تأثير إضافة (بذور الحلبة) على صفات السائل المنوي في ذكور الأرانب

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الملخص العربي

أجريت هذه الدراسة بهدف تقييم تأثير إضافة مستويات مختلفة من بذور الحلبة إلى علائق ذكور الأرانب البالغة في صفات السائل المنوي، استخدمت 32 ذكر من الأرانب البالغة (بعمر 8 أشهر) خلال الفترة من ديسمبر إلى فبرائر، وقسمت إلى أربعة مجاميع وبواقع 8 أرانب لكل معاملة والمعاملات كانت كالأتي: C = معاملة السيطرة غذيت على عليقة قياسية (بدون اي اضافة) ، T1 = غذيت على عليقة السيطرة+ 5 جرام / كجم علف من مسحوق بذور الحلبة، T2 = غذيت على عليقة السيطرة+ 10 جرام / كجم علف من مسحوق بذور الحلبة ، T3= غذيت على عليقة السيطرة+ 15 جرام / كجم علف من مسحوق بذور الحلبة. تم جمع عينات السائل المنوي بعد مرور أربعة أسابيع من المعاملة بواسطة المهبل الأصطناعي لدراسة صفات السائل المنوي من حيث حجم القذفة والحركة الفردية والجماعية للحيوانات المنوية وحيوية الحيوانات المنوية وقيم pH ،PSV ، تركيز الحيوانات المنوية، العدد الأجمالي للحيوانات المنوية لكل قذفة إضافة إلى صفات بلازما السائل المنوي والمتمثلة في مستوى الكوليستيرول، البروتين الدهني العالي الكثافة، البروتين الدهني المنخفض الكثافة، الليبيدات الكلية، الجليسيريدات الثلاثية، ومستوى الفركتوز، TAC ، MDA ، ونشاط انزيمات AST ، ALT ، ALP .

أظهرت النتائج انخفاض معنوي للمعاملات المحتوية على بذور الحلبة في كل من حجم القذفة والحركة الفردية والجماعية وحيوية الحيوانات المنوية و PSV وتركيز الحيوانات المنوية والعدد الكلي للحيوانات المنوية لكل قذفة وانخفاض في مستوى الكوليستيرول و LDL ، والليبيدات الكلية والجليسيريدات الثلاثية وانزيمات ALP ، AST ، ALP و MDA مقارنة بمجموعة السيطرة. وزيادة معنوية في الحيوانات المنوية الميتة و LDL ، TAC مقارنة بمجموعة السيطرة في حين لاتوجد اختلافات في قيم pH مابين مجموعة السيطرة والمعاملات المحتوية على بذور الحلبة. نستنتج من هذه الدراسة أن إضافة مستويات مختلفة من بذور الحلبة وخاصة مستوى 15 جرام/ كجم يؤدي إلى أنخفاض معنوي في صفات السائل المنوي لذكور الأرانب.

EFFECT OF NATURAL ADDITIVE (FENUGREEKSEEDS) ON SEMEN TRAITS IN BUCKS

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ABSTRACT This study was conducted to evaluate the effect of the addition of fenugreek seeds (F.S) to the rabbit bucks ration on semen quality and semen plasma traits. A total number of 32 male New Zealand rabbits(8 month old) divided into four groups (8 males/group) and treated as follows T1 =control group reared on standard ration, T2 = standard ration supplemented with 5gm F.S /kg, T3= standard ration supplemented with 10gm F.S /kg, T4= standard ration supplemented with 15gm F.S /kg. Semen samples were assessed after four weeks of treatment as regards ejaculation volume, mass activity, Individual motility, pH, PSV, semen concentration and totalsperm count. However, the second semen collection was used for determine seminal plasma concentrations of cholesterol, LDL, HDL, total lipids, triglycerides, fructose level, MDA, TAC, and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes.Results revealed that the treatment (T2, T3 and T4) showed that a significant ($p \leq 0.05$) decrease in ejaculation volume, mass activity,individual motility, PSV,semen

concentration and total sperm count and seminal plasma cholesterol, LDL, total lipids, triglycerides, ALP, AST, ALT enzyme and MDA,and significantly (P< 0.05) increase in dead spermand seminal plasma concentrations of HDL, TAC as compared with the control group. There was no significant difference in pHof semen among the control and experimental groups (T1,T2 and T3). In conclusion, the results of this study showed that supplementing fenugreek seeds in the diet of buck rabbits resulted in significant decreased in semen quality and seminal plasma characteristics.

Keywords: Fenugreek seeds, Rabbit bucks, semen quality.

INTRODUCTION

Feed additive are important materials that can improvethe efficiency of feed utilization and animal performance. The possibility of using new natural alternative additives instead of antibiotics and hormone in animal diets is being recently used. Some plants, containing various essential oils, have been used as alternative remedies

by some researchers **(Ceylan *et al*., 2003).**Fenugreek (*Trigonella foenum graecum* L.) is an annual plant from the family of Leguminosae,originate in India and native cultivated in south Europe, Northern Africa, India and Egypt and south of Iraq. It have many uses in medical purposes, fenugreek seeds have a great effect in relation to increasing lactation; also it helps to increase urine and menstruation in women. It helps also in rickets and anemia. Owing to the existence of mucilage, it helps relieve sore throttle and is useful in the treatment of asthma and difficult breathing/Fenugreek is considered as an appetizer and helps in digestion.Fenugreek seeds have also been recognized as a potential source of diosgenin, a basic compound in the hemisynthesis of steroidal sapogenins such as cortisone and sex hormones (**Brenac and Sauvaire1996a,b**).It use as astrangents materials and antibloat (**Chopra *et al*,1982**) , and used in animal feed (**Cheij ,1984**). However it used as a supplement to poultry feeding to lowering plasma total lipids and total cholesterol in Hubbard broiler chicks (**Azouz,2001**) and improve antioxidant status and production performance in laying hens (**Alkatan, 2006**).Fenugreek seeds improve the reproductive and physiological performance of broiler breeder males (**Taha, 2008**) , and revealed positive significant results of semen traits in aged broiler breeder males (**Abdul –Rahman *et al* 2010**).

Fenugreek like other legumes is a good source of dietary protein (20-30%) for consumption by human and animals, the fatty acids from5-10% which are predominantly linoleic, linolenic, olic and palmatic acids. It had 45-65% total carbohydrates with 15% of galactomannan (**Schryver, 2002**).Fenugreek seeds has been used extensively to prepare extract and powder for medicinal uses (**Basch *et al*., 2003**). It's reported to have anti- dialetic, anti-fertility, anti-cancer, anti- microbial, anti-parasitic, hypo- cholesterolaemic effects (**Al-Habori and Roman, 2002**). Also, it contains many minerals and vitamins (**Micheal and Kumawat**, **2003**).**(Seleem, 2008)** found that supplementation of 0.3% fenugreek to rabbit diet showed a great role in enhancing the immune systems, improved growth performance, blood metabolites and reproductive performance.A study showed that in rabbits, a fenugreek seed powder containing diet (30%) for 3 months could significantly decrease male testis weight [~25%] and sperm concentration [~43%], indicating a toxic effect of fenugreek seeds on seminiferous tubules and the interstitialtissue (Leydig cells).The negative impact of fenugreek seeds on the male structural and functional integrity of testicular tissues was evidenced by the histopathological data highlighting the damage of interstitial tissue, showing a decrease in the number of seminiferous tubules with mild spermatogenesis hypoplasia when compared with that in the control group **(Kassem *et al.,* 2006)**. Therefore the present study was designed to examine the ability of fenugreek seeds to decreasedin semen quality and semen plasma characteristics in the rabbit bucks.

MATERIALS 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 2016 to February 2017,to determine the effect of addition of fenugreek seeds to the rabbit bucks ration on semen quality and the seminal plasma traits.

A total of 32 males of New Zealand rabbit bucks 8 months old of proven fertility, with an average initial live body weight of 2.85 ± 0.03 kg, were randomly distributed into were allocated for the 4 treatment groups containing 8 bucks each. Treatment groups were as follows: Control diet (free from fenugreek seeds), T1: Control diet + 5 g/kg fenugreek seeds, T2: Control diet + 10g/kg fenugreek seeds; T3: Control diet + 15 g/kg fenugreek seeds. Bucks were allowed to become accustomed to treatment for a preliminary period of 28day during December 2016.Bucks were housed individually in galvanized batteries (50Lx50Wx40Hcm) provided with feeders and automatic drinkers in a windowed rabbitary.

Semen samples were collected weeklyover 8 weeks using an artificial vagina and the samples of the each week were subjected to chemical analysis. Semen collection and handling were carried out and evaluated according to the international guidelines of **(IRRG, 2005)**. Ejaculated volume was measured to the nearest 0.01 ml. A weak eosin- formalin (10% formalin) solution was used for evaluation of sperm concentration by the improved Neubauer hemocytometer slide method as described by **Smith and Mayer (1995)**. Total sperm out- put was calculated by multiplying semen ejaculation volume by semen concentration. Semen mass motility was given an arbitrary score from 0 to 3 based on the following assessment and the following variables were estimated: 0=No current, (0.5) = Very few slow current, 1 = Few slow current, 1.5= Many moderate waves, 2= Many sweeping waves, 2.5= numerous vigorous waves, 3= numerous rapid and vigorous waves, as described by **Moule (1995)**. Individual sperm motility was estimated at 400x magnification (**Kamar, 1960**). Evaluation of seminal initial fructose was carried out immediately after collection according to **Mann (1948)**. Assessments of dead and abnormal spermatozoa were performed using an eosin-aniline blue staining mixture (**Shaffer and Almquist, 1948**). Initial hydrogen ion concentration (pH) of semen sample was determined just after collection using pH meter. Packed sperm volume (PSV) was recorded using Micro-AID® microhematocrit tubes and microhematocrit-centrifuge which centrifuged for 5 min at 4000 RPM. Seminal plasma was separated by centrifugation at 3000 rpm for 20 minutes and was stored at -20°C in Eppendorf tubes for further analysis of total lipids, cholesterol, LDL, HDL, total antioxidants capacity, malondialdehyde,triglycerides,alkaline phosphatase, ALT and AST which were determined in seminal plasma colorimetrically using commercial kits obtained from (BIO- DIAGNOSTICS, Egypt) according to the procedure outlined by the manufacturer.

Statistical analysis

Results are expressed as mean ± standard error (SE). Differences between means in different group were tested for significance using a one- way analysis of variance (ANOVA) followed by Duncan's multiple range test (**Duncan, 1955**). The *P* value of 0.05 or less was considered significant using **SPSS (2004)**.The model wed was:

Yij= μ + Ti + eij where Yij = an observation treatment; μ = the overall mean; Ti = effect of treatments groups and eij = the random error component assumed to be normally distributed.

RESULTS AND DISCUSSION

Fenugreek seeds supplementation caused a significant decrease ($p \leq 0.05$) in ejaculation volume, mass and individual motility, spermatozoa concentration and total sperm count in comparison with the control group, and a significant increase in dead sperm percentage as compared with the control group, while there was no significant differences among treatment groups in relation to the pH of

semen **(Table 1).**Including fenugreek seeds in the diet resulted in significant ($P \leq 0.01$) decrease in ejaculate volume, mass activity, individual sperm motility, PSV%, sperm concentration and total sperm count in comparison with the control group. While dead sperm percentage was numerically increased in comparison with the control group.**Kassem *et al.*, (2006)** found that feeding diets containing 30% fenugreek seeds to male and female white New Zealand rabbits clearly demonstrate an antifertility effect of fenugreek seeds in the female rabbits and more of a toxicity effect in the male rabbits. The results showed that diets containing 30% fenugreek seeds significantly reduced male testis weight (~25%) as well as sperm concentration (~ 43%), indicating a toxic effect of fenugreek seeds on seminiferous tubules and the interstitial tissue (Leydig cells). Furthermore, feeding fenugreek seeds at 30% to male rabbits lowered circulating androgen (testosterone) by 65.8%. Showed that the negative impact of fenugreek seeds on the male structural and functional integrity of testicular tissues was evidenced by the histopathological data highlighting the damage of interstitial tissue, showing a decrease in the number of seminiferous tubules with mild spermatogenesis hypoplasia when compared with that in the control animals.The latter finding may support the hypothesis that a component of fenugreek seeds might have a direct toxic effect on the cells responsible for synthesis of androgens **(Kamal *et al.,* 1993).** This is further supported in the report of **Kassem *et al.*, (2006)**, by the lack of differences in the number of litter size when treated males were mated with control females, despite the decrease in androgen levels and sperm concentration, suggesting a toxicity effect rather than an antifertility effect in the male rabbits.**AlYahya, (2013)** reported that oral administration of fenugreek at the higher doses of 305 and 610 mg/kg body weight per day has caused male reproductive toxicity followed by reduced fertility, decreased motility, sperms count and an increase in the proportion of abnormal sperms in mice. The reduction observed in the fertility of male mice and the abnormal shapes of the sperms observed might be related with the increased accumulation of free radicals. Previous studies **(Farag *et al.,* 2010; Al-Majed *et al.,* 2006)**also showed that the depletion of glutathione cause spermatotoxicity and produce abnormal shapes of the sperms. Furthermore, the endocrinological study in plasma of male mice showed increase of estradiole and reduction of testosterone, which are known to affect the fertility. These results confirms the previous reports **(Kassem *et al.,* 2006; Khare *et al.,* 1983)** which showed fenugreek seeds to demonstrate an anti-fertility effect in both male and female rabbits. **Khalki *et al.*, (2010)** reported that aqueous seeds extract of *Trigonella foenum- graecum* affectedreproduction in mice and showed teratogenic and foetotoxic effect. Steroidal saponins and alkaloids have beenshown to beteratogenic. **Araee *et al.*, (2009)** has considered that in view of the presence of the steroidal saponin diosgenin in fenugreek seeds, it is likely that the administration of fenugreek in high doses adversely influences bone marrow cell proliferation. These changes are attributed to the toxic constituents present in fenugreek, such as quinones and flavonoids **(Skibola and Smith, 2000; Wu *et al.,* 1997). Mohammed *et al.*, (2013)** reported that *Trigonella foenum-graecum* seeds ethanolic extract decreased serum testosterone concentration in the treated rats and both mass and individual motility of the sperms in the treated cocks, while it insignificantly increased abnormal sperm percentage. Histopathological examination of rat's treated with *Trigonella foenum-graecum* seeds ethanolic extract showed presence of inactive seminiferous tubules and oedema formation between the seminiferous tubules. This effect may also contribute to the potential antifertility effects of this extract. The primary function of testosterone is the maintenance of spermatogenesis and hence fertility also testosterone is important in the maturation of spermatozoa as they pass through the epididymis and vasdeferens. A low testosterone level is one of the best indicators of hypogonadism of hypothalamic or pituitary origin, although very high levels of testosterone suppress sperm autogenesis **(McLachlan *et al.,* 2002). Jankorvaa *et al.*, (2003)** had reported that 12.6% of male infertile patients have hypotestosteronaemia. The decrease of sperm concentration in cocks may be due to the fact that *Trigonella foenum-graecum* increases the prolactin levels and higher levels of prolactin tends to inhibit production of G nRH leading to decrease in both FSH and LH and thereby decrease spermatogenesis and effects, in male, that mentioned by previous study of **Kassem *et al.*, (2006)** which reported that high concentrations of fenugreek in the diet significantly reduces fertility of

both male and female rabbits and reduced testis weight, with evident damage to the seminiferous tubules and interstitial tissues. **Embark (2016)** found that the supplementation of male rabbit's diets with 6g/kg fenugreek seeds decreased reproductive performance.

The effect of fenugreek seeds on seminal plasma total lipids, triglycerides, total cholesterol, high densitylipoprotein (HDL), low density lipoprotein (LDL), AST, ALT, ALP, fructose level,TAC, and MDA of bucks are presented in **Table (2).**

Seminal plasma total lipids, triglycerides and cholesterol were significant ($P \leq 0.01$) decreased in rabbits fed diets containing fenugreek seeds. fenugreek seed decreased seminal plasma total lipids, triglycerides and cholesterol in comparison with the control group. Fenugreek seeds used in the present study as phytobiotics resulted in decreasing seminal plasma LDL levels in comparison with the control group,while seminal plasma HDL was significantly increased in these treatments when compared with the control group.**Tavilani *et al.,* (2014)** studied the correlation between serum lipid concentrations and variations in seminal lipid parameters in infertile men. They found no relationship between the concentration of cholesterol, phospholipids and triacylglycerols in serum, spermatozoa or seminal plasma of the infertile men, which is consistent with the findings of several other authors (**Grizard *et al.,* 1995). Grizard *et al.*, (1995)** compared the effect of hypercholesterolemia and normocholesterolemia on the spermatozoa and seminal content of cholesterol andphospholipids.They suggested that hypercholesterolemia has no effect on cholesterol and phospholipid levels in spermatozoa and seminal plasma (**Grizard *et al.,* 1995)**. Since cholesterol has a major role in the sperm membrane, which is essential for sperm cell function, it can be assumed that an increase of cholesterol level in the blood will also increase the cholesterol content of semen. This hypothesis was not confirmed in the study of **Tavilani *et al.,* (2014).** There appears to be no correlation between the amount of cholesterol in the serum and in sperm or seminal plasma, suggesting that sperm cholesterol content is regulated locally within the male reproductive tract (**Travis and Kopf, 2002; Saez *et al.,* 2011; Maqdasy *et al.,* 2012)**. For proper function of spermatozoa, cholesterol and phospholipids should be regulated accurately. In the male reproductive tract lipid homeostasis is done by testicular and post-testicular function (**Maqdasy *et al.,* 2012, Lobaccaro *et al.,* 2012).** The results of **Tavilani *et al.*, (2014)** showed no correlation between serum lipids with sperm parameters, which is consistent with the findings of some other authors. **Khalili *et al.* (2009)** reported that the concentrations of serum lipids were not generally related to the quality of semen parameters. Nonetheless, the results of **Tavilani *et al.*, (2014)** were not consistent with the results of some other studies, in which animals were fed with a high-fat diet (**Shalaby *et al.,* 2004; Saez Lancellotti *et al*., 2010**). These results suggest that serum cholesterol, phospholipids and triacylglycerols have no effect on the levels of cholesterol, phospholipids and triacylglycerolsin spermatozoa and seminal plasma, and in addition, they do not cause any alteration of semen parameters.Seminal plasma alkaline phosphatase (ALP), ALT and AST showed significant ($P \leq 0.01$) decrease in rabbits fed diets containing fenugreek seeds comparison with the control group. These results get along with the finding of **(Roussal** and **Stalleup**, **1965)** who found negative correlation between AST activity of seminal plasma and with each of ejaculation volume, sperm motility, sperm concentration and percent live cells. Also **Chauban *et al.,* (1993)** found a positive correlation between enzyme release and sperm acrosomal damage.The results presented in **Table (2**) showed significant increase ($P \leq 0.01$) in seminal plasma fructose level and this increase in rabbits fed diets containing fenugreek seeds as compared with control group. In seminal vesicle, the seminal plasma is synthesized which is a medium for sperms. It consists of proteins, fructose, mucus, vitamin c, flavins, phosphoryl choline and prostaglandins. The high fructose concentration provides nutrient energy for the spermatozoa(**Wilke et al., 2009**), which reflects testosterone action and better quality of semen **(Taha, 2008).**

In the present study, seminal plasma total antioxidant capacity increased significantly ($P \leq 0.01$) as a result of feeding rabbits on diet containing fenugreek seed as compared with the control group.In support of this finding, lipid peroxidation (malondialdehyed) levels of rabbits fed fenugreek seed in significant ($P \leq 0.01$) decrease as compared with the control group.Reactive

oxygen species are metabolites of oxygen, including hydrogen peroxide and the superoxide and hydroxyl radicals (**Mostafa *et al.,* 2006**). The mamalian spermatozoon can generate ROS under physiologic conditions to mediate signal transduction, as well as to regulate the sperm function (**Naughton *et al.,* 2001**). On the other hand, like most cells, spermatozoa are equipped with antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase to detoxify harmful excessive ROS and to prevent cell injury (**Suleiman *et al.,* 1996**). The antioxidant-rich secretions of the seminal vesicles, including both enzymatic and nonenzymatic antioxidant systems, also assist in protecting the spermatozoa from ROS (**Werthman *et al.,* 2007**). Reduced glutathione (GSH) is an important component of antioxidant systems. Under high stress conditions, GSH regeneration is retarded and the antioxidant capacity of the tissue is impaired. Under such circumstances, ROS production surpasses the antioxidant capacity and causes increased oxidative stress (**Naughton *et al.,* 2001**). In support of this account, exogenous glutathione is known to ameliorate the effects of varicocele and partially to restore fertility (**Lenzi *et al.,* 1994**). Owing to the low amount of cytoplasm and abundance of polyunsaturated fatty acids in the plasma membrane of spermatozoa, spermatozoa are susceptible to damage by oxidative stress through ROS, especially lipid peroxidation (**Chen *et al.,* 2008; Gonzales, 2001; Zalata *et al.,* 2004**). This may result in acidification by some or all of the following mechanisms. Malondialdehyde, produced by the peroxidation of polyunsaturated fatty acids, may be oxidized to malonic acid, which potentially can be decarboxylated to acetate, as in the liver (**Siu and Draper, 1982; Davydov, 1993**). Polyamines, particularly spermine, are essential for spermatozoal activity (**Atmar *et al.,* 1981**). Malondialdehyde will react with spermine and other amines, forming Schiff bases. This will further decrease the intracellular pH, as well as directly impairing spermine-dependent cellular functions. The optimum pH for ROS scavenging by the enzymatic antioxidant systems ranges between neutral and slightly alkaline (**BRENDA, 1999**). Thus, lowering of the pH markedly depresses the antioxidant enzyme activities, which indeed are significantly impaired in varicocele cases (**Barbieri, 1999**). Therefore, there is a directly proportional relationship between acidification and increasing ROS levels.

Conclusion

Conclusion, the results of this study showed that supplementing fenugreek seeds in the diet of buck rabbits resulted in significant decreased in semen quality and seminal plasma characteristics.

Table1: Effect of dietary supplementation with different levels of fenugreek seeds on semen traits (Mean ± SE) of buck rabbits

Traits	Treatments				*P*.Value
	Control	T1	T2	T3	
Ejaculate volume(ml)	0.71 ± 0.003^{a}	0.67 ± 0.003^{b}	0.62 ± 0.003^{c}	0.57 ± 0.004^{d}	0.0001
Mass activity (1-3)	2.33 ± 0.003^{a}	2.27 ± 0.003^{b}	2.22 ± 0.003^{c}	2.17 ± 0.004^{d}	0.0001
Individual motility %	77.06 ± 0.19^{a}	75.05 ± 0.22^{b}	73.19 ± 0.33^{c}	70.87 ± 0.35^{d}	0.0001
Dead sperm %	20.16 ± 0.29^{d}	21.84 ± 0.21^{c}	23.39 ± 0.28^{b}	25.36 ± 0.15^{a}	0.0001
pH	7.95±0.007	7.96±0.005	7.94±0.003	7.96±0.004	0.876
*PSV %	12.03 ± 0.18^{a}	11.61 ± 0.12^{b}	10.23 ± 0.07^{c}	10.18 ± 0.03^{c}	0.0001
Sperm concentration ($10^{6}mm^{3}$)	263.26 ± 1.45^{a}	257.52 ± 1.22^{b}	254.70 ± 1.16^{c}	249.86 ± 1.23^{d}	0.0001
Total sperm (10^{6}/ ejaculate)	187.89 ± 1.62^{a}	174.14 ± 1.35^{b}	159.82 ± 1.12^{c}	144.61 ± 1.25^{d}	0.0001

Each value represented the mean of 8 semen evaluations; C: Control group; T1, T2 and T3: Diet supplemented with 5, 10 and 15 g/kg of fenugreek seeds; a-d Values within rows followed by different letters differ significantly ($P<0.05$).

*PSV = Packed sperm volume.

Table2: Effect of dietary supplementation with different levels of fenugreek seeds on seminal plasma traits (Mean ± SE) of buck rabbits

Traits	Treatments				
	Control	T1	T2	T3	*P*.Value
Cholesterol(mg/dl)	54.71±0.42^{a}	52.05±0.52^{b}	49.54±0.48^{c}	45.74±0.38^{d}	0.0001
LDL (mg/dl)	19.19±0.27^{a}	17.13±0.21^{b}	15.89±0.38^{c}	13.63±0.27^{d}	0.0001
HDL mg/dl)	20.240±0.44^{c}	22.757±0.43^{b}	23.83±0.32^{ab}	24.91±0.34^{a}	0.0001
Total lipids (mg/dl)	140.15±0.47^{a}	137.40±0.30^{b}	135.15±0.47^{c}	128.78±0.69^{d}	0.0001
Triglyceride (mg/dl)	132.27±0.86^{a}	124.97±0.52^{b}	121.40±0.24^{c}	114.97±0.48^{d}	0.0001
ALP (IU/L)	44.31±0.25^{a}	39.45±0.27^{b}	37.82±0.51^{c}	37.19±0.30^{c}	0.0001
AST (U/L)	25.69±0.23^{a}	24.14±0.20^{b}	24.12±0.38^{b}	24.21±0.30^{b}	0.001
ALT (U/L)	23.52±0.39^{a}	22.13±0.28^{b}	22.86±0.37^{b}	22.56±0.22^{b}	0.001
Fructose (mg/100ml)	193.86±1.79^{c}	209.35±3.18^{b}	214.08±1.16^{b}	220.65±1.62^{a}	0.0001
TAC mmol/l	0.94±0.002^{d}	1.05±0.01^{c}	1.12±0.01^{b}	1.18±0.01^{a}	0.0001
MDA nmol/ml	5.43±0.01^{a}	5.31±0.06^{b}	5.20±0.04^{c}	4.89±0.04^{d}	0.0001

Each value represented the mean of 8 semen evaluations; C: Control group; T1, T2 and T3: Diet supplemented with 5, 10 and 15g/kg of fenugreek seeds; a-d Values within rows followed by different letters differ significantly ($P<0.05$).

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