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Effects of *Zingiber officinale* Aqueous Extract on Semen Characteristic and Some Blood Plasma, Semen Plasma Parameters in the Broilers Breeder Male

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Abstract: To investigate the effects of *Zingiber Officinale* on male reproductive functions and study the mechanisms underlying these effects, aqueous extract of *Zingiber officinale* were administered in drinking water to two groups of male broilers breeder (24wk age) at 5% and 10%. A third group served as control and received the treatment vehicle, distilled water. Treatment lasted for 28, 32, 36, 40 and 44 wk age. Ejaculate volume, sperm concentration, counts, movements, motility and abnormality, semen plasma cholesterol, protein and glucose, the antioxidant malonhydialdehyde, glutathione and blood serum LH, FSH and testosterone, were determined. The treatment caused a significant increase ($p<0.05$) in the weight of the testis and There were dose and duration dependent increases 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 semen plasma cholesterol, glucose and a significant decrease ($p<0.05$) in protein. Antioxidant malonhydialdehyde were significantly reduced ($p<0.05$), glutathione and blood serum LH, FSH and testosterone serum level were significantly increase ($p<0.05$). Our results indicated that extract of *Zingiber officinale* possesses pro-fertility properties in male broiler which might be a product of both its potent antioxidant properties and androgenic activities.

Key words: *Zingiber officinale*, semen characteristic, broilers breeder male

INTRODUCTION

Zingiber officinale commonly called ginger belongs to the family Zingiberaceae. The plant is a knotted, thick, beige underground stem (rhizome) that has been used in traditional medicine to aid digestion and treat stomach upset, diarrhea, nausea and arthritis for centuries. In addition to these medicinal uses, ginger continues to be valued around the world as an important cooking spice and is believed to help the common cold, flulike symptoms, headaches and even painful menstrual periods. Today, ginger root is widely used as a digestive aid for mild stomach upset and is commonly recommended by health care professionals to help prevent or treat nausea and vomiting associated with motion sickness, Pregnancy and cancer chemotherapy (Bone *et al*., 1990; Sripramote and Lekhyananda, 2003). Ginger is used as support in inflammatory conditions such as arthritis (Altman and Marcussen, 2001) and may even be used in heart disease (Bhandari *et al*., 1998) or cancer (Katiyar *et al*., 1996). The important active components of the ginger root are thought to be volatile oils and pungent phenol compounds such as gingerols, shogaols, zingerone and gingerols (Sekiwa *et al*., 2000; Zancar *et al*., 2002). Although the beneficial effect of ginger has been exploited, little research has been conducted on its activity on male reproductive functions except a study that reported that *Z. officinale* possess androgenic property (Kamtchouing *et al*., 2002). This work was therefore carried out in view of the paucity of literature on the action of *Z. officinale* extract on reproductive functions and antioxidant activities in male broiler.

MATERIALS AND METHODS

Animals: Adults of 56 male broiler breeder (24wk age) were housed in a well ventilated animal house under standard condition of humidity, temperature and a constant 16 hour light:8 hour dark lighting schedule. The animals were randomly housed in clear polypropylene cages (50*150*40)cm(6bird each) in lined with wire chip beddings. They were fed with standard diet (Table 1) and water was made available at all times. The health and reproductive status of the animals were assessed and only healthy animals were selected for the experiment.

Extraction of plant material: The *Z. officinale* rhizome was purchased from local commercial sources and shade dried at room temperature before being pulverized with an electric grinder. The extracts were then obtained by maceration method with distilled water for 48 h to obtain a final aqueous concentration of 1 mg/ml.

Experimental design: Fifty six adult male were randomly divided into 3 groups of 18 animals each (3 replicate

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Table 1: Composition of starter (0 to 2 wk), grower (3 to 24 wk) and laying (25 to 64 wk) diets in experiments

	Broiler breeder diet (%)		
Ingredient and analysis	Starter	Grower	Laying
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	0.0	0.0	0.05
Lysine HCl	0.0	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; antothenic 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

each). Group 1 (control group) was administered with the vehicle (distilled water) while groups 2 and 3 were given an aqueous suspension of *Z. officinale* at 5% and 10%. five cocks from each experimental group were randomly sacrificed after 36 wk of extract administration to determine the different in testes weight. Treatment was done daily.

Sperm function analysis: Semen was collected by the massage method (Gee and Temple, 1978; Howe, 1981; 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 appears. This process takes about 15 to 30 s. 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, 1995), 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 (Haung *et al*., 1995; Khaki *et al*., 2009).

Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA) concentration measurement in semen plasma: A TAC detecting kit was obtained from Nanjing Jiancheng Bioengineering Institute-China. According to this method, the antioxidant defense system, which consists of enzymatic and non-enzymatic antioxidants, is able to reduce Fe^{3+} to Fe^{2+}. TAC was measured by the reaction of phenanthroline and Fe^{2+} using a spectrophotometer at 520 nm. At 37°C, a TAC unit is defined as the amount of antioxidants required to make absorbance increase 0.01 in 1 mL of serum. Free radical damage was determined by specifically measuring Malondialdehyde (MDA). MDA was formed as an end product of lipid peroxidation which was treated with thiobarbituric acid to generate a colored product that was measured at 532 nm (MDA detecting kit from Nanjing Jiancheng Bioengineering Institute-China) (Quintanilha *et al*., 1982).

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.

Table 2: The effect of the 5% and 10% ginger aqueous extract on testis weight and sperm parameters of control and experimental groups in the broiler breeder male

Parameter's	G1 Control	G2 Ginger rhizome 5%	G3 Ginger rhizome 10%
Testes weight (gr)	17.710±0.97C	20.930±0.45B	27.100±0.37A
Ejaculated volume(ml)	0.393±0.02C	0.475±0.04B	0.724±0.05A
Sperm concentration [$x10^9mL^{-1}$]	2.890±0.33C	3.840±0.12B	4.980±0.12A
Motility (%)	60.000±4.35C	70.000±2.33B	79.340±1.32A
Dead sperm (%)	24.210±0.68A	19.270±1.23B	12.120±1.54C
Abnormal sperm (%)	15.980±43A	13.120±1.43B	8.660±0.64C

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% ginger aqueous extract on concentration of LH, FSH and Testosterone (ngr/ml) of control and experimental groups in the broiler breeder male

Parameter's	G1 Control	G2 Ginger rhizome 5%	G3 Ginger rhizome 10%
LH	9.55±0.23B	9.55±0.23B	12.53±0.35A
FSH	10.06±0.24B	11.83±0.15A	12.38±0.72A
Testosterone	1.17±0.04B	1.45±0.05B	2.34±0.04A

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

Table 4: The effect of the 5% and 10% ginger aqueous extract on concentration of MDA and TAC of control and experimental groups in the broiler breeder male

Parameter's	G1 Control	G2 Ginger rhizome 5%	G3 Ginger rhizome 10%
MDA	0.42±0.04A	0.19±0.01B	0.16±0.031B
TAC	0.73±0.06C	0.94±0.04B	1.18±0.08A

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

RESULTS

Weight of individual male testis: The obtained results in this study are illustrated in Table 2. There was significant (p<0.05) difference in testes weights between the groups and it were higher for G3 (27.1±0.37). G2 (20.93±0.45) compared to G1 (control group) (17.71±0.97).

Ejaculate volume, sperm concentration, motility, dead and abnormal: Administration of 50 mg/kg/cock and 100 mg/kg/cock ginger for 28, 32, 36, 40 and 44wk consecutive significantly (p<0.05) increased ejaculate volume, sperm concentration, motility, dead and abnormal in both experimental groups as compared with the control group (Table 2). The mean of ejaculate volume was (0.393±0.02) ml concentration was (2.89±0.33) $x10^9$, motility, dead and abnormal were (60±4.35% and 24.21±0.68, 15.98±43) % in G1 and the corresponding value in G2 were (0.475±0.04) ml and (3.84±0.12) $x10^9$, (70±2.33, 19.27±1.23, 13.12±1.43)% and in G3 was (0.724±0.05) ml and (4.98±0.12) $x10^9$, (79.34±1.32, 12.12±1.54, 8.66±0.64)%.

Results of serum total testosterone, LH and FSH hormones measurement: Administration of 50 mg/kg/rat 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 control (G1) and G2 and G3 groups. The concentration of LH, FSH and Testosterone were (9.55±0.23, 10.06±0.24 and 1.17±0.04) in G1 the corresponding value in G.2 were (9.55±0.23, 11.83±0.15 and 1.45±0.05) and in G3 were (12.53±0.35, 12.38±0.72 and 2.34±0.04) (Table 3).

Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA) concentration measurement in Serum: The mean concentration of Malondialdehyde (MDA) level was significantly (p<0.05) lower in G3 (0.16±0.031) and G2 (0.19±0.01) in comparison to control group (0.42±0.04). Total Antioxidant Capacity (TAC) was significantly higher (p<0.05) in G3 (1.18±0.08) and G2 (0.94±0.04) as compared with control group (0.73±0.06) (Table 4).

DISCUSSION

The main pharmacological actions of ginger and compounds isolated there from include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. 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. Cellular damage in the semen is the result of an improper balance between ROS generation and scavenging activities. Excessive ROS production that exceeds critical levels can overwhelm all antioxidants defense strategies of spermatozoa and seminal plasma causing oxidative stress (De *et al*., 1997; Sikka, 1996). Therefore, ROS production and Total Antioxidant Capacity (TAC) can be used as a marker of oxidative stress in seminal fluid and is correlated with male infertility. Infertile men with male factor or idiopathic diagnoses had significantly lower ROS-TAC scores than controls (Sharma and Agarwal, 1996). Besides, Said *et al*. (2005) 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 of polyunsaturated fatty acids within their plasma membrane. The lipid peroxidation 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% ginger for twenty consecutive weeks significantly increased sperm motility and viability in both experimental groups as compared with 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 is reflected by the decrease of malonaldehyde level and increase in total anti oxidants capacity (Table 3). 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). Others reported that *Z. officinale* extracts have a potent androgenic activity in male rats (Amr and Hamza, 2006). In agreement with these reports; the present study showed an increase in the testes weight, serum testosterone levels. In conclusion, the present study has demonstrated that, ginger aqueous extract possess an antioxidant and androgenic activity in doses of 5% and 10% and have a useful effects on spermatogenesis and sperm parameters in broiler breeder males.

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