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Effects of *Zingiber officinale* Powder on Semen Characteristic and Blood Serum Sex Hormones Concentration in Broilers Breeder Male

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Abstract: This study was conducted to examine the effects of *Zingiber officinale* (ginger) powder on male broiler reproductive system. *Zingiber officinale* was administered in the feed of two groups of male broiler breeder (30 wk age) at levels of 5 and 10 kg/ton, Third group was a control group (no additive). Treatments were ejaculate volume, sperm concentration, counts, movements, motility and abnormality, blood serum LH, FSH and testosterone concentration. The ginger caused a significant increase ($p<0.05$) in ejaculate volume, sperm concentration, counts, movements and a significant decrease ($p<0.05$) in motility and abnormality. There was also a significant increase ($p<0.05$) in blood serum LH, FSH and testosterone levels. Our results indicated that *Zingiber officinale* powder possesses pro-fertility properties in male broiler which might be a product of androgenic activities.

Key words: *Zingiber officinale*, male broiler, reproductive system

INTRODUCTION

Zingiber officinale commonly called ginger belongs to the family of Zingiberaceae, it has been used in medicine for centuries. Today, ginger root is broadly used to prevent or treat pregnancy and cancer chemotherapy (Sripramote and Lekhyananda, 2003). Ginger is used in heart disease (Bhandari *et al*., 1998). The important active components of the ginger roots are thought to be volatile oils and pungent phenol compounds such as gingerols, shagols, zingerone and gingerols (Sekiwa *et al*., 2000; Zancan *et al*., 2002). Although the useful result of ginger has been subjugated, its activity on male reproductive functions except a study that reported that *Z. officinale* possess androgenic property (Kamtchouing *et al*., 2002). This employment was as a result carried out in sight of the scarcity of literature on the action of *Z. officinale* on reproductive functions activities in male broiler.

MATERIALS AND METHODS

Birds: A 72 male broiler breeder habrid classic (30 wk age) were housed in a birds house in typical condition with 16 h light:8 h dark lighting program. The birds were housed in bens (250*200) cm (8 birds each) in wood chip. Birds fed diet (Table 1), water offered all times.

Experimental design: Seventy two mature males were randomly divided into 3groups of 24 birds each (3 replicate each). Group 1 (control group) given a standard diets (no additive) while groups 2 and 3 were given a diet with an *Z. officinale* powder at 5 and 10 kg per ton. Treatments was given daily.

Sperm occupation analysis: Semen was collected by the massage method (Gee and Temple, 1978; Zhang and Zheng, 2002), which is performed in the following sequential steps. 1) Simultaneously massaging the outer wall of the cloaca and the root of the tail untilthe rudimentary copulation organ of the bird suddenly

Table 1: Composition of diets in experiment

	Broiler breeder diet		
	Starter	Grower	Laying
Ingredient and analysis	(%)		
Corn	66.11	68.00	66.40
Soybean meal (48% CP)	22.21	17.00	19.20
Wheat	7.64	10.87	6.00
Dicalcium phosphate	1.62	1.60	1.20
Limestone	1.24	1.28	6.10
Mineral premix[1]	0.20	0.20	0.05
Vitamin premix[2]	0.10	0.10	0.10
Salt (NaCl)	0.45	0.58	0.41
Coccidiostat	0.05	0.05	0.05
D,L-Methionine	0.08	0.03	0.07
Selenium premix[3]	0.10	0.10	0.10
Mold inhibitor	-	-	0.05
Lysine HCl	-	0.08	0.05
Choline chloride	0.20	0.20	0.12
Total	100.00	100.00	100.00
Calculated analysis[4]			
Crude protein (%)	17.00	15.00	16.03
AME (kcal/kg)	2,925.00	2,925.00	2,918.00
Lysine (%)	0.88	0.75	0.82
Methionine + cystine (%)	0.70	0.80	0.63
Calcium (%)	0.90	0.90	2.70
Available phosphorus (%)	0.45	0.45	0.42

[1]Mineral premix contained the following in milligrams per kilogram of diet: manganese, 120; zinc, 120; iron, 180; copper, 10; iodine, 2.5; cobalt, 1.0.

[2]Vitamin premix contained the following per kilogram of diet: vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; vitamin E, 66 IU; vitamin B12, 34.6 ug; riboflavin, 13.2 mg; niacin, 110 mg; pantothenic acid, 22 mg; vitamin K, 4 mg; folic acid, 2.2 mg; thiamine, 4 mg; pyridoxine, 8 mg and biotin, 252 ug.

[3]Selenium premix contained sodium selenite (Na_2SeO_3), providing 0.3 mg/kg.

[4]Data expressed on a percentage of dry matter basis. Formulations confirmed by proximate analyses

appears. This process takes about 15 to 30s. 2) Extruding the cloaca wall and the root of the penis moderately. The semen will be ejected along the longitudinal groove of penis at the same time. 3) Collecting the ejaculate with a 5-cc tube (fine scale is 0.01 mL). The ejaculate volume is read from the tube directly. To avoid expending more of the pheasant's energy, care is taken during capturing, restraining, and AI. Furthermore, semen collection would be abandoned if the ejaculate were not obtained after 1-min massaging. The semen collection experiment would be stopped if the ejaculate volume were regularly less than 0.10 ml.

The standard hemacytometer and light field microscope (Olympus Corp., Tokyo, Japan) were used to determine sperm concentration, motile sperm at a magnification of 400x. To measure the ratio of dead and abnormal spermatozoa, the smears of fresh semen were made on microscope slides. After preservation in 95% alcohol for 1 min and staining with 0.05% gentian violet solution for 3 min (Yang *et al*., 2006), 200 spermatozoa per smear were checked under the inner-light-field Olympus microscope at the magnification of 1,000x. Video playing was also used to check the dead and abnormal sperm. The JVC microscope-video system and Panasonic monitor (Victor Co., Japan) were used during the whole measuring process to improve the accuracy of sperm counting.

Serum FSH, LH total testosterone hormone measurements: Serum concentration of FSH and LH were determined in duplicated samples using Radioimmunoassay (RIA). Cocks FSH/LH kits obtained from Biocode Company-Belgium, according to the protocol provided with each kit. The sensitivities of hormone detected per assay tube were 0.2 ng/ml and 0.14 ng/ml for FSH and LH respectively. Serum concentration of total testosterone was measured by using a double antibody RIA kit from immunotech Beckman Coulter Company-USA. The sensitivities of hormone detected per assay tube were 0.025 ng/ml (Huang *et al*., 1995; Khaki *et al*., 2009).

Statistical analysis: Statistical comparisons were made using the ANOVA test for comparison of data in the control group and the experimental groups. The results were expressed as mean±SEM (standard error of means). Significant difference is written in parentheses.

RESULTS AND DISCUSSION

Results of ejaculate volume, sperms concentration, motility, dead and abnormal: Results of 5 and 10 kg/ton of ginger for 28, 32 and 36 wk significantly ($p<0.05$) increased ejaculate volume, concentration, motility, dead and abnormal sperms in both experimental groups as compared to the control group (Table 2). The mean of ejaculate volume was 0.275±0.07 ml. Concentration was 3.22±0.21 X 10^9, motility, dead and abnormal were 73±5.45, 18.45±2.25, 10.85±0.77% in T1 and the corresponding value in T2 were 0.345±0.05 ml and 3.93±0.33 X 10^9, 80±3.65, 13.75±0.45, 7.45±0.65% and in T3 was 0.455±0.08 ml and 4.54±0.32 X 10^9, 88.54±5.45, 9.66±0.85, 4.67±0.37%, the cause of this result may be due to the fact that ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals.

It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Ali *et al*., 2008). Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. It has become clear that constant generation of pro-oxidants, including oxygen free radicals, is an essential attribute of aerobic life (Sies, 1991). A disturbance in the pro-oxidant/antioxidant system has been defined as oxidative stress. Reactive Oxygen Species (ROS) are very reactive molecules ranked as free radicals owing to the presence of one unpaired electron such as a superoxide ion (O-2), Nitrogen Oxide (NO) and hydroxyl radical (HO-). Even though naturally present in the organism, they are mainly confined to cell compartments and counterbalanced by natural antioxidant molecules, such as glutathione, glutathione peroxidase, superoxide dismutase, vitamin E and vitamin C, acting as free radical scavengers (Aruoma *et al*., 1994; Miller *et al*., 1993). Ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities (Ahmed *et al*., 2000) found that ginger significantly lowered lipid per oxidation by maintaining the activities of the antioxidant enzymes-super oxide dismutase, catalase and glutathione peroxides in rats. Besides, Sies (1991) suggested that abnormal sperm morphology combined with elevated ROS production may serve as a useful indicator of potential damage to sperm DNA. On the other hand, spermatozoa are highly susceptible to damage by excessive concentrations of ROS due to the high content

Table 2: The effect of the 5 and 10 kg/ton ginger on sperm parameters of control and experimental groups in the broiler breeder male

Parameter's	T1	T2	T3
Ejaculated volume (ml)	0.275±0.07C	0.345±0.05B	0.455±0.08A
Sperm concentration X(10) 9	3.220±0.21C	3.930±0.33B	4.540±0.32A
Motility (%)	73.000±5.45C	80.000±3.65B	88.540±5.45A
Dead sperm (%)	18.450±2.25A	13.750±0.45B	9.660±0.85C
Abnormal sperm (%)	10.850±0.77A	7.450±0.65B	4.670±0.37C

Data are presented as mean ± SE. Significant different at $p<0.05$ level (compared with the control group)

Table 3: The effect of the 5 and 10 kg/ton of ginger powder on concentration of LH, FSH (Miu/ml) and Testosterone (ngr/ml) of control and experimental groups in the broiler breeder male

Parameter's	T1	T2	T3
LH	10.38±0.35B	13.23±0.43B	18.34±0.76A
FSH	103.40±3.45B	119.50±2.23A	132.20±4.43A
Testosterone	0.88±0.02B	1.23±0.04B	1.76±0.08A

Data are presented as mean ± SE. Significant different at $p<0.05$ level (compared with the control group)

of polyunsaturated fatty acids within their plasma membrane. The lipid per oxidation destroys the structure of lipid matrix in the membranes of spermatozoa and it is associated with loss of motility and impairment of spermatogenesis (Sharma and Agarwal, 1996). In the present study, administration of 5 and 10 kg/ton ginger for twenty consecutive weeks significantly increased sperm motility and viability in both experimental groups as compared to the control group (Table 2). These results are supported by the finding of Aitken *et al.* (1995), who reported that the conventional basic semen characteristics other than motility are not obviously influenced by the oxidative state of semen. This increase in sperm motility of experimental groups in comparison to control group could be due to the protective effect of ginger rhizoma administration. Besid, these productive effects are reflected by the decrease of malonaldehyde level and increase in total anti oxidants capacity (Table 3). In accordence with these results, Amr and Hamza (2006) have demonstrated that *Z. officinale* treatment increased the activities of testicular antioxidants enzyme and restore sperm motility of cisplatin-treated rats. Amr and Hamza (2006) reported in animal models that *Z. officinale* have protective effects against cisplatin-induced testicular damage and oxidative stress in rats. Ginger rhizome contains a wide variety of both antioxidative (Sekiwa *et al.*, 2000) and androgenic activity (Kamtchouing *et al.*, 2002). The major active phenolic ingredients isolated from *Z. officinale* (Zingerone, Gingerdiol, Zingibrene, gingerols and shogaols) have antioxidant activity (Zancan *et al.*, 2002; Kamtchouing *et al.*, 2002; Jorsaraei *et al.*, 2008).

Results of serum total testosterone, LH and FSH hormones measurement: Administration of 50 mg/kg/cock and 100 mg/kg/cock ginger for twenty consecutive weeks had significant effect ($p<0.05$) on LH, FSH and Testosterone concentration in the serum between the T1, T2 and T3 groups. The concentration of LH, FSH (Miu/ml) and Testosterone (ngr/ml) were 10.38±0.35, 103.4±3.45 and 0.88±0.02 in T1 the corresponding value in T2 were 13.23±0.43, 119.5±2.23 and 1.23±0.04 and in T3 were 18.34±0.76, 132.2±4.43 and 1.76±0.08 (Table 3). These findings showed that *Z. officinale* extracts have a potent androgenic activity (Amr and Hamza, 2006). In agreement with these reports; the present study showed an increase in the sex hormones levels.

In conclusion, the present study has demonstrated that, ginger aqueous extract possess an antioxidant and androgenic activity in doses of 5 and 10 kg/ton and have a useful effects on spermatogenesis and sperm parameters in broiler breeder males.

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