Short Communication

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Dietary organic zinc and selenium supplementation improves semen quality and fertility in layer breeders

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Trace minerals as a part of many enzymes are necessary for normal biochemical reactions. Supplementation of minerals in organic form is more bioavailabe than in inorganic form due to absence of any interaction or antagonism between minerals or other dietary constituents. Furthermore, organic trace minerals are efficiently absorbed in the gut necessitating lower level of supplementation in the diet (Rao *et al*. 2013). In pullets organic Zn supplementation increased gonadal maturation at placement in broiler breeder males (Suchý *et al.* 1998). Shanmugam *et al.* (2014) found that organic Zn supplementation at 100 ppm improved the sperm metabolism in layers. In chicken, studies on organic Se supplementation have focussed on sperm morphology (Edens and Sefton, 2009) and semen quality during high ambient temperature (Ebeid 2009). Apart from the effect of supplemental organic Zn and Se on rooster semen quality there are no report of supplemental trace minerals in males on fertility and hatchability. The objective was to investigate the dietary supplementation of organic Zn and Se on semen quality, fertility and hatchability in layer breeders.

The experiment was conducted at the experimental poultry farm of the Institute located at Hyderabad, India with the approval of the Institute Animal Ethics Committee. Thirty Dahlem Red roosters (29 weeks) were assigned to 3 groups in individual cages in an open-sided house. A practical male breeder diet was prepared to contain 2,665 kcal/kg ME, 16.4% crude protein and other nutrients (Table 1). The diets of experimental groups were supplemented with organic Zn (zinc proteinate) and Se (selenomethionine in yeast protein) as follows - group 1 (0 Zn/0 Se), group 2 (100 mg/kg Zn/0.15 mg/kg Se), group 3 (100 mg/kg Zn/ 0.35 mg/kg Se) for 8 weeks. The birds were provided with weighed quantity of feed (100g/day) and had free access to water.

By abdominal massage (Burrows and Quinn 1937) semen was collected from the birds at fourth and eighth weeks after selenium supplementation. The semen collected was evaluated for different gross and biochemical parameters. Immediately after collection semen was diluted 4 times using high temperature diluent (Chaudhuri and Lake 1988) and used for further analysis. The volume of the ejaculated semen was assessed by using a 1 ml syringe. The appearance of raw semen was scored 1 to 5 visually (McDaniel and Craig 1959). Percentage of progressively motile sperm was assessed subjectively by placing a drop of diluted semen on a Makler chamber and examining at 20 × magnifications. Sperm concentration was determined in a colorimeter at 540 nm of wavelength (Taneja and Gowe

Table 1. Ingredients and nutrient composition of basal diet

Ingredient	g/100g
Yellow maize	70.535
Soybean meal	24.993
Salt	0.444
Shell grit	1.611
Di-calcium phosphate	1.866
DL-methionine	0.133
Choline chloride, 50%	0.111
Vitamin premix[A]	0.022
B complex	0.028
$MnSO_4$	0.010
$FeSO_4$	0.013
$CuSO_4$	0.001
Mycotoxin binder	0.111
Antibiotic	0.056
Tylosine	0.056
Nutrient composition (Analysed)	
Metabolizable energy (ME) (kcal/kg)	2665
Crude protein (%)	16.4
Lysine (%)	0.71
Methionine (%)	0.37
Calcium (%)	1.02
Available phosphorus (%)	0.41

[A]Supplied (mg/kg diet): thiamin 1; pyridoxine, 2; cyanocobalamine, 0.01; niacin, 15; pantothenic acid, 10; a tocopherol, 10; riboflavin, 10; biotin, 0.08; menadione, 2; retinol acetate, 2.75; cholecalciferol, 0.06; choline, 650.

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Table 2. Effect of combined dietary supplementation of organic zinc and selenium on semen parameters, fertility and hatchability in Dahlem Red roosters

Parameters	0 mg/kg organic Se and Zn	100 mg/kg organic Zn		SEM
		0.15 mg/kg organic Se	0.35 mg/kg organic Se	
Volume (ml)	0.59	0.55	0.52	0.06
Appearance	3.70	3.53	3.60	0.21
Initial sperm motility (%)	59.25	63.68	65.88	2.46
Sperm concentration (million/ µl)	4.64	4.90	4.74	0.32
MTT dye reduction test (nM of MTT Formazan /min/million sperm)	23.75[b]	24.84[ab]	27.56[a]	0.91
Live sperm (%)	79.41[b]	82.98[b]	94.02[a]	2.24
Abnormal sperm (%)	5.28	4.64	3.85	0.98
Seminal plasma lipid peroxidation (nmol MDA/mg protein)	2.88	2.29	1.74	0.44
Fertility (%)	39.10[b]	71.40[a]	65.32[a]	3.40
Hatchability on FES (%)	69.36	67.77	70.67	4.69

Figures bearing different superscripts in a row differ significantly ($P \leq 0.05$).

1961). Tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction test was carried out and absorbance was recorded using a colorimeter at 570 nm (Hazary *et al.* 2001). Percent live sperm was estimated by differential staining technique using eosin–nigrosin stain (Campbell *et al.* 1953). The slides were used for estimating the percent abnormal sperm on the basis of observable abnormalities. Seminal plasma was separated by centrifugation of raw semen samples at 1,500 × g and assessed level of lipid peroxidation (LP) (Hsieh *et al.* 2006).

A fertility trial to assess the organic mineral supplementation on fertility and hatchability was conducted after eight weeks of feeding the supplements. In each treatment group semen from 5 birds was pooled (2 replicates / group) and samples were uniformly diluted to have 100 million sperm in 0.1 ml semen. This fixed dose semen was then inseminated into hens of 29 weeks age (10 hens / replicate). Insemination was done once in a week for 4 continuous weeks. Eggs collected on each day was marked to maintain group identity, stored for a maximum of 10 days and incubated. The eggs were candled on eighteenth day of incubation for observing developing embryos and per cent fertility calculated. Infertile eggs were broke opened for confirmation. Fertile eggs were transferred in to hatcher incubator. On 21st day of incubation the number of chicks hatched was recorded.

Statistical analysis to determine differences among treatments in semen parameters was done by repeated measures ANOVA, fertility and hatchability was done by one-way ANOVA (SAS 9.2) and Tukeys post hoc test. The treatment means were compared at $P < 0.05$. Individual birds were considered as replicates in a group and analysed accordingly. Percent values were arcsine transformed before analysis of data. Values given are least squares means±SEM.

The metabolic activity and percent live sperm of roosters supplemented with 100 mg/kg organic Zn and 0.35 mg/kg organic Se in the diet was significantly higher ($P < 0.05$) compared to other groups (Table 2). Other parameters like semen volume, appearance, sperm concentration and abnormal sperm per cent were not affected by supplementation of organic minerals in this study. The percent fertility but not hatchability was affected by organic mineral supplementation. No difference between weeks of experiment was observed in any of the parameters.

Zinc has important role in organisation of nucleic acids and is a part of many metalloenzymes that are involved in different cellular metabolism. Zinc is present in the active site of matrix metalloproteases that degrade components of the extracellular matrix. Zinc as part of superoxide dismutase enzyme protects biological structures against free radical damage. Zinc as well as selenium have role in anatomical development of reproductive organs and in spermatogenesis (Yamaguchi *et al.* 2009, Wu *et al.* 1973). Selenium is a component of many selenoproteins and glutathione peroxidise that has antioxidant activity. Thus both these minerals are required for normal semen production and sperm activity. In the present study organic selenium supplemented at 0.35 mg/kg produced higher metabolic activity of sperm and higher live sperm percent but no effect on other semen parameters. This is in contrast to the that reported by (Ebeid 2009) where lower level of organic selenium supplementation (0.1 mg/kg) lead to higher sperm count and lower dead sperm percent. Similarly, the semen parameters studied (volume, motility, sperm concentration and live sperm) were reported to be improved by the lower level of organic selenium supplementation in local cocks (Maysa *et al.* 2009). Furthermore, the fertility and hatchability of the selenium supplemented birds were also improved (Maysa *et al.* 2009). Selenium supplementation at 0.3 mg/kg diet improved sperm concentration and motility in turkey (S³owiñska *et al.* 2011). In the results of this study the abnormal sperm percent and seminal lipid peroxidation showed a decreasing trend in the highest selenium supplemented group though there was no statistical significance. Though there was not much change in the semen parameters in the group supplemented with 0.15 mg/kg selenium, the percent fertility was higher than the control. The results indicated that supplemental zinc and selenium, after absorption, gets incorporated in many enzymes and other proteins at molecular level and have played role in bringing about higher fertility. However, higher level of selenium (0.35 mg/kg) supplementation does not provide any additional advantage in terms of fertility. Since hatchability depends on the quality of embryo and incubation conditions, mineral supplementation and semen quality is of less important role in this regard. In conclusion supplemental zinc and selenium improves semen quality and fertility in layer breeders.

SUMMARY

The objective was to evaluate the effect of supplementation of organic zinc and selenium in breeder male diet on semen quality and fertility parameters. Thirty Dahlem Red males, 29 weeks old, were equally distributed into three treatment groups and supplemented with organic zinc (zinc proteinate) and organic selenium (yeast source) - group 1 (0 Zn / 0 Se), group 2 (100 mg/kg Zn / 0.15 mg/kg Se), group 3 (100 mg/kg Zn / 0.35 mg/kg Se) for 8 weeks. Dietary supplementation of 100 mg/kg Zn / 0.35 mg/kg Se combination significantly ($P < 0.05$) improved the MTT dye reduction value and percent live sperm. However, the percent fertility was significantly improved in both the treatment groups supplemented with zinc and selenium. The hatchability was not affected by mineral supplementation. In conclusion, supplementation of organic zinc and selenium improved the semen quality and fertility in layer breeders.

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