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Effects of *Thymus vulgaris* and *Zingiber officinale* Aqueous on Semen Parameters, Testes Weight and Histology Measurements of Broiler Breeder Male

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Abstract: To examine the effect of Ginger rhizome and thyme leaf aqueous extract on male reproductive system and the mechanisms of these effects the aqueous were add two drinking water to two groups of male broilers breeder ROSS308, the addition start in 24wk and end in 44wks age at levels 5% and 10% for each plant aqueous where the other group was a control (received a distilled water). ejaculate volume, sperm concentration, counts, movements, motility, abnormality, testes weight and histology measurements were the mean characteristics studied. There were a significant increase ($P<0.05$) in ejaculate volume, sperm concentration, counts, movements and a significant decrease ($P<0.05$) in motility and abnormality, also there were a significant increase ($P<0.05$) in testes weight, percentage and histology measurements, there for my results refer to that extract of ginger and thyme conceder as pro-fertility properties in male broiler which might be a product of both its potent antioxidant properties and androgenic activities.

Key words: Ginger, broiler breeder male, reproductive

INTRODUCTION

Since 1950 till 1980 the number of studies focus and pay attention on the use of herbs and medicinal plants for improving the reproductive system, All these works aimed to minimized or prevent the use of certain antibiotics and hormones as stimulant for growth in the animal feed in many European countries and America as a result of the harmful effect of them on human health due to the accumulation of remnants of these substances in animal products, as well as consumer awareness of health towards the use of natural materials of vegetable origin (Castanon, 2007). Ginger and thyme was used in a variety in human nutrition as a spice and food flavor and as anti-oxidant for treatment of many diseases (Mathur, 2003). Also, it was used in animal feed and poultry as antioxidants and as stimulant for growth (Omage *et al*., 2007). Also, it was found that ginger has the properties of sex hormones and in particular hormones androgenic properties (Sekiwa *et al*., 2000; Kamtchouing *et al*., 2002). Sultin *et al*. (2008) used thyme leave in reducing the age of puberty in female of domestic rabbits. The ginger oil's has a role in the conservation and protection of the DNA from oxidation by hydrogen peroxide (H_2O_2) and the protection from the harmful effects of the reactive oxygen species (Rajeev *et al*., 2006 and Yang *et al*., 2006). The importance action of antioxidants by protecting the DNA from damage is by improving the quality of sperm and thus increase the rate of fertility in humans. In a recent study it was found by Khaki *et al*. (2009) that the addition of ginger had significant effect in semen increased and That thyme leaf, ginger roots did not appear so far to have a negative impact on animal or human health when it was used by human on daily basis

MATERIALS AND METHODS

Extraction of plant material: The *Zingiber officinale* rhizome and *Thymus vulgaris* leafs were grind with an electric grinder, then extracts were made by adding 150gm with 1 later of distilled water and left to 48 hours to obtain a final aqueous concentration of 150 mg/ml (Shanoon, 2011).

Birds and Experiment design: The experiment was conducted in poultry farm by using 30 male (6 male /treat) of the broiler breeder Ross308 at the age of 22 weeks and the experiment period were from 22 to 44 wks of old. The birds were numbered, use of cages of dimensions (100×50×55) cm for the purpose of breeding birds therein and the distribution & classification of birds and distribution of treatments are shown below:

1: The first group was a control were The birds provided with a drinking water (without any addition)
2: The 2nd group was treat with 5% the aqueous extract of thyme leaves
3: The third group was treat with addition of 10% of aqueous extract of thyme leaves in drinking water
4: The fourth group was treat with 5% of aqueous extract of ginger in the drinking water
5: The 5th group was treat with addition of 10% of aqueous extract of ginger tubers.

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The experiment was start after two weeks of adaptation of birds to the new environment and this period was considered as the adjustment period and at the age of 24 weeks the plant extracts was introduce by adding them in the drinking water from 8 am to 8 pm and then after that the drinking water without any addition was given.

Semen collection: Semen samples were collected from birds five times per week and for two consecutive weeks to ensure daily production estimate of the actual sperm (Shanoon, 2011) at the same time (8 am). The semen samples were collected at the age of (28, 32, 36, 40, 44, 48, 52 and 56) wk.

Semen samples from birds were collected by standard procedure described by Shanoon (2011).Semen parameters (ejaculate volume, sperm count, massive movements, alive and dead sperm %) were done according to standard procedure.

Histopathology: After the end of the experiment (44wk old) all males were sacrificed and testis were weighted and fixed in 10% formalin and embedded in paraffin. Five-micron thick sections were prepared and stained with Hematoxylin and Eosin (H and E). The specimens were examined under Olympus/3H light microscope-Japan and the measurements were taken by six samples each replicates of treatments.

Statistical analysis: Data from experiment 1 were analyzed as a generalized randomized complete block design with the three replicates(6 male each) as blocks. Means were separated using Fisher's protected least significant difference (P<0.05). the results were expressed as mean±S.E.M (standard error of means).

RESULTS

Ejaculate volume (ml): Effect of aqueous extract of ginger and thyme on ejaculate volume of broiler breeder males Ross308 were shown in Table 1, there was a significant increase in the rate of ejaculate volume compared with control group, especially the group of aqueous extract of ginger 10% compared to other treatment groups (5% ginger, 5 and 10% thyme groups and control group) in all experiment periods While there were no significant difference between the groups but it show significant increase in ejaculate volume as compare to control group. As far as Table 1 indicated a significant differences (P<0.05) in ejaculate volume between periods, it show increase in volume from 28 to 44 weeks except control group.

Sperm concentration (X10 9): The results in Table 2 indicated the scale described the existence of significant differences (P<0.05) in the concentration of sperm in the semen parameters between the aqueous extracts of ginger, thyme and the control group. Ginger treatment 10% results a significant increased of sperm concentration in all periods (3.98, 4.22, 4.55, 4.78 and 4.92) Billion/ml compared to other 10% of thyme (3.77, 3.88, 3.95, 4.22 and 4.45) Billion/ml, ginger 5% (3.71, 3.80, 3.88, 4.15 and 4.55) and thyme 5% (3.68,3.74,3.83,3.97 and 4.33) and control (3.33, 3.40, 3.56, 3.65 and 3.67) Billion/ml, respectively. There were no significant difference between treatment of thyme 10%, ginger, thyme 5% but there were a significant differences with the treatment of control (P <0.05).

As indicated in Table 2 also for the existence of significant differences between the periods age for all transactions of the experiment, have taken the average concentration of sperm superiority with age in the

Table 1: Effect of aqueous extract of thyme and ginger on ejaculate volume (ml)

Age		Thyme conc. (%) ginger conc. (%)			
Wk	Control	5	10	5	10
28	0.354±0.015D a	0.421±0.058B b	0.466±0.056B b	0.435±0.043B b	0.510±0.056A c
32	0.366±0.032C a	0.457±0.022B ab	0.490±0.044B ab	0.465±0.056B ab	0.0570±0.033A b
36	0.365±0.053C a	0.460±0.053B ab	0.510±0.036B ab	0.470±0.035B ab	0.610±0.052A ab
40	0.370±0.023C a	0.470±0.032B a	0.515±0.043B a	0.475±0.062B ab	0.625±0.012A a
44	0.375±0.022D a	0.476±0.033C a	0.520±0.033B a	0.480±0.054BC a	0.650±0.037A a

Data are presented as mean ± SE; Significant different at p< 0.05 level (compared with the control group). Letter A,B,C and D refer to different between treatments. Letter a,b and c refer to different between periods

Table 2: Effect of aqueous extract of thyme and ginger on sperm concentration (109 Ml-1)

Age		Thyme conc. (%) ginger conc. (%)			
Wk	Control	5	10	5	10
28	3.33± 0.03C b	3.68 ± 0.05B b	3.77±0.05AB b	3.71±0.06AB c	3.98±0.04A d
32	3.40±0.06C b	3.74±0.04BC b	3.88±0.07B b	3.80±0.04B c	4.22±0.03A c
36	3.56±0.04C ab	3.83±0.06BC b	3.95±0.04B b	3.88±0.06B bc	4.55±0.07A b
40	3.65±0.05C ab	3.97±0.03BC ab	4.22±0.08B a	4.15±0.05B b	4.78±0.08A ab
44	3.67±0.05C a	4.33±0.07B a	4.45±0.05B a	4.55±0.05B a	4.92±0.05A a

Data are presented as mean ± SE. Significant different at p<0.05 level (compared with the control group). Letter A,B,C and D refer to different between treatments. Letter a,b and c refer to different between periods

Table 3: Effect of aqueous extract of thyme and ginger on sperm massive movement (%)

		Thyme conc. (%) ginger conc. (%)			
Age Wk	Control	5	10	5	10
28	70.33±3.22B b	71.54±4.66B c	75.43±5.34AB b	73.33±3.65B c	77.44±4.76A c
32	73.54±4.55C ab	76.43±6.37BC b	77.65±6.87B b	77.43±7.67B bc	81.23±6.45A b
36	74.76±3.76C a	78.46±5.65B a	79.51±4.33B ab	78.34±7.53B b	83.55±2.32A b
0	75.45±3.32C a	79.54±5.43B a	81.55±3.44B a	80.22±2.43B ab	85.44±7.56A ab
44	76.22±6.82C a	80.43±7.43B a	82.55±4.66B a	82.43±2.44B a	87.45±3.65A a

Data are presented as mean ± SE. Significant different at p<0.05 level (compared with the control group). Letter A,B,C and D refer to different between treatments. Letter a,b and c refer to different between periods

Table 4: Effect of aqueous extract of thyme and ginger on dead sperm (%)

		Thyme conc. (%) ginger conc. (%)			
Age Wk	Control	5	10	5	10
28	19.33±1.32A a	18.33±2.85A a	16.33±2.54A a	17.33±2.41A a	13.36±0.76B a
32	17.65±2.65A b	14.44±1.77A b	14.86±1.33A ab	14.34±1.54A a	10.54±1.65B ab
36	16.32±2.21A b	12.43±1.65A bc	12.15±0.47A b	12.68±1.87A b	8.55 ± 0.35B b
40	14.64±1.76A b	11.33±1.65A c	11.21±1.33A b	11.92±1.34A b	8.45 ± 0.76B bc
44	15.43±2.43A b	11.12±0.55B c	10.33±1.38 B b	10.65±1.43B b	7.54 ± 0.43C c

Data are presented as mean ± SE. Significant different at p< 0.05 level (compared with the control group). Letter A,B,C and D refer to different between treatments. Letter a,b and c refer to different between periods

Table 5: Effect of aqueous extract of thyme and ginger on abnormal sperm (%)

		Thyme conc. (%) ginger conc. (%)			
Age Wk	Control	5	10	5	10
28	14.24±0.45A a	14.74±1.43A a	11.22±1.48AB a	12.35±1.36AB a	10.42±1.34B a
32	13.43±0.47A b	11.13±1.23AB b	10.44±1.33B ab	11.45±1.66B a	9.54 ± 1.65B ab
36	13.12±1.34A b	11.65±1.32A bc	9.88±1.58BC b	10.44±1.38B b	7.32 ± 0.58C b
40	12.23±1.54A b	10.23±1.83AB c	8.78±1.45B b	9.74±1.32B b	6.43 ± 0.53C bc
44	11.28±1.56A b	9.43±1.28AB c	8.12 ±1.87B b	8.76±1.54B b	5.32 ± 0.36C c

Data are presented as mean ± SE. Significant different at p< 0.05 level (compared with the control group). Letter A,B,C and D refer to different between treatments. Letter a,b and c refer to different between periods

Transactions of control and all of the treatment of ginger 5 and 10 % and thyme 5 and 10% massive movement of sperm (%). From the results of Table 3 the existence of significant differences (P<0.05) in the percentage rate of the movement's collective sperm between the totals of aqueous extracts of ginger, thyme, compared with the treatment of control, the treatment of ginger 10% had a higher rate of massive movement compared to other aggregates which amounted (77.44, 81.23, 83.55, 85.44 and 87.45)% compared to (73.33, 77.43, 78.34, 80.22 and 82.43)% for ginger 5%,(75.43, 77.65, 79.51, 81.55 and 82.43)% for thyme 10% and (71.54, 76.43, 78.46, 79.54 and 80.43) to thyme 5% treatment and all treatment were significant differences (P <0.05) in the percentage rate of the movement's compared to control which recorded (70.33, 73.54, 74.76, 75.45 and 76.22).

Each of the 10% treatment thyme, ginger and 5% and control, respectively had seemed a significant differences (P <0.05) in the percentage rate of the movement's with age periods tall the end of experiment.

dead sperm (%).

Table 4 shows the existence of significant differences (P<0.05) in the proportion of dead sperm between transactions aqueous extracts of ginger and another treatments of ginger 5%, thyme 5, 10% and the control group, as recorded treatment Ginger 10% showed the lowest reached in all periods (13.36±0.76, 10.54±1.65, 8.55±0.35, 8.55±0.35, 8.45±0.76 and 7.54±0.43) compared to all treatments, while the periods showed that ginger 10% are become a significant decrease (P<0.05) in the percentage of dead sperm and lowest when age are become bigger but another treatments showed a significant decrease (P<0.05) in the percentage of dead sperm just in the first period (32wk) and it were not a significant decrease (P<0.05) in the percentage of dead sperm for the periods of experiment.

Abnormal sperms (%).

The results shown in Table 5a significant differences (P<0.05) in the proportion of abnormal sperms in the semen between transactions aqueous extracts of ginger, thyme and the control group being of recorded treatment Ginger 10% show lower rate of sperm abnormal, amounting to (10.42±1.34, 9.54±1.65, 7.32±0.58, 6.43±0.53, 5.32±0.36) in while the control treatment recorded the highest percentage of abnormal sperm (14.24±0.45, 13.43±0.47, 13.12±1.34, 12.23±1.54, 11.28±1.56). As indicated in Table 5 a significant differences (P<0.05) between the periods in

Table 6: Effect of aqueous extract of thyme and ginger on testes weight (gm), testes weight (%) and some histology measurements

		Thyme conc. (%) ginger conc. (%)			
	Control	5	10	5	10
Testes weight (gm)	18.53±0.44C	20.44±0.33BC	21.33±0.65B	23.32±0.87B	28.54±0.55A
Testes%	1.1±0.02B	1.3±0.04B	1.5±0.08B	1.5±0.05B	1.8±0.04A
Seminiferous tubes bowl area (*M)2*	2540±22A	2100±44B	1950±33B	1880±65C	1650±54D
seminiferous tubes thickness (*M)2*	5.22±0.66C	5.66±0.43B	5.88±0.37B	5.78±0.75B	6.33±0.34A
Seminiferous germinal thickness (*M)*	24.22±2.43C	30.54±3.54B	33.85±2.25B	35.34±3.43B	42.63±2.43A
Seminiferous germinal area (*M)2*	7233.1±255C	9353.3±323B	9734.3±433B	9876.5±243B	11643±373A

Data are presented as mean ± SE. Significant different at p< 0.05 level (compared with the control group). Letter A,B,C and D refer to different between treatments

all the transactions had registered the highest percentage of deformed sperm during the periods of age (28wk) compared to another for the treatment of control and treatment of ginger 5 and 10 %, thyme 5% and 10%, with the survival results of the recorded results and less moral superiority to the rest of the transaction for treatments on control group.

Histopathology: In this experiment results improve the effect of ginger aqueous in testes (weight and percentage) seminiferous tubes bowl, seminiferous tubes thickness, seminiferous germinal thickness and area of testes measurements (Table 6), the results of treatments ginger 10% has a significant differences (P<0.05) in the testes weight (28.54gm), testes weight percentage from body weight(1.8%) compared to another treatments and control. But there were a significant increase (P <0.05) in the seminiferous tubes bowl area for control treatment (2540 m^2) compared to other treatments but it seemed that ginger treatments (especially 10%) have a significant differences (P<0.05) in the seminiferous tubes thickness, seminiferous germinal thickness (42.63) *M.* seminiferous germinal area (11643)(*M)2*.

DISCUSSION

In this study, the result of adding water extracts of each of ginger and thyme produce a significant increase in semen ejaculate volume, number of sperm in the ejaculate and massive and individual sperm movement, dead sperm % and abnormal sperm morphology (P<0.05), testes weight and weight% and histopathology may be attributed to the reason that ginger thyme contain a broad spectrum of nutrients and chemical compounds that have a positive effect on the vital functions of a bird, It was found that ginger contains Capsaicin, Zingerone, Shagaols, gingerol, phenolic, curcumin, proteolysis, vitamin C and E (Zancan *et al*., 2001; Sekiwa, 2005; Grzanna, 2005 and Belewu, 2009). Also, thyme contains flavonoids, terponoids, thymol, carvacol and eugenol and vitamin E (Guillen *et al*., 1998 Radwan, 2003 and Bölükbasi *et al*., 2006) and all so there were in observed in many laboratory studies the ability of these compounds to break the chain reaction of oxidation from free radicals and scavenged for many types of free radicals such as superoxide and hydroxyl and peroxy radial (Halliwell *et al*., 2005). As well as the poly phenols prevent the oxidation of enzymes that inhibit the formation free radicals (Oleary *et al*., 2004). Also, the protection of DNA and plasma membrane and mitochondria of the sperm. Because the sperm is more vulnerable and sensitive to free radicals, because of the high concentration of unsaturated fatty acids in sperm membrane (Al-Daraji *et al*., 2007).

This explains the low percentage of dead sperm and abnormal sperm morphology and increase the rate of massive movement of sperm in all groups treated with water extract of ginger and thyme. The present finding is similar to the result of Khaki *et al*. (2009) in rats by adding of 100 mg/kg body weight/day cause an increase in the percentage of living sperm and massive movement of sperm. Also increase in testosterone concentration. These findings are consistent with previous studies, each of Rajeev *et al*. (2006) and Yang *et al*. (2006), who emphasized the act of antioxidants in protecting the DNA from oxidative damage, as well as improve the quality of semen and thereby increase the fertility of mice and humans, as noted by Amin and Hamza (2006) increase the activity of antioxidants in the testis and the movement of sperm in treated mice by alcohol extract of ginger at dose of 10 mg/kg of body weight for 26 days. The ginger had a real antioxidant activity than that done by vitamin E and C (Bölükbasi *et al*., 2006). Also, an increase in the resistance of glutathione (Dickinson *et al*., 2003), thereby reducing the lipid oxidation which lead to the deterioration of semen quality (Sekiwa, 2000). In a study conducted by Jarsia *et al*. (2007) who noted that the addition of ginger extract at concentration of (0,1 and 0.2 and 0.4 and 0.6%) led to significant improvement in the qualities of the semen particularly significant decrease in the proportion of dead sperm and concluded that Ginger actually brake or prevent the Lytic activity within semen fluid as antioxidant, especially camphene which is one of the active compounds in ginger which works antioxidant through his union with the free radicals. On the other hand it was found that water extracts of plants that rich in phenolic substances have a role in the

prevention of decomposition oxidative fat as ginger and thyme (Kamtchouing *et al*., 2002; Radwan *et al*., 2008).

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