry Research Station B_2 (PRS B_2) broiler breeder cocks from 32 to 38 weeks of age were randomly divided into four groups 1) Control: Standard broiler ration, 2) Treatment – I: Broiler ration + Supplementation of 1 per cent amla powder, 3) Treatment – II: Broiler ration + Supplementation of 1 per cent grape seed powder and 4) Treatment – IV: Broiler ration + Supplementation of 0.5 per cent amla powder and 0.5 per cent grape seed powder. Semen samples were collected at weekly intervals and semen characterstics were recorded during pre treatment period (0 weeks), treatment period (1-4 weeks) and post-treatment period (last 2 weeks). The results of the present study revealed that the birds supplemented with the combination of amla and grape seed each at 0.5 per cent level significantly (P = 0.01) improved the semen volume, sperm concentration, percentage of motile sperms and live sperms and significantly (P = 0.01) reduced the sperm abnormality*

Key words: Amla (*Emblica officinalis*), grape (*Vites venifera*) seed, semen characterstics -broiler breeder cocks.

INTRODUCTION

Indian poultry industry ranks fourth in egg production and fifth in broiler production in the world, contributing two per cent of Gross Domestic Product (GDP). Modern intensive poultry industry demands more rapid growth in a confined housing environment leads to great susceptibility to stress in broiler birds. Stress related changes in the semen characterstics contribute infertility problems in broiler breeders (Karaca *et al.*, 2002). Rapid growth rate in broilers accelerated the metabolic rate and make them vulnerable to oxidative stress owing to increased free radical generation. 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

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the sperm cell membrane is the primary cause of infertility (Aitken *et al.,* 1989; Cecil and Bakst, 1993). Amla (*Emblica officinalis*) is as potent source of antioxidants such as carotenoids, phenolic acids and flavonoids. It has the ability to stimulate natural antioxidant enzyme systems including catalase, superoxide dismutase and glutathione peroxidase (Rajak *et al.,* 2004 and Battacharya *et al.,* 2000). Proanthiocyanins, the biologically active constituents of grape (*Vites venifera*) seed had a potent antioxidant activity (Nakamura *et al.,* 2003). Trans – resveratrol of grape seed exhibited a protective 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).

MATERIALS AND METHODS

The trial was conducted in twenty four PRS B_2 broiler breeder cocks for a period of seven weeks from 32 to 38 weeks of age. Experimental birds were randomly divided into four groups as follows

Group	Experimental feeding	No. of birds
Control	Standard broiler ration	6
Treatment - I	Broiler ration + 1% amla powder	6
Treatment - II	Broiler ration + 1% grape seed powder	6
Treatment - III	Broiler ration + 0.5% amla + 0.5% grape seed powders	6
	Total	**24**

The birds were reared in deep litter system under standard managemental practices throughout the experimental period.

This experiment was approved by the Institutional Animal Ethical Committee.

The experimental birds were fed with normal breeder ration for a week and assessed the pre treatment effect. Feeding trial was conducted for a period of six weeks. Experimental rations were fed to the respective treatment groups for four weeks (treatment period). Subsequently the experimental birds were fed with normal breeder ration without any supplementation for the next two weeks (post – treatment period).

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 samples were then transferred to sterile test tubes kept in a water bath at 18-20^0**C.**

Immediately after the semen collection semen volume of each bird was measured directly from the tuberculin syringe

Sperm concentration was estimated according to the procedure of Allen and Champion, (1955).

Sperm motility and sperm abnormality were 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 Snedecor and Cochran, (1994).

RESULTS AND DISCUSSION

Effect of dietary supplementation of amla and grape seed on semen characterstics are presented in the table 1.

Highly significant (P = 0.01) increase in semen volume was noticed in birds supplemented with amla and grape seed powder each at 0.5 per cent level (Treatment III) followed by treatment I and II.

The mean semen volume of the present study (0.86 ± 0.03 ml at 4th week of treatment) was comparatively higher than the reports of Ramamurthy (1983) in IC_2 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 *el al.* (2001) in local breeder cocks. Significant improvement in the semen volume could be attributed to higher concentration of ascorbic acid and grape seed which increase testosterone synthesis and therefore accessory glands secretions.

Treatment III revealed significantly higher (P = 0.01) sperm concentration (3091 ± 97.54 x 10^6/ µl) in the fourth week of treatment 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 fourth week of treatment.

The mean sperm motility of the present study indicated higher percentages than the observations of Ramamurthy (1983) in White Cornish cocks and Bha ***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 to the 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 fourth week of treatment 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 Bha *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.

Significant improvement in the sperm viability and decrease in sperm abnormality might be due to synergistic action of antioxidants of amla and grape seed which reduced the oxidative damage and maintained the integrity of cell membrane (Luck *et al.,* 1995). The reduced sperm abnormality might also be due to the ability of antioxidants in the supplementations to resist the oxidative DNA damage and genetic alterations in the spermatozoa (Luck *et al.,* 1995; Bagachi *et al.,* 1997).

Effect of dietary supplementation of amla and grape seed powder on semen characterstics of broiler breeder cocks

Group	Semen volume (ml) Mean ± SE						
	Pre Treatment period	Treatment period				Post- treatment period	
	0 week	1st week	2nd week	3rdweek	4th week	1st week	2nd week
Control	0.18^{Aa} ± 0.01	0.21^{Aa} ± 0.02	0.23^{Aa} ± 0.02n	0.28^{Aa} ± 0.02	0.28^{Aa} ± 0.02	0.26^{Aa} ± 0.01	0.26^{Aa} ± 0.02
Treatment - I	0.21^{Aa} ± 0.01	0.33^{Bab} ± 0.01	0.43^{Cbc} ± 0.01	0.55^{Cc} ± 0.01	0.63^{Cc} ± 0.03	0.60^{Cd} ± 0.04	0.50^{Cc} ± 0.04
Treatment - II	0.16^{Aa} ± 0.01	0.30^{ABab} ± 0.02	0.38^{Bcb} ± 0.04	0.50^{Bcb} ± 0.04	0.55^{Bcb} ± 0.04	0.48^{Bb} ± 0.04	0.43^{Bcb} ± 0.04
Treatment - III	0.23^{Aa} ± 0.01	0.38^{Bb} ± 0.01	0.55^{Dc} ± 0.04	0.75^{Dde} ± 0.04	0.86^{De} ± 0.03	0.81^{De} ± 0.02	0.75^{De} ± 0.03
Group	Sperm concentration x 10^6/µl Mean ± SE						
	Pre Treatment period	Treatment period				Post- treatment period	
	0 week	1st week	2nd week	3rdweek	4th week	1st week	2nd week
Control	1860^{Aa} ± 89.83	1843^{Aa} ± 78.03	1855^{Aa} ± 85.74	1861^{Aa} ± 85.29	1880^{Aa} ± 85.74	1858^{Aa} ± 79.62	1865^{Aa} ± 85.74
Treatment - I	1934^{Aa} ± 130.66	2320^{Cbc} ± 130.66	2478^{Cc} ± 129.75	2835^{Ce} ± 118.41	2840^{CDe} ± 132.02	2853^{Ce} ± 88.47	2720^{Cde} ± 97.99
Treatment - II	1953^{Aa} ± 99.36	2178^{BCbc} ± 99.81	2198^{Bc} ± 102.53	2218^{Bc} ± 99.81	2241^{Bc} ± 98.90	2260^{Bc} ± 96.63	2256^{Bc} ± 90.73
Treatment - III	1968^{Aa} ± 158.56	2641^{Dbc} ± 123.17	2773^{Dcd} ± 96.63	2953^{Dd} ± 93.00	3091^{Dd} ± 97.54	2991^{Dd} ± 94.59	2925^{Cd} ± 89.15
Group	Sperm motility (%) Mean ± SE						
	Pre Treatment period	Treatment period				Post- treatment period	
	0 week	1st week	2nd week	3rdweek	4th week	1st week	2nd week
Control	52^{Aa} ± 0.05	58^{Aa} ± 0.03	58^{Aa} ± 0.03	62^{Aa} ± 0.03	60^{Aa} ± 0.02	60^{Aa} ± 0.02	58^{Aa} ± 0.03
Treatment - I	53^{Aa} ± 0.04	68^{Bbc} ± 0.03	78^{CDde} ± 0.02	85^{CDe} ± 0.03	85^{CDe} ± 0.03	80^{Ce} ± 0.03	70^{Ccd} ± 0.03
Treatment - II	50^{Aa} ± 0.04	63^{ABbc} ± 0.05	73^{Bcde} ± 0.04	82^{BCe} ± 0.01	83^{BCe} ± 0.01	80^{BCe} ± 0.01	70^{BCcd} ± 0.03
Treatment - III	52^{Aa} ± 0.03	60^{Bbc} ± 0.03	83^{Def} ± 0.02	90^{Df} ± 0.00	90^{Df} ± 0.00	82^{Cde} ± 0.01	75^{Ccd} ± 0.02
Group	Live sperms (%) Mean ± SE						
	Pre Treatment period	Treatment period				Post- treatment period	
	0 week	1st week	2nd week	3rdweek	4th week	1st week	2nd week
Control	64^{Aa} ± 0.01	65^{Aa} ± 0.01	66^{Aa} ± 0.00	68^{Aa} ± 0.01	68^{Aa} ± 0.01	66^{Aa} ± 0.01	67^{Aa} ± 0.01
Treatment - I	67^{Aa} ± 0.01	72^{Aab} ± 0.02	78^{Bb} ± 0.02	81^{Bb} ± 0.03	84^{Db} ± 0.03	84^{Bcb} ± 0.04	81^{Cb} ± 0.02
Treatment - II	65^{Aa} ± 0.01	71^{Aa} ± 0.01	74^{Ba} ± 0.01	78^{ABa} ± 0.01	81^{BCab} ± 0.01	84^{Cb} ± 0.01	78^{BCa} ± 0.02
Treatment - III	67^{Aa} ± 0.01	74^{Aab} ± 0.02	80^{Bb} ± 0.01	85^{Bb} ± 0.02	90^{Cb} ± 0.02	87^{Cb} ± 0.02	84^{Cc} ± 0.02
Group	Sperm abnormality (%) Mean ± SE						
	Pre Treatment period	Treatment period				Post- treatment period	
	0 week	1st week	2nd week	3rdweek	4th week	1st week	2nd week
Control	14^{Ba} ± 0.01	14^{Da} ± 0.01	13^{Ca} ± 0.01	12^{Ca} ± 0.01	12^{Ba} ± 0.01	13^{Ba} ± 0.01	13^{Ba} ± 0.02
Treatment - I	13^{Be} ± 0.02	10^{Cde} ± 0.02	8^{Bcd} ± 0.02	6^{Bab} ± 0.02	4^{Aa} ± 0.02	5^{Aa} ± 0.02	7^{Abc} ± 0.01
Treatment - II	9^{Ab} ± 0.01	7^{Aab} ± 0.01	6^{Aa} ± 0.01	5^{Aa} ± 0.01	4^{Aa} ± 0.01	5^{Aa} ± 0.01	5^{Aa} ± 0.01
Treatment - III	12^{ABe} ± 0.01	10^{BCde} ± 0.01	7^{ABc} ± 0.01	5^{Aa} ± 0.01	4^{Aa} ± 0.02	5^{Aab} ± 0.01	7^{Abc} ± 0.01

Same superscript (Capital letters) between treatments does not differ significantly at 1% level.

Same superscript (Small letters) between weeks does not differ significantly at 5% level

CONCLUSION

The synergistic effect of amla and grape seed significantly improved semen characterstics by alleviating antioxidative stress in broiler breeder cocks.

REFERENCES

Aitken, R.J., J.S. Clarkson and S. Fishel, 1989. Generation of ROS lipid peroxidation and human sperm function. *Biol. Reprod*., 40: 183 –197.

Ajit Vaze., 2007. Double- blind comparative trial of herbomineral antioxidant formulation with ubiquinone in oligoasthenospermia. *Lancet. Infect.Dis.*, 7:1057-1058.

Akmal, M.,J.Qadri, N.S. Al-waili, S. Thangal, A. Haq and K.Y.Shaloom, 2006. Improvement in human semen quality after oral administration of vitamin C. *J. Med. Food*., 9 (3): 440-442.

Allen, C.J. and L.R. Champion, 1955. Competitive fertilization in the fowl. *Poult . Sci*., 34: 1332-1342.

Aziz, M.H., R. Kumar and N. Ahmad, 2003. Cancer chemopreventive by resveratrol: *in vitro* and *in vivo* studies and the underlying mechanisms. *Int. J. Oncol*., 23: 17-28.

Bagachi D., A. Garg, R. Krohn, M. Bagachi, M. Tran and S. Stohs, 1997. Oxygen free radical scavenging abilities of vitamin C and E and a grape seed proanthocyanidin extract *in vitro*. *Res. Commun. Mol. Pathol. Pharmacol*., 95: 179 – 90.

Bah G.S., S.U.R.Chaudhari and J.D. Al- Amin, 2001. Semen Characteristics of local breeder cocks in the Sahel region of Nigeria. *Revue. Med. Paystrop*., 54 (2):153-158.

Battacharya, A., S. Ghosal and S.K. Bhattacharya, 2000. Antioxidant activity of tannoids principles of *Emblica officinalis* (amla) in chronic stress induced changes in rat brain. *Indian. J. Exp. Biol*., 38: 877-880

Bhat K.P.L., J.W. Kosmeder. and J.M. Pezutto, 2001. Biological effects of resveratrol. *Antioxid. Redox . Signal*., 3: 1041-1064.

Burrows, W.H., and J.P.Quinn, 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poult. Sci.*, 16:19-24.

Cecil H.C and M.R. Bakst 1993. In vitro lipid peroxidation of turkey spermatozoa. *Poult. Sci*., 72: 1370-1378.

Emilia Juan M., E.G. Pons, T. Munuera, J. Ballester, J. E. Rodriguez – Gil and J.M. Planas, 2005. trans – Resveratrol a natural antioxidant from grapes increases sperm output in healthy rats. *J. Nutr*., 135: 757-760.

Eskenazi,B., S.A.Kidd , A.R. Marks , E. Sloter, G. Block and A.J. Wyrobek, 2005. Antioxidant intake is associated with semen quality in healthy men. *Hum*. ***Reprod.,*** 20 **(**4): 1006 –1012.

Kamar, G.A.R., M.K. Khalifa, S.A. Raid and A.A.M. Sarhan, 1984. Studies on semen characteristics, fertility and hatchability of Fayoumi, Plymouth Rock and Rhode island Red cocks. *Egyptian Journal of Animal*

Production, 24: 41-50. *Poult. Abstr.*,1349, 13 (7): 165.

Karaca, A.G., H.M. Parke and C.D. McDaniel, 2002. Elevated body temperature directly contributes to heat stress infertility of broiler breeder males. *Poult. Sci.*, 81 :1892-1897.

Lake, P.E. and J.M. Stewart, 1978. Artificial insemination in poultry. HMSO bulletin 213. London Ministry of Agriculture, Fisheries and Food.

Lillie, R.J., S.J. Harris, H.C. Cecil and Joel Hitman, 1974. Normal reproductive performance of mature cockerels fed. Aroclar. 1248. *Poult. Sci*., 53 : 604-1607.

Luck M.R., I. Jeyseelan, and R.A.,Scholes,1995. Ascorbic acid and fertility. *Biol. Reprod*., 52: 262-266.

Monsi, A. and D.O. Onitchi, 1990. Effects of ascorbic acid (vitamin C) supplementation on ejaculated semen characteristics of broiler breeder chickens under hot and humid tropical conditions. *Animal Feed Science and Technology*., 34: 1-2.

Nakamura. Y., S. Tsuji and Y. Tonogai, 2003. Analysis of proanthocyanidins in grape seed extract, health foods and Grape seed oils. *J. Health. Sci.*, 49 (1): 45-54.

Parker, J.E., F.F. McKenzie and H.L. Kempster, 1942. Development of the testes and combs of White Leghorn and New Hampshire cockerels. *Poult. Sci*., 21: 35- 44.

Petrovska, E., F. Spacek and Z. Cada, 1987. The selection of cocks for artificial insemination. *Zivocisna Vyroba.*, 32(2): 123-127. cited in *Anim . Breed. Abstr*., 55 (7): 582, 4744.

Rajak, S., S.K. Banerjee, S. Sood, A.K. Dinda, Y.K. Gupta, S.K. Gupta and S.K. Maulik , 2004. *Emblica officinalis* causes myocardial adaptation and protects against oxidative stress in ischemic reperfusion injury in rats. *Phytother Res*., 18 (1): 54-60.

Ramamurthy, N., 1983. Association of semen characteristics with production and reproduction traits in broiler. M.V.Sc Thesis submitted to Tamil Nadu Agricultural University.

Roemer, K, and M. M. Roemar 2002. The basis for the chemopreventive action of resveratrol. *Drugs Today*., 38:571-580.

Snedecor, G.W and E.G. Cochran, 1994. Statistical methods, 8th edn., Iowa State University Press, USA. 564 p.

Stephens, J.A.A., 1986. A comparison of diluents for Cryopreservation of rooster semen. Ph.D thesis submitted to Tamil Nadu Agricultural University.

Tienhoven V. A., and R.G.D. Steel, 1957. The effect of different diluents and dilution rates on fertilizing capacity of turkey semen. *Poult. Sci*., 36: 473-479.