EFFECT OF GINGER EXTRACT ON REPRODUCTIVE PERFORMANCE OF MALE RABBITS

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ABSTRACT: Thirty 7-month old V-line rabbit bucks, with average initial weight of 3.325 Kg were used in the trial. The rabbits were homogeneously divided into 3 groups (10 males/group). The groups were fed the same commercial diet and submitted to the following dietary treatments: control group (without additives); G150 and G250 groups fed an aqueous extract of Z. Officinale at 150 and 250 mg/kg body weight, respectively twice per week for 12 weeks. Semen was collected weekly over the time of the study and blood samples were collected every two weeks. Males of each group were bred with receptive nulliparous female rabbits for recording same parameters such as: kindling rate, litter size at birth (total born and total born alive), and at weaning. Also, bunny weights at birth and at 28 days were recorded.

Main results obtained can be summarized as follows:

1- Supplementating the bucks with both concentrations of ginger extract increased \( P<0.05 \) each of ejaculate volume, sperm motility, sperm concentration, total sperm output, total motile sperm, live sperm and total functional sperm fraction.

2- Seminal plasma total protein, albumen and acid phosphatase were increased \( P<0.05 \) in ginger groups compared with control one.

3- Seminal plasma antioxidant activities were improved \( P<0.05 \) with the increase of ginger extract level. Also, plasma thiobarbituric acid-reactive a substance was decreased compared to control one.

4- Oral administration of 150 and 250 mg/kg BW extract of ginger had no significant effect on serum LH and FSH concentrations compared with the control group. In addition, serum total testosterone level was increased \( P<0.05 \) in animals received both low and high doses in comparison to control group

5- Conception rates of females mated with rabbit bucks supplemented with both concentrations of ginger were improved \( P<0.05 \) compared with the control group. The total and live litter sizes at birth, litter size at 28 days were also higher \( P<0.05 \) than those of the control group but without significant change in the ginger supplementation groups except bunny weight at 28 days.

6- Protein band with molecular weight (100.7 kd) has the highest intensity in seminal plasma protein for high dose of ginger extract group followed by low one compared to control.

7- Histopathological study showed that the cycle of spermatogenesis is regular in all experimental and control groups.
INTRODUCTION

Ginger rhizome (Zingiber officinale R., family Zingiberaceae) is used worldwide as a spice. Both antioxidative (Khaki et al., 2009) and androgenic activity (Kirtikar and Basu 1991) of Z. officinale were reported in animal models. The importance of ginger root is thought to contain some active phytochemical components such as volatile oils, gingerol, gingerone, piperine, shogaols and zingerone (Zancan et al., 2002). All major active ingredients in these components have antioxidant activity (Nassiri et al., 2009).

The natural antioxidants can protect DNA and other molecules from cell damage induced by oxidation, improving sperm quality and increasing reproductive efficiency of men (Yang et al., 2006). Ahmed et al. (2000) found that ginger significantly lowered lipid peroxidation by maintaining the activities of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxides in rats. Also, Ganiyu et al. (2010) found that the higher protective effect of red ginger may be due to presence of higher antioxidant phytochemicals. Besides, the productive effects are reflected by the decrease of malonaldehyde level and increase the total antioxidants capacity (Khaki et al., 2009). Also, the intake of ginger significantly decreased the concentration of thiobarbituric acid-reactive substances (TBARS), lipid peroxidation and the formation of malonaldehyde in rats (Ippoushi et al., 2005). While, the conventional basic semen characteristics rather than motility are not obviously influenced by the oxidative state of semen; the increase in sperm motility could be due to the protective effect of ginger rhizomes administration (Aitken et al., 1995).

Cellular damage in the semen is the result of an improper balance between reactive oxygen species (ROS) generation and scavenging activities. Excessive ROS production that exceeds critical levels can overwhelm all antioxidants defense strategies of spermatozoa and seminal plasma which causing oxidative stress (Sikka, 1996) and decreases phosphorylation of axonemal proteins and consequently causes transient impairment of motility (De Lamirande et al., 1992).

Other studies have suggested that ROS attacks the integrity of DNA in sperm nucleus through causing base modification (Aitken and Baker, 2006). Moreover, DNA damage induced by excessive levels of ROS could accelerate the process of germ cell apoptosis, and leading to decline in sperm counts regarding with male infertility (Agarwal and Allamaneni, 2004). Motility is indispensable for the spermatozoa, as it has migrated in the female reproductive tract. Also, the levels of ROS are correlated with the motility of spermatozoa (Armstrong et al., 1999). Peroxidative damage to the sperm membrane and axonemal proteins appears to be the cause of permanent impairment in sperm motility (Agarwal and Allamaneni, 2004). The aim of this study was studying the effect of aqueous ginger extract on semen quality, hormonal profile and antioxidant status of male rabbits.

MATERIALS AND METHODS

Experimental Animals and Design:

Thirty 7-month old V-line rabbit bucks with average initial weight of 3.325 Kg were used in the trial. The rabbits were homogeneously divided into 3 groups (10 males/group). The groups were fed the same commercial diet and submitted to the following dietary treatments: control group (without additives); G150 and G250 groups fed an aqueous extract of Z. Officinale at 150 and 250 mg/kg body weight, respectively twice per week for 12 weeks.
All male rabbits were individually housed in a naturally ventilated building and kept in individual Italian wire galvanized cages (60 × 55 × 40 cm) and subjected to 14 hours of daily light. Fresh water and diet were offered ad libitum during the experimental period. Rabbits were fed on balanced pelleted diets composed of 18.43% crude protein, 12.70% crude fiber and 2502 kcal ME/ kg diet according to NRC (1984).

Preparation of Ginger Aqueous Extract:

Ginger (Z. Officinale Roscoe) rhizome was purchased from Egyptian market. One kilogram fresh ginger rhizome was cleaned, washed under running tap water, cut into small pieces, air dried and 125 g of this powder were macerated in 1000 ml of distilled water for 12 h at room temperature, filtered and the concentration of the extract is 24 mg/ml. Each experimented animal in the present study was orally given 1 ml of the final aqueous extract as recommended by Kamtchouing et al. (2002). The description and identification of ginger composition are reported by Jolad et al. (2004).

Data Collected:

Semen collection occurred weekly over the 12 weeks of this study, so 120 ejaculates were obtained per treatment (10 male rabbits × 12 weeks). Ejaculates were collected using an artificial vagina maintained at 45-46°C and a teaser doe. Reaction time (RT) was the interval from the introduction of the teaser doe into the rabbit buck cage to ejaculation. It was measured in seconds with a stopwatch and considered as an indication of libido. Immediately after collection, semen was kept at 35 °C in water bath in order to be evaluated. Semen volume of each ejaculate was recorded after removal of the gel mass. Immediately following semen collection, for measuring mass motility rate, two drops of fresh semen were placed on a warmed slide and covered with a cover slip (20×20mm). Mass motility from at least three fields was examined at 37 °C under a phase microscope at 40x and assessed from 0 to 100%. A weak eosin solution was used at a rate of 1:99 before counting the cells, for evaluation of sperm concentration (×10⁶/ml) according to Smith and Mayer (1955) by the haemocytometer slide. Total sperm output was calculated multiplying semen ejaculate volume by semen concentration. Assessment of live and abnormal spermatozoa was performed using an eosin-nigrosine blue staining mixture (Blom, 1950). The percentage of live spermatozoa was determined by using stains that penetrate cells with damaged membranes. Total number of motile sperm was calculated as multiplying percentage of motile sperm by total sperm outputs. The total functional sperm fraction (TFSF) was then calculated as the product of total sperm output multiplied by percent of motile sperms times percent normal sperms (Correa and Zavos, 1996). Seminal plasma was obtained by centrifugation of semen samples at 860 ×g for 20 min at 4 °C, and stored at -20 °C until analysis.

Blood sample were collected from the ear vein of each buck every two weeks (10 rabbits × 6 collections). The blood samples were centrifugation at 860 ×g for 20 minutes to separate the serum and kept in a deep freezer at -20 °C until biochemical analysis.

Seminal plasma samples were analyzed biweekly for total protein (TP), Albumin (Alb), total lipid (TL), the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (AlP) activity, acid phosphatase (AcP) activity and cholesterol were measured using commercial kits purchased from bio-diagnostic company (Recycling Crusher-SBM®). Thiobarbituric acid-reactive substances (TBARS) were measured in the seminal plasma using the method of Tappel and Zalkin (1959). Seminal plasma glutathione content (GSH) was determined...
using commercial glutathione reductase kits according to the method of Beutler et al. (1963). Glutathione peroxidase (GPx) activity was assayed using the method of Chiu et al. (1976). Superoxide dismutase (SOD) activity was assayed according to Misra and Fridovich (1972). Glutathione S-transferase (GST) activity was determined according to Habig et al. (1974) using P-nitrobenzylchloride as a substrate.

**Serum FSH, LH and Total Testosterone Hormones Measurements:**

Serum concentrations of FSH and LH were determined in duplicated samples using radioimmunoassay (RIA). FSH and LH kits obtained from Biocode Company-Belgium, according to the protocol provided with each kit of serum. The sensitivities of hormones detected per assay tube were 0.2 ng/ml and 0.14 ng/ml for FSH and LH respectively (Khaki et al., 2009).

Serum testosterone concentration was measured using immunoassay (Biosource-Europe S.A. 8, rue de L’Industrie.B-1400 Nivelles. Belgium).

**Bucks Reproductive Performance**

Thirty receptive nulliparous female rabbits were divided into three groups and housed in individual cages. Females from each group were artificially inseminated from the pooled semen of each male group. Parameters of kindling rate and litter size at birth (total born and total born alive) and at weaning were recorded. Also, bunny weights at birth and at 28 days were recorded.

**Protein Pattern and Free Amino Acids Determination:**

Free amino acids in seminal plasma were extracted according to the method described by Hamilton (1962) and the individual free amino acids were measured using a method described by Spackman et al. (1958) using amino acid analyzer system (model: SYKAM S 7130). Polyacrylamide gel electrohoresis (PAGE) of seminal plasma proteins methods were carried out according to the methods of Sambrook et al. (1989).

**Histopathology and Light Microscopy:**

The testis was fixed in 10% formalin and embedded in paraffin-wax. Five-micron thick sections were prepared and stained with Hematoxylin and Eosin (H&E). The specimens were examined under light microscope.

**Statistical Analysis:**

Data were analyzed as a randomized design using the General Linear Model procedure of SAS (2002). Dunnett Post hoc analysis was used to compare means of treatment groups against the control. P values <0.05 were accepted as significant.

**RESULTS AND DISCUSSION**

**Semen Quality:**

Data in Table 1 indicate that rabbit bucks received different doses of ginger extract exhibited an increase (P<0.05) with increase of ginger supplementation in the ejaculate volume, sperm motility, sperm concentration, total sperm output, the total motile sperm, live sperm and TFSF. The opposite trend is shown in the RT. Normal sperm % was increased (P<0.05) as the ginger supplementation increased compared with the control.

These results suggest that ginger extract had beneficial effects on male reproductive functions of rabbits. The increase in sperm motility of experimented groups in comparison to the control group could be due to the protective effect of ginger extract. These results are supported by the findings of Morakinyo et al. (2008) who found a significant increase in both sperm count and motility after 14 and 28 days treatment with ginger extract in a dose and duration dependent manner compared with the controls. Also, Khaki et al. (2009) reported that administration to rats of 50 mg/kg and 100 mg/kg of ginger for twenty consecutive days increased (P<0.05) sperm motility and viability as compared to the control group. Hafez (2010) reported that
oral administration of either ginger extract at 250 and 500 mg/kg BW for 65 days to diabetic rats induced increases (P \leq 0.05) in the sperm progressive motility, sperm count and viability as well as decreases in the percentage of sperm cell abnormality. While, Aitken et al. (1995) mentioned that the conventional basic semen characteristics other than motility are not obviously influenced by the oxidative state of semen.

**Seminal Plasma Biochemical Constituents:**

Data in Table 2 showed that seminal plasma TP, AlP and AcP were increased (P<0.05) in ginger groups compared with control one. The increase in seminal plasma TP and AlP was maximized (P< 0.05) at high dose of ginger, while there was no significant difference between low and high dose groups in seminal plasma AcP value. The opposite trend was shown in seminal plasma TL, ALT and AST for ginger supplemented groups compared with control one. Also, no significant differences were observed in seminal plasma TL and AST values between both low and high doses of ginger. On the other hand, ginger had no significant effect on ALb value.

Seminal plasma of mammal is a physiological secretion from multiple glands of the male reproductive tract that play an important role in the final maturation of the spermatozoa through hormonal, enzymatic and surface-modifying events, and it functions as a vehicle for ejaculated spermatozoa (Mann and Lutwak-Mann, 1981). Generally, the improvement found in biochemical constituents of seminal plasma for rabbit bucks supplemented with ginger extract is in accordance with those reported by Al-Daraji et al. (2010) who supplemented quail males with fish oil as antioxidant and decreased AST and ALT activities. Also, the increase in seminal plasma AlP and AcP in the present results is in agreement with those presented by Al-Daraji et al. (2001) who reported that both alkaline and acid phosphatase are involved in the metabolism of spermatozoa via the hydrolysis of carbohydrates.

**Seminal Plasma Antioxidant Status:**

Table 3 shows that seminal plasma GSH, SOD and GST activities were increased (P≤0.05) with the increase of ginger extract level. On contrary, seminal plasma TBARS was significantly decreased compared to control group. These improvements in seminal plasma antioxidant enzymes were maximized at high dose group for GSH, SOD and GST activities. Also, there were no significant differences between low and high doses of ginger in seminal plasma GPx value.

Oxidative damage in tissues can be limited by the defense system of the host and defined as an imbalance between the cellular antioxidant defense systems and the production of reactive oxygen species ROS (Sies, 1986). Seminal plasma has an antioxidant system that may be relevant to the protection of sperm, sperm oxidative defense enzymes predominantly include SOD, CAT, GPx and glutathione reductase (De Lamirande et al., 1992). Inhibition of xanthine oxidase activity responsible for the generation of reactive oxygen species, such as superoxide anion has been documented with gingerol (Chang et al., 1994).

In vitro antioxidant activity of gingerol and other constituents of ginger have previously reported by Kikusaki and Nakatani (1993). Moreover, Agarwal et al. (1994) reported that increased formation of ROS is correlated with the reduction of sperm motility. Ginger extract seems to confer a protective antioxidant defense capacity on the treated rabbits. Protective effects of antioxidant enzymes, consumption of dietary antioxidants through the diet play an important modulatory role against endogenous oxidative damage. Excessive ROS production that exceeds critical levels can
overwhelm all antioxidants defense strategies of spermatozoa and seminal plasma causing oxidative stress (De Lamminardi et al., 1997). Furthermore, Ahmed et al. (2000) found that ginger significantly lowered lipid peroxidation by maintaining the activities of the antioxidant enzymes SOD, CAT and GPx.

Cellular damage in the semen is the result of an improper balance between ROS generation and scavenging activities. This effect is reflected by the decrease of TBARS level and increase in anti-oxidants enzymes levels. In accordance with these results, Kim et al., (2007) demonstrated that ginger plant is endowed with strong antioxidant action both in vivo and in vitro, in addition to strong anti-inflammatory and anti-apoptotic actions. Also, it has been shown that the major active phenolic ingredients from ginger have antioxidant activity (Jorsaraei et al., 2008).

**Blood Serum FSH, LH and Testosterone Levels:**

Oral administration of 150 and 250 mg/kg BW ginger extract had no significant effect on serum FSH and LH concentrations compared with the control group (Table 4). However, serum total testosterone level increased (p≤0.05) in animals received both low and high doses in comparison to control one. The data of the present study showed an increase in serum testosterone levels with the increase of ginger concentration. This conclusion have been supported by Aitken et al. (1995) who found that ginger extracts have a potent androgenic activity in male rats. Moreover, Hamza et al. (2006) showed that ginger treatment increased the activities of testicular anti-oxidant enzymes in rats and have protective effects against testicular damage and oxidative stress.

**Reproductive Performance:**

Table 5 indicates that conception rate of females mated with rabbit bucks supplemented orally with either concentrations of ginger extract was improved (p≤0.05) compared with the control group. The total and live litter size at birth and litter size at weaning were also higher (p≤0.05) for groups of ginger supplementation than those of the control group but without significant difference between ginger groups. Moreover, ginger extract had no significant influence on bunny weight at birth whereas it had significant influence on bunny weight at 28 days compared to control with concentration of 250 mg only.

In agreement with the current results presented in Table 2, Raji et al. (2003) showed that low sperm count, motility and high percentage of abnormal spermatozoa level have been associated with fertility reduction. Also, Brun et al. (2002) found that the mass motility significantly influenced the kindling rate. Furthermore, they found that litter size (total born) was significantly influenced by concentration and number of total motile sperms. Also, results in the current study represented enhancement of semen quality of ginger supplemented groups as reflection of seminal plasma constituent improvement. These improvements are accompanied with better reproductive performance (kindling rates), and litter size up to weaning than the control group. The positive effect of ginger as an enhancer of reproductive capacity of rabbit bucks could be attributed to its ability to protect mammal cells from oxidation (Ulkowski et al., 2005).

**Seminal Plasma Free Amino Acids Components:**

Table 6 showed that total free amino acids for seminal fluids of groups supplemented with high or low level of ginger extract were higher (p≤0.01) compared with those for control group. Seminal fluid of high level of ginger extract group represented increase (p≤0.01) of all amino acids compared with those for control. Whereas, the significant increase was not observed in
some free amino acids in the low level of ginger extract (glycine, cysteine, isoleucine, leucine and tyrosine) compared with control.

Increasing total free amino acids content in seminal plasma of the low and high doses groups was associated with semen quality improvement and seminal plasma antioxidant enzymes activities. Oltjen et al. (1971) showed that high amounts of total free amino acids are important for the semen quality and the fertility of the animals. Also, Ibrahim and Boldizsár (1981) noted that there is some evidence that the amino acids present in the seminal plasma play an important role in survival of spermatozoa. The function of seminal plasma free amino acids is shown to act as fuels for the spermatozoa, to create favorable conditions for cell survival and to be probably involved in detoxifying functions. Moreover, mammalian cells can only utilize thiolic compounds such as cysteine and GSH, which have been shown to penetrate the cell membrane easily, for the intracellular GSH biosynthesis in vitro and in vivo.

Glutathione content protects the membrane lipids and proteins by direct radical-scavenging properties (Michael et al., 2007). Moreover, 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. Besides, cysteine has been shown to improve motility and morphology of post thawed of ram sperm (Uysal and Bucak, 2007) and to maintain the viability, the chromatin structure and membrane integrity of boar sperm. Sariozkan et al. (2009) reported that cysteine may enhance intracellular glutathione synthesis.

**SDS Polyacrylamide Gel Electrophoresis of Seminal Plasma Proteins:**

Figure 1 represents SDS PAGE of seminal plasma proteins for low and high doses of ginger extract samples compared to control. It is obvious from protein bands pattern that the protein band with molecular weight 100.7 kd has the highest intensity in high dose seminal plasma proteins samples followed by low dose of ginger extract compared to control sample. As well as, the bands with molecular weight 78.8 kd was found in both doses of samples but it was not found in the control group. It can be seen that the protein with molecular weights (47.5, 40.7 and 36 kd) which was expressed in high dose group was completely absent from the low dose and control groups. Butler (1989) found that osteopontin is an acidic glycoprotein of about 41.5 kd that has been isolated from rat, human and bovine bone. It is rich in aspartic acid, glutamic acid and serine and contains about 30 monosaccharides, including 10 sialic acids. The 55 kd protein, shown to be more prevalent in higher-fertility males, was determined to be osteopontin (Cancel et al., 1997).

The protein at molecular weight of 33 kd showed high expression protein with both high and low samples. As well as, the protein at 16.5 kd was expressed in both high and low ginger samples but it was highly expressed in the high dose than the low dose sample. Supporting to our results, Mohan et al. (1995) reported that chicken seminal plasma contains a high molecular weight protein (>100 kd) which neutralizes the motility inhibiting property of spermatozoa motility inhibiting factor. This result is in accordance with that in the present study, which demonstrated that the protein band with molecular weight 100.7 kd has the highest intensity in high dose sample which associated with increase total motile sperm. This protein band seems to be similar to the peptide band having 105 kd which was detected in chicken seminal plasma by Aly and El-Sahm (2006). It can be observed that the protein bands at molecular weight (78.8, 47.5, 40.7 and 36 kd) that were expressed in the high dose of ginger extract only may have an enzymes
role which is involved with maintaining
sperm motility.

Based on the previous studies and matching with our electrophoresis of rabbits seminal plasma proteins, Moura, et al. (2006) reported that bulls with the highest fertility scores had 2.3 times more of a 55 kDa osteopontin than bulls with above-average fertility and at least 4 times more than bulls with below average. A secreted form of phospholipase A2 (PLA2) 58 kDa present in the accessory gland fluid was more prevalent in bulls of high fertility (Moura et al., 2006).

**Histological Studies on Testis:**

Histopathological sections shown in Figure 2 illustrated that the cycle of spermatogenesis is regular in all experimental and control groups. However, lumen of seminiferous tubules were seen in accumulated sperm in all animals exposed to 150 and 250 mg/kg BW. The regularity and the accumulation of the sperm as demonstrated in this figure and their relations with testicular antioxidant enzyme and testosterone level as previously detected are in harmony with those reported by different authors. Amr and Hamaza (2006) demonstrated that Z. officinale treatment increased the activities of testicular antioxidant enzymes and restore sperm motility of cisplatin-treated rats. Also, the same authors reported in animal models that Z. officinale have protective effects against cisplatin-induced testicular damage and oxidative stress in rats.

The increase in semen testosterone level in the present study as observed in Table 4 due to administration of aqueous ginger extract could be reason for representing the accumulation of aqueous ginger extract could be reason for representing the accumulation of semen in the lumen of seminiferous tubules as reported by Aitken et al. (1995).

**CONCLUSION**

The oral administration of aqueous ginger extract with concentrations of 150 and 250 mg/kg BW is recommended for improving reproductive performance of male adult rabbits.

**ACKNOWLEDGEMENT**

The authors wish to thank Prof. Dr. Raouf E. Rizk, Professor of Poultry Husbandry, Animal Production Research Institute, Agricultural Research Center, Egypt for his kind comments and preparing this study.

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**Table (1):** The overall means of semen characteristics of V-Line male rabbits supplemented with aqueous ginger extract

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Control</th>
<th>Ginger (mg/kg BW)</th>
<th>150</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejacula te volume (ml)</td>
<td>0.76±0.012</td>
<td>0.94b±0.016</td>
<td>1.11a±0.020</td>
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</tr>
<tr>
<td>Reaction time (sec.)</td>
<td>10.9±0.20</td>
<td>5.9b±0.29</td>
<td>5.0c±0.28</td>
<td></td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>69.4±0.72</td>
<td>76.5b±0.75</td>
<td>86.5a±0.75</td>
<td></td>
</tr>
<tr>
<td>Sperm concentration (x10^6/ml)</td>
<td>235.3c±4.03</td>
<td>346.4b±7.82</td>
<td>407.9a±7.59</td>
<td></td>
</tr>
<tr>
<td>Total sperm output (x10^6)</td>
<td>179.5c±5.58</td>
<td>330.9b±11.53</td>
<td>454.1a±14.17</td>
<td></td>
</tr>
<tr>
<td>Total motile sperm (x10^6)</td>
<td>124.5c±4.64</td>
<td>253.7b±10.40</td>
<td>394.6a±13.17</td>
<td></td>
</tr>
<tr>
<td>Live sperm (%)</td>
<td>72.5±0.71</td>
<td>75.7b±0.69</td>
<td>88.5a±0.84</td>
<td></td>
</tr>
<tr>
<td>Normal sperm (%)</td>
<td>77.5c±0.28</td>
<td>86.1b±0.42</td>
<td>88.9a±0.45</td>
<td></td>
</tr>
<tr>
<td>TFSF*</td>
<td>96.5c±3.66</td>
<td>219.7b±8.81</td>
<td>351.1a±11.44</td>
<td></td>
</tr>
</tbody>
</table>

a,b,c means having different superscripts in the same row are significantly different (P≤0.05);
Rabbits, ginger, semen, hormones and antioxidant status

*TFSF: Total functional sperm fraction.

Table (2): The overall means of seminal plasma TP, Alb, TL, ALT, AST, AlP and AcP as affected by aqueous ginger extracts

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Control</th>
<th>Ginger (mg/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>5.7±0.09</td>
<td>5.8±0.08</td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td>3.70±0.10</td>
<td>3.67±0.09</td>
</tr>
<tr>
<td>TL (mg/l)</td>
<td>408.3±1.06</td>
<td>382.4±1.08</td>
</tr>
<tr>
<td>ALT (IU)</td>
<td>19.4±0.40</td>
<td>20.3±0.56</td>
</tr>
<tr>
<td>AST (IU)</td>
<td>31.1±0.50</td>
<td>29.2±0.91</td>
</tr>
<tr>
<td>AlP (U/L)</td>
<td>56.6±0.73</td>
<td>60.4±1.26</td>
</tr>
<tr>
<td>AcP (U/L)</td>
<td>32.5±0.23</td>
<td>35.3±0.30</td>
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</tbody>
</table>

Table (3): The overall means of seminal plasma antioxidants TBARS, GSH, GPx, SOD and GST as affected by aqueous ginger extracts

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Control</th>
<th>Ginger (mg/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>TBARS (nmol/ml)</td>
<td>1.120±0.008</td>
<td>0.976±0.017</td>
</tr>
<tr>
<td>GSH (g/dl)</td>
<td>17.30±0.39</td>
<td>20.50±0.77</td>
</tr>
<tr>
<td>GPx (mg/l)</td>
<td>4.20±0.08</td>
<td>4.91±0.09</td>
</tr>
<tr>
<td>SOD (IU)</td>
<td>6.99±0.09</td>
<td>7.37±0.07</td>
</tr>
<tr>
<td>GST (µmol/hr)</td>
<td>1.061±0.17</td>
<td>1.396±0.05</td>
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</table>

Table 2 and Table 3: The overall means of seminal plasma TP, Alb, TL, ALT, AST, AlP and AcP as affected by aqueous ginger extracts. The means having different superscripts in the same row are significantly different (P < 0.05); TP: total protein; Alb: albumin; TL: total lipids; ALT: alanine transaminase; AST: aspartate transaminase; AlP: alkaline phosphatase and AcP: acid phosphatase.
Table (4): Effect of aqueous extract of ginger on serum testosterone, FSH and LH hormone level

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Control</th>
<th>Ginger (mg/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>3.60±0.091</td>
<td>5.51±0.449</td>
</tr>
<tr>
<td>FSH (ng/ml)</td>
<td>18.37±2.09</td>
<td>19.09±2.12</td>
</tr>
<tr>
<td>LH (ng/ml)</td>
<td>2.41±0.119</td>
<td>2.56±0.356</td>
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</table>

\(^{a,b,c}\) means having different superscripts in the same row are significantly different (P<0.05);
FSH= Follicle-stimulating hormone; LH= Luteinizing hormone.

Table (5): Fertility indices of bucks supplemented with ginger extracts

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Control</th>
<th>Ginger (mg/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>Conception rate</td>
<td>65.08±2.98</td>
<td>82.12±1.76</td>
</tr>
<tr>
<td>Litter size (kits/litter) at birth(n)</td>
<td>7.7(^b)±0.52</td>
<td>9.3(^a)±0.69</td>
</tr>
<tr>
<td>Live kits/litter at birth (n)</td>
<td>6.2(^b)±0.37</td>
<td>8.9(^a)±0.43</td>
</tr>
<tr>
<td>Litter size (n) at weaning</td>
<td>5.1(^b)±0.34</td>
<td>7.9(^a)±0.35</td>
</tr>
</tbody>
</table>

\(^{a,b,c}\) means having different superscripts in the same row are significantly different (P<0.05).
Table (6): Free amino acids contents and ammonia (mg /100ml) in seminal plasma V-line male rabbits supplemented with aqueous ginger extracts

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Control</th>
<th>Ginger (mg/kg BW)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>150</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.721±0.103</td>
<td>0.961b±0.102</td>
<td>1.124a±0.087</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>1.235c±0.115</td>
<td>1.552b±0.102</td>
<td>1.845a±0.105</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>1.016c±0.076</td>
<td>1.225b±0.065</td>
<td>1.341a±0.041</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.226±0.106</td>
<td>3.313b±0.103</td>
<td>4.551a±0.101</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>1.165±0.10</td>
<td>1.812b±0.102</td>
<td>2.213a±0.103</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>5.033±0.103</td>
<td>5.145b±0.105</td>
<td>10.821a±0.101</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.915±0.085</td>
<td>1.134b±0.104</td>
<td>1.225a±0.075</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>34.480b±0.110</td>
<td>36.391b±0.971</td>
<td>47.223a±0.113</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>0.385c±0.125</td>
<td>1.772b±0.112</td>
<td>2.641a±0.121</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>0.155c±0.115</td>
<td>1.545b±0.105</td>
<td>1.662a±0.122</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.081c±0.531</td>
<td>1.473b±0.703</td>
<td>3.245a±0.685</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>0.285b±0.115</td>
<td>0.271b±0.141</td>
<td>0.421a±0.111</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.175b±0.125</td>
<td>1.185b±0.145</td>
<td>3.132a±0.132</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.392c±0.112</td>
<td>3.176b±0.126</td>
<td>12.354a±0.144</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.913c±0.093</td>
<td>1.936b±0.106</td>
<td>4.912a±0.112</td>
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<tr>
<td>Lysine</td>
<td>1.065c±0.115</td>
<td>7.282±0.102</td>
<td>10.331a±0.121</td>
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</tr>
<tr>
<td>Arginine</td>
<td>2.165c±0.125</td>
<td>4.754b±0.114</td>
<td>23.142a±0.122</td>
<td></td>
</tr>
<tr>
<td>Amonia</td>
<td>1.561c±0.091</td>
<td>1.993b±0.103</td>
<td>2.562a±0.106</td>
<td></td>
</tr>
<tr>
<td><strong>Total free amino acids</strong></td>
<td>56.968±2.718</td>
<td>76.920±3.160</td>
<td>134.745±4.715</td>
<td></td>
</tr>
</tbody>
</table>

\(a,b,c\) means having different superscripts in the same row are significantly different (\(P\leq0.05\)).
Figure (1): SDS polyacrylamide gel electrophoresis (PAGE) of rabbit seminal plasma proteins in low, high and control groups:

- M = protein marker, GLD = Low dose of ginger, GHD = High dose of ginger and GC = No ginger (Control)
(A): Regular seminiferous tubule with normal germinal epithelium morphology (control group), (× 640).

(B): Regular seminiferous tubule with normal germinal epithelium morphology in 150 mg/kg/rabbit of ginger (× 640).

(C): Regular seminiferous tubule with normal germinal epithelium morphology and sperm presences in lumen in 250mg/kg/rabbit of ginger (× 640).

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Rabbits, ginger, semen, hormones and antioxidant status

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