University of Benghazi
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جامعة بنغازي
كلية التربية – المرج
ISSN 2518-5845

Global Libyan Journal
المجلة الليبية العالمية

العدد الثاني والخمسون / يوليو / 2021

Effect of Dietary Ginger Rhizome Powder Addition on Reproductive Performance of Rabbit Bucks

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الملخص :

أجريت هذه الدراسة لتقييم تأثير إضافة مسحوق جذور الزنجبيل إلى ذكور الأرانب البالغة على جودة السائل المنوي وصفات بلازما السائل المنوي. تم استخدام 28 ذكر من الأرانب النيوزيلندية (بعمر 7 أشهر) تم تقسيمها إلى أربع مجموعات؛ الأولى كانت ضابطة (0٪ زنجبيل) ؛ والثانية والثالثة والرابعة تم إضافة مسحوق الزنجبيل إلى العليقة بنسبة 0.5٪ و1.0٪ و1.5٪ على التوالي. أظهرت النتائج التي تم الحصول عليها عدم وجود تغير في حجم القذفة وزيادة معنوية في حركة الحيوانات المنوية، وتركيز الحيوانات المنوية، والعدد الكلي للحيوانات المنوية، وإجمالي الحيوانات المنوية المتحركة، والحيوانات المنوية الحية، والجزء الوظيفي الكلي للحيوانات المنوية. يقلل الزنجبيل الغذائي من درجة حموضة السائل المنوي، ونسبة الحيوانات المنوية غير الطبيعية. كان لدى المجموعات التي حصلت على 1.0٪ أو 1.5٪ من مسحوق الزنجبيل تركيزات عالية من البروتينات الكلية في البلازما المنوية، الألبومين، الجلوبيولين، وزيادة نشاط الفوسفاتيز القلوي والفوسفاتيز الحمضي. على العكس من ذلك، انخفض معنوياً نشاط الإنزيمات الناقلة لمجموعة الأمين (الأسبارتات والآلانين) بشكل ملحوظ في البلازما المنوية مقارنة بالمجموعة الضابطة. من الناحية العملية، يمكن الاستنتاج أن الزنجبيل هو إضافة مناسبة لتحسين جودة السائل المنوي وبلازما السائل المنوي في ذكور الأرانب البالغة.

الكلمات المفتاحية: جذور الزنجبيل، ذكور الأرانب، جودة السائل المنوي.

ABSTRACT: This study was conducted to evaluate the effect of the addition of ginger rhizome powder (GRP) to the rabbit bucks ration on semen quality and semen plasma traits. A total of 28 male New Zealand rabbits (7- month- old), divided into four groups; the first treatment served as a control (Con; 0% ginger); the second, third, and fourth treatments were supplemented with 0.5%, 1.0%, and 1.5% ginger powder in the diet, respectively. The results revealed no change in ejaculate volume, a significant increase in the mass sperm motility, sperm concentration, total sperm output, total motile sperm, live sperm, and Total functional sperm fraction. Dietary ginger reduced the reaction time, semen pH, and the percentage of abnormal sperm. Bucks that received 1.0% or 1.5% dietary ginger had a higher seminal plasma total proteins, albumin, globulin concentrations, alkaline phosphatase and acid phosphatase activities. Conversely, seminal plasma aspartate aminotransferase and alanine aminotransferase activities were significantly decreased compared to the control group. Practically, it can be concluded that ginger is a suitable supplement for improving semen quality and semen plasma in adult male rabbits.
Keywords: Ginger rhizome, rabbit bucks, semen quality.

INTRODUCTION

Ginger rhizome *(Zingiber officinale*), is used worldwide as a spice antioxidative **[1]**، and an androgenic activator **[2]** as 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 **[3].** All major active ingredients in these components have antioxidant activity [4]. The natural antioxidants can protect DNA and other molecules from cell damage induced by oxidation, improving sperm quality, and increasing reproductive efficiency of men. **[5]** Found that ginger significantly lowered lipid peroxidation by maintaining the activities of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxides in rats [6]**.** Also, found that the higher protective effect of red ginger may be due to the presence of higher antioxidant phytochemicals **[7].** Besides, the productive effects are reflected by the decrease of malonaldehyde level and increase the total antioxidants capacity **[1].** In addition, the intake of ginger significantly decreased the concentration of thiobarbituric acid-reactive substances (TBARS), lipid peroxidation and the formation of malonaldehyde in rats **[8].** While, the conventional basic semen characteristics rather than motility are not influenced by the oxidative state of semen; the increase in sperm motility could be due to the protective effect of ginger rhizomes administration **[9]**. 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 **[10]**, decreases phosphorylation of axonemal proteins, and consequently causes transient impairment of motility **[11].** Infertility is one of the major health problems in life, and approximately 30 % of infertilities are due to a male factor **[12], [13].** Several conditions can interfere with spermatogenesis and reduce sperm quality and production. More factors such as drug treatment, chemotherapy, toxins, air pollutions, and insufficient vitamins intake have harmful effects on spermatogenesis and sperm normal production **[14]**. Several studies have reported that antioxidants and vitamins A, B, C, and E in the diet can protects perm DNA from free radicals and increase blood-testis barrier stability **[15],[16].** Nowadays ginger rhizome (*Zingiber officinale R., family: Zingiberaceae*),

is used worldwide as a spice. Both antioxidative **[17]** of Z. officinale were reported in animal models. All major active ingredients of Z. officinale, such as Zingerone, Gingerdiol, Zingibrene, gingerols, and shogaols, have antioxidant activity **[3].** Besides, other researches showed that ginger oil has a dominative protective effect on DNA damage induced by H_2O_2, might act as a scavenger of oxygen radical, and might be used as an antioxidant **[18].** Antioxidants protect DNA and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men **[19], [5]**. Peroxidative damage to the sperm membrane and axonemal proteins appears to be the cause of permanent impairment in sperm motility **[20]**.

The aim of this study was to investing the effect of the addition of ginger rhizome powder (GRP) to the rabbit bucks ration on semen quality and semen plasma traits.

MATERIAL AND METHODS

The present study was carried out at the Rabbit Research Laboratory, Department of Animal Production, Faculty of Veterinary Science, Agriculture, Zawia University during the period from December 2019 to February 2020.

A total of 28 males of New Zealand rabbit bucks 7 months old proven fertility, with an average initial live body weight of 2.95±0.05 kg, were randomly distributed among the four treatment groups containing 7 bucks each. The first treatment served as a control (Con; 0% ginger); the second, third, and fourth treatments were supplemented with 0.5%, 1.0%, and 1.5% ginger powder in the diet, respectively (Table 1). The diets were formulated based on the National Research Council **[21]** to meet rabbits' nutrient requirements. The rabbits bucks were individually housed in galvanized wire cages, offered free access to fresh water and feed and subjected to a 14:10 L:D cycle.

Semen samples were collected weekly over 8 weeks using an artificial vagina and the samples of each week were subjected to chemical analysis. Semen collection and handling were carried out and evaluated according to the international guidelines of **[22].** Ejaculated volume was measured to the nearest o.o1 ml. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH paper (Universalindikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Mass sperm

motility from at least three fields was examined at 37 ºC under a phase microscope at 40× and assessed from 0 to 100%. A weak eosin solution was used at a rate of 1:99 before counting the cells.

Table (1): Proximate analysis of pelleted basal diet.

Items	Ginger rhizome powder supplementation (% on a dry matter basis)			
Ingredients (%)	0.0	0.5	1.0	1.5
Berseem hay	35	35	35	35
Yalow corn	12	12	12	12
Barley	15	15	15	15
Wheat bran	15	14.5	14	13.5
Soybean meal	17.3	17.3	17.3	17.3
Ginger rhizome powder	0.0	0.5	1.0	1.5
Molasses	03	03	03	03
Di- calcium phosphate	1.0	1.0	1.0	1.0
Sodium chloride	0.3	0.3	0.3	0.3
Vit &Min mix*	0.3	0.3	0.3	0.3
DL- Methionine	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0
Calculated analysis**				
Digestible energy, Kcal/Kg	2560	2570	2587	2588
Crude protein, %	17.2	17.3	17.3	17.4
Crude fiber,%	13.8	14.2	14.3	14.5
Ether extract,%	2.75	2.95	3.05	3.25

*The vitamin and mineral premix/kg contained the following IU/gm of vitamin or minerals: A-4,000,000, D3-5000,000, E-16,7 g, K-0.67 g, B1-0.67 g, B2-2 g, B6-0.67 g, B12-0.004 g, B5-16.7 g, Pantothnic acid-6.67 g, Biotein-0.07 g Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co) [23].

** Calculated according to NRC (1984).

For evaluation of sperm concentration (×106/ml) by the haemocytometer slide. Total sperm output was calculated by multiplying semen ejaculate volume by semen concentration. Assessment of live and abnormal spermatozoa was performed using aneosin-nigrosine blue staining mixture **[23]**. The percentage of live spermatozoa was determined by using stains that penetrate cells with damaged membranes. The total number of motile sperm was calculated as a multiplying the 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 **[24].** Seminal plasma was obtained by centrifugation of semen samples at 860 ×g for 20 min at 4 ºC and stored at -20 ºC until analysis. Seminal plasma samples were analysed biweekly for total protein (TP), Albumin (Alb), globulin (Glob), the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AlP) activity, and acid phosphatase (AcP) activity. All parameters were measured using commercial kits purchased from bio-diagnostic company (Recycling Crusher-SBM®).

For statistical analysis results are expressed as mean ± standard error. Differences between means in different group were tested for significance using a one way analysis of variance (ANOVA) followed by Dancan's test **[25]** and *p* value of 0.05 or loss was considered significant using that statistical analysis system SPSS.

RESULTS AND DISCUSSION

The results in this study are illustrated in (**Table 2)**. Where there was no change in ejaculate volume between the control and the ginger treated group. The addition of 0.5%, 1.0% and 1.5% ginger powder significantly ($P<0.05$) increased mass sperm motility, sperm concentration, total sperm output, the total motile sperm, live sperm, and TFSF. However, the opposite trend was shown in the pH and abnormal sperm percentage; although the difference among the different concentrations of supplemented sperm significantly decreased progressively when 1% ginger was added. These results suggest that ginger had beneficial effects on the male reproductive functions of rabbits, which are supported by the findings of **[26]**, 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

control **[1].** It was reported that administration to rats of 50 mg/kg and 100mg/kg of ginger for twenty consecutive days increased (P<0.05) sperm motility and viability as compared to the control group **[27].** It was also reported that oral administration of either ginger extract at 250 and 500 mg/kg BW for 65 days to diabetic rats induced increases (P<0.05) in the sperm progressive motility, sperm count, and viability as well as decreases in the percentage of sperm cell abnormality.

Table 2: Effect of ginger rhizome powder addition on semen quality of New Zealand rabbit bucks

(Mean ±SE).

Items	Control	Ginger Addition			*P*.Value
		0.5%	1.0%	1.5%	
Ejaculate volume (ml)	0.69±0.004	0.70±0.009	0.70±0.005	0.71±0.003	**0.213**
Semen pH	8.33 ± 0.03^{a}	7.56 ± 0.03^{b}	7.60 ± 0.03^{b}	7.63 ± 0.04^{b}	**0.0001**
Sperm motility (%)	69.31 ± 0.30^{d}	76.71 ± 0.20^{c}	84.66 ± 0.30^{b}	88.76 ± 0.31^{a}	**0.0001**
Sperm concentration ($\times10^{6}$/ml)	207.66 ± 1.36^{d}	241.68 ± 1.11^{c}	276.26 ± 2.87^{b}	331.80 ± 3.12^{a}	**0.0001**
Total sperm output ($\times10^{6}$)	145.01 ± 1.33^{d}	190.11 ± 2.15^{c}	249.56 ± 3.17^{b}	331.31 ± 2.59^{a}	**0.0001**
Total motile sperm ($\times10^{6}$)	100.51 ± 0.92^{d}	145.85 ± 1.82^{c}	211.33 ± 3.29^{b}	276.34 ± 2.60^{a}	**0.0001**
Live sperm (%)	66.58 ± 0.74^{d}	77.80 ± 0.63^{c}	83.43 ± 0.44^{b}	88.20 ± 0.50^{a}	**0.0001**
Abnormal sperm (%)	28.55 ± 0.26^{d}	19.26 ± 0.27^{c}	14.81 ± 0.34^{b}	11.40 ± 0.28^{a}	**0.0001**
TFSF*	73.69 ± 1.07^{d}	117.70 ± 1.19^{c}	180.20 ± 2.63^{b}	246.74 ± 2.92^{a}	**0.0001**

$^{a, b, c, d}$ means having different superscripts in the same row are significantly different (P<0.05).

*TFSF: Total functional sperm fraction.

The higher libido scores recorded and the rest of semen parameters improvement in **(Table 2)**for bucks received ginger powder could be attributed to ginger rhizome powder that contained bioactive principle improves the activities of 3β-hydroxysteroid dehydrogenase (3β-OHSD) and Δ5,4-isomerase;17α-hydroxylase and 17,20- lyase and 17β-hydroxysteroid

dehydrogenase (17β-OHSD) in the testis **[28]**. Which will likely enhance extra- gonadal testosterone production since maintenance of sex libido in males and improvement in semen characteristics that attributed to testosterone concentration.

Data presented in **(Table 3)** showed that seminal plasma TP, Alb, glob and AlP were significantly increased (P<0.05) with the inclusion of ginger in bucks diets compared with the control group, and this increasing was level independent. On the other hand, seminal plasma AST and ALT decreased significantly decreased due to ginger supplementation compared with unsupplemented group.

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 **[29].** Generally, the most improvement found in biochemical constituent concentrations of seminal plasma of rabbit bucks supplemented with ginger as an antioxidant factor is in accordance with those report by **[30],** who reported that supplemented quail males with fish oil decreased seminal plasma AST and ALT activities. The results showed significant increase (P<0.05) in the concentration of the ALP and AcP enzymes in the seminal plasma of bucks rabbit-fed diet contained ginger compared to the control group. The alkaline phosphatase from the enzymes that cause loss of the phosphorus group, which is effective in several tissues, including bone, liver, kidney, bowel, lung, and placenta in addition to the reproductive system **[31].** The place and the amount of secretion of the ALP enzyme different according to the type of organism varies from one species to another **[32].** The studies conducted with humans have indicated that the secretion of ALP enzyme from the prostate and testis **[33]**, while another study revealed that this enzyme in rabbits and dogs is secreted by the epididymis **[34].** The significant increase of AlP and AcP enzymes in this study may be due to the improvement induced by ginger on the epididymis cell in rabbit testis. The strains of rabbits that have high fertility with higher levels of seminal phosphatase enzymes compared to those that have low fertility **[35]**. In a similar trend, **[36]** reported that both alkaline and acid phosphatase are involved in the metabolism of spermatozoa via the hydrolysis of carbohydrates.

Table 3: Effect of ginger rhizome powder addition on seminal plasma TP, Alb, Glob, ALT, AST, ALP, and AcP of New Zealand rabbit bucks (mean ±SE).

Items	Control	Ginger Addition			*P*. Value
		0.5%	1.0%	1.5%	0.0001
TP (g/dl)	5.67±0.11^{d}	6.13±0.09^{c}	6.56±0.69^{b}	6.83±0.31^{a}	0.0001
Alb (g/dl)	2.86±0.02^{d}	3.08±0.06^{c}	3.50±0.02^{b}	3.75±0.05^{a}	0.045
Glob (g/dl)	2.81±0.01^{b}	3.04±0.06^{a}	3.05±0.08^{a}	3.08±0.08^{a}	0.0001
ALT (IU)	28.50±0.47^{a}	26.55±0.10^{b}	25.43±0.11^{c}	22.46±0.08^{d}	0.0001
AST (IU)	34.44±0.11^{a}	31.08±0.29^{b}	29.71±0.07^{c}	26.30±0.31^{d}	0.0001
ALP (U/L)	53.47±0.43^{d}	58.88±0.04^{c}	62.47±0.23^{b}	68.01±0.31^{a}	0.0001
AcP (U/L)	46.57±0.15^{d}	50.68±0.11^{c}	54.50±0.17^{b}	56.61±0.16^{a}	0.0001

^{a,b,c,d} Means within a row with different superscripts are significantly different (P<0.05).

TP= total proteins; Alb=albumin; Glob=globulins; ALT=alanine aminotransferase; AST=aspartate aminotransferase; AlP=alkaline phosphatase; AcP =acid phosphatase.

CONCLUSION

In conclusion, the present study suggested that ginger has a positive effect on the male fertility in rabbits. Therefore, it is recommended to use ginger for improving the semen quality, fertility and reproductive performance of male rabbits.

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