Semen Quality, Lipid Peroxidation of the Seminal Plasma, And Serum Testosterone Profile of Zaraibi Goat Bucks Administrated Chitosan with Normal or High-Fat Diets

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Abstract

This study aimed to determine the effect of dietary inclusion of chitosan (CTZ) on semen variables, sperm apoptosis and DNA fragmentation, and seminal plasma antioxidant status of Zaraibi goat bucks fed high-fat diet.Total of 15 mature bucks (39.42±0.48 kg LBW and 18-30 mo old) were divided into 3 groups (n= 5); the control (CG) and two of the groups received the control diet in addition to2.5 g CTZ/kg concentrate (CTZ) or 2.5 g CTZ+30 g dry fat diet/kg concentrate(CTZ+FAT), respectively for 8 weeks. Results showed that ejaculate volume, percentages of progressive motility, livability, and abnormality of spermatozoa, sperm concentration, as well as sperm counts (total, motile, live, normal and functional) per ejaculate were improved (P<0.05) by CTZ-diet. CTZ+FAT diet improved (P<0.05) all previous traits, except percentage of sperm abnormality. The viable sperm percentage was higher (P<0.05), while the percentage of necrotic spermatozoa was lower (P<0.05) with CTZ or CTZ+FAT diets. apoptotic spermatozoa percent was not affected significantly by treatments. The percentage of sub G1 was decreased (P<0.05) while haploid percentage increased (P<0.05) by CTZ and CTZ+FAT diets. The diploid and spermatid percentages were not affected by CTZ. Peroxidation marker (MDA) was decreased (P<0.05) while activity of GSH, CAT, and GPxwereincreased (P<0.05) by CTZ alone. CTZ+FAT dietrestore (P<0.05) the depleted levels of antioxidant enzyme and MDA to their values in CG. Serum testosterone did not differ, but testosterone level was higher (P<0.05) in CTZ than in CTZ+FAT in the seminal plasma. Insignificant differences in serum total protein, albumin, globulin, and urea concentrations while serum activity of AST and ALT was significantly (P<0.05) increased by CTZ+FAT treatment as compared to CG.

The dietary chitosan supplementation at a level of 2.5 g/kg for 8 week-interval pre-semen collection has a vital role on scavenging the negative effects of oxidative stress in the seminal plasma of bucks fed control or high-fat diets. Thus, chitosan could be safely used in Zaraibi buck's diets to improve semen quality and antioxidant defense system and to potentiate the reproduction of goat males.

***Keywords**: Goat, chitosan, high-fat diet, semen, oxidative stress.*

INTRODUCTION

Zaraibi goat breed (related to the Egyptian Nubian breed) present in Nile valley and delta of Egypt **(Galal et al., 2005),** and considered as a local goat breed specified for milk and meat production **(Galal et al., 2005; Dwidar et al., 2018)**. Reproduction is an important factor that affects the goat productivity **(Song *et al.*, 2006).**Reproductive efficiency of males during the breeding season is essential for successful mating **(Ford *et al.*, 2009).** Therefore, quality of the semen is the main limitation of reproductive performance of goat bucks**(Mahal*et al.*,2013)**. Suitable choice of goat bucks for the natural mating or collection of semen used in artificial insemination (AI)is necessary for the quality of breeding stock **(Ngoma*et al.* 2016),** and for good herd fertility. Therefore, application of good reproductive management of goats including semen evaluation (Semen quality and sperm DNA fragmentation), as in AI breeding program are related to male fertility **((Gore *et al.*, 2020;Fernández et al., 2005; Esteves et al., 2012)**.

To date, the production of good quality semen in dairy goats to enhance the breeding strategies have been reported by **Vakka et al.,(2018)**.It was reported that high-fat diet given to increase weight gain may develop hypercholesterolemia(**Bunnoy etal.,2015),** abnormality in lipid homeostasis, and may cause liver dysfunction (**Ouchietal.,2011).**However, natural products are used to control hypercholesterolemia as reported by **Chiu et al., (2019).**Natural antioxidant compounds in diets of animals exert a protective action on the possible oxidative stress **(Fadl et al., 2019; Dawood et al., 2021).** This might be related to increasing the activity of antioxidant enzymes **(Wu et al., 2005),** and has beneficial effects on spermatogenesis in males **(Atta et al., 2017)**. Chitosan is a new organic product used as a natural antioxidant and anti-obesity agent in the diet of the animals **(Li etal.,2018, 2021).**It is a nontoxic rich natural polysaccharide, the N-deacetylates product of chitin **(Tu etal.,2017). It** contains β-(1-4)-2-acetamido-D-glucose and β-(1-4)-2-amino D-glucose units **(Okawa et al. 2021),** and commonly used as a safe feed additive**(Shah**

etal.,2022). Also, it plays an important role as an antimicrobial agent **(Seankamsorn et al., 2020).**

Since reduced fertility is a global health problem, therefore, the aim of the current study was to investigate the effects of the use of chitosan as a dietary additive to improve performance of goat bucks. Also, to evaluate the possible mechanisms of chitosan in regulating antioxidant capacity, testosterone profile, and liver function to improve semen quality of Zaraibi goat bucks.

Materials and methods

The experimental animals used in this study belongs to the herd of Sakha Experimental Station, Animal Production Research Institute (APRI), Agricultural Research Center. The analytical procedures were conducted in Faculty of Veterinary Medicine, Kafr el sheikh University, Egypt.

Animals

At the beginning of this experiment (1st April), we used a total of 15 mature Zaraibi goat males having an average of 39.42±0.48kgLBW and 18-30mo. old. The experimental animals were kept in a hygienic pen. They fed a ration consisted of concentrates and corn silage, while drinking water was available all day. The experimental animals were randomly divided into three groups, five animals in each, fed three experimental diets. The control group (CG), chitosan group (CTZ), and chitosan with fat (CTZ+FAT) group.

Experimental diets

Three experimental diets included corn silage plus concentrates with out supplementation (control, CG), concentrates supplemented with chitosan at a level of 2.5 g/kg (CTZ), and concentrates supplemented with 2.5 g CTZ and 30 g fat per kg (CTZ+FAT). The treatment period was 8 weeks (April-May) followed by semen collection period for other 8 weeks (June and July). Each buck in all groups fed individually balanced ration. Chemical analysis of concentrate feed mixture and corn silage are shown in Table 1. The amounts of feeds offered were adjusted based on body weight changes and the physiological status of bucks.

Table 1: Chemical composition of concentrates and corn silage

Item	Concentrates (%)	Corn silage (%)
DM	89.55	31.20
OM	87.35	93.65
CP	14.28	7.82
CF	13.11	26.53
EE	2.34	2.83
NFE	57.62	56.47
ASH	12.65	6.35

Collection and evaluation of the semen

At the beginning of the semen collection period, ejaculates were collected from Zaraibi bucks by using an artificial vagina. Semen was collected twice weekly for eight weeks and evaluated for semen volume, progressive motility**,** livability (Eosin and Nigrosin stain), in addition to the abnormality percentage of spermatozoa. Sperm cell concentration was estimated microscopically using a Neubauer hemocytometer.

Sperm production in terms of counts of total, motile, live, normal, and functional spermatozoa per ejaculate was calculated according to the following equations:

Total count= ejaculate volume x sperm cell concentration

Motile sperm count = total count x progressive motility percentage

Live sperm count = total count x live sperm percentage

Normal sperm count = total count x (100- abnormal sperm percentage)

Functional sperm count = total count x motility% x live sperm% x normal sperm%.

At the last week of semen collection period, seminal plasma was obtained from each ejaculate in each group after centrifugation of the semen ejaculates(3000 rpm for 20 min), and stored at -20 ◦C until analysis.

Blood sampling and biochemical analyses

At the end of semen collection period (8 weeks), blood samples were taken from the jugular vein of each buck in to test tubes, left for clot (2-3 h), and centrifuged at 3000 rpm for 15 min, then serum samples were collected and stored at -20 °C for further biochemical and hormonal analysis. Serum samples were analyzed for total protein (TP) and albumin (AL) according to **Henry et al. (1974),** and globulin concentration (GL) was computed by subtracting albumin concentration from total protein concentration. Enzyme activity of aspartate (AST) and alanine (ALT) aminotransferase**)** was assayed (**Reitmanand Frankel, 1957)**. Determination of blood biochemical and enzyme activity was performed by commercial kits (Bio diagnostics, Cairo, Egypt).

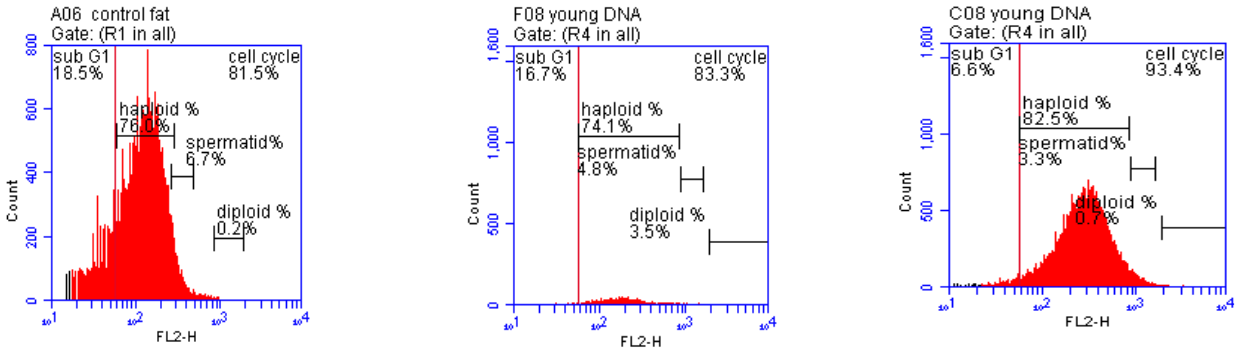
Lipid peroxidation, antioxidant enzyme, and testosterone assay

Lipid peroxidation was evaluated in the seminal plasma by measurement of malondialdehyde (MDA) content according to **Ohkawa et al. (1979)**.Catalase (CAT) was estimated according to **Aebi (1984),**while glutathione peroxidase activity (GPx) was evaluated according to **Paglia and Valentine (1967)** and reduced glutathione (GSH) was evaluated according to the method of **Beutler et al. (1963)**.

Testosterone concentrations in serum and seminal plasma of bucks were determined using a commercial kit (Biodiagnostics, Cairo, Egypt).

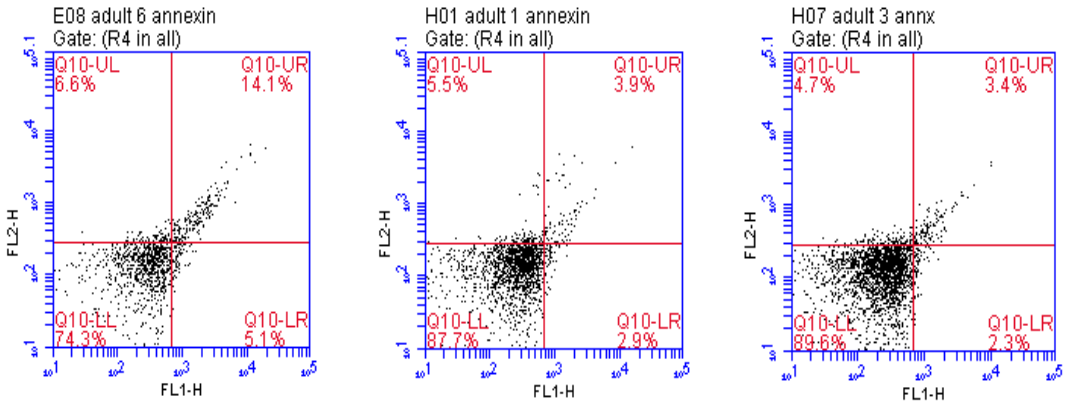
Spermatic cell cycle method (DNA fragmentation)

DNA ploidy is a measurement of the cellular content of DNA relative to the percentage of the normal (diploid) control. Mathematical models have been developed to predict the proportion of cells in different phases of the cell cycle (G0/1, S-phase, G2/M), diploid and aneuploid cycles, CV and the percentage of death.Cells reaching the termination of their life cycle include apoptotic sperm cells, DI, percent diploid, and percent aneuploidy **(Spyratos, 1993)**.Subtype G1 is diagnosed as apoptotic and the longest phase of the cell cycle. Lymphocytes or tissue cells were fixed by 1 cc of ice-cold 100% alcohol/tube, then stored at4 °C until analysis. Post-fixation, the excess ethanol was isolated by centrifugation of the material for 12 h **(Vindelov, 1977)**. Data analyzed by MODFIT DNA analysis program (verity software house, Inc. Po Box 247, Topsham, ME 04086USA, version: 2.0 power Mac with 131072 KB Registration number: 42000960827-16193213) to determine the variation coefficient (CV) around the G0/G1 peak, apoptosis percent, the index of DNA (DI), and the percentage of cells in each phase (G0/G1, S, and G2/M) of the DNA cell cycle for each sample, as well as percentage of diploid and aneuploidy cycle (Fig. 1)**.**



Control **CTZ** **CTZ+FAT**

Fig. 1. Flow cytometry contour plots of buck spermatozoa in control, CTZ, and CTZ+FAT labelled with propidium iodide (PI) fluorescence. *Percentage of sperm cells at each cell cycle phase relative to total phases.* ***Sub-G1*****:** DNA damage percent**;** ***haploid percent*****:** sperm cells with DNA content**;** ***spermatid percent*****:** round spermatid and very closed in the content of DNA**;** ***diploid percent*****:** includes diploid cells (2N) of sperm cells, lymphocytes and two sticky sperm cells (N) represents as one sperm.



Control **CTZ** **CTZ+FAT**

Fig. 2: Flow cytometry contour plots of sperm cells labelled with AV and PI in control, CTZ, and CTZ+FAT. *The lower left quadrant***(LL)** *includes viability percent (A-/PI-), lower right quadrant* **(LR)***reveals early apoptosis (A+/PI-), upper right quadrant* **(UR)***shows apoptosis (A+/PI+), and upper left***(UL)** *quadrant mentions necrosis (A-/PI+).*

Statistical analysis

The normality before analysis of the obtained data was checked by Shapiro-Wilk's test, while the homogeneity was tested before analysis by Shapiro-Wilk's and Levene's test. Data were statistically analyzed by one-way Analysis of Variance to evaluate the effect of treatment. The significant differences were separated by Tukey's Multiple Comparison test. An arcsine transformation was used before processing percentage values.

RESULTS AND DISCUSSION

Sperm production

The obtained results showed that ejaculate volume, percentages of progressive motility, livability,

and abnormality of spermatozoa, sperm concentration (Table 2), as well as sperm counts (total, motile, live, normal and functional) per ejaculate (Fig. 3) were significantly (P<0.05) improved in bucks fed CTZ-diet as compared to control. Also, feeding CTZ+FAT significantly (P<0.05) improved all previous traits, except sperm abnormality percent.

Table 2: Semen characteristics of bucks in the experimental group during semen collection period.

semen characteristics	CG	CTZ	CTZ + FAT	*P-value*
Ejaculate volume (ml)	0.91 ± 0.05^{b}	1.08 ± 0.05^{a}	1.17 ± 0.04^{a}	0.000
Progressive motility (%)	76.0 ± 0.58^{c}	80.9 ± 0.65^{b}	83.0 ± 0.66^{a}	0.000
Live sperm (%)	78.1 ± 0.86^{b}	81.8 ± 0.77^{a}	83.6 ± 0.59^{a}	0.000
Sperm abnormality (%)	10.9 ± 0.44^{a}	8.8 ± 0.41^{b}	9.8 ± 0.48^{ab}	0.000
Sperm concentration ($x10^{9}$/ml)	2.31 ± 0.057^{b}	2.59 ± 0.045^{a}	2.61 ± 0.071^{a}	0.042

Groups with different superscripts arestatistically significant(P<0.05).

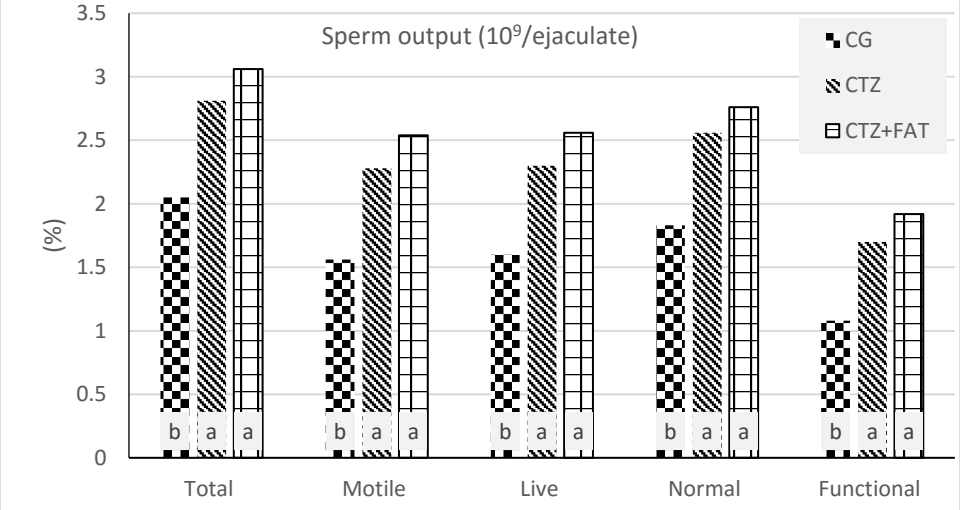


Fig. 3: Sperm output per ejaculate of bucks in the experimental groups during semen collection period.
Groups with different superscripts are statistically significant (P<0.05).

During clinical and rological examinations, evaluation of spermatozoa is of paramount importance, since sperm 'quality' provides an indication of the normality of testicular function in terms of sperm numbers, their viability and the normality of morphological features. As well, it provides an idea of the normality of the epididymal function, i.e. sperm maturation, with the display of proper morphological features and of activated motility after ejaculation. The present results revealed that feeding bucks on CTZ or CTZ+FAT diets has beneficial impact on semen quality and sperm count per ejaculate. Chitosan provides protective effects upon male reproductive organs that exposed to lead.

Many investigators observed a reduction in sperm concentration and motility in relation to oxidative stress by feeding a high-fat diet **(Vigueras-Villasenor et al., 2011; Chen et al., 2013**). Improving semen parameters by CTZ or CTZ+FAT diet may be attributed to the antioxidant and inflammatory actions of CTZ. This enhancement may beparallel to higher activity of the endogenous antioxidant enzymes (GSH, CAT, and GPx) in the seminal plasma. In this respect, we determined the antioxidative action of CTZ on the oxidative stress marker (MDA), then on sperm quality that may occur via protecting the testis and preserving the sperm quality. It was reported that Chitosan restored the decrease in testicular weight with the increased incidence of sperm abnormalities (abnormal sperm morphology) and the decrease in the number of spermatozoa caused by lead-toxicity (**Marianti et al., 2019)**. Therefore, chitosan, which is also nontoxic, has the potential for further development as alternative natural compound to overcome the negative effects to lead exposure**.** It is of interest to note that sperm abnormality not affected by feeding CTZ+FAT-dietin comparing with control (Table 2). In this respect, **Liu et al., 2014** reported increase in abnormal testicular structures, such as epithelial disruption, after 6 week of 20% fat feeding for Sprague-Dawley rats.

Sperm flow cytometry parameters

Sperm flow cytometry parameters of the control and treated bucks are shown in Table 3. Results revealed that viable sperm percentage was significantly (P<0.05) higher, while the percentage of necrotic spermatozoa was significantly (P<0.05) lower in CTZ treated groups either alone or combined with fat compared with the control one. However, the percentage of early apoptotic and apoptotic spermatozoa was not affected significantly by treatments (Table 3).

Feeding high-fat diets can affect spermatogenesis via several mechanisms and processes, among others via hormonal changes leading to a reduction in sperm quantity and quality (**Akingbemi, 2005;Davidson et al., 2015**). Apoptosis, is a programmed cell death, which delimits a sequence of events, subsequently leading to the destruction of cells without releasing harmful substances into its surrounding area **(Hossainetal.,2011).** Some studies showed a reduction in sperm viabilityin association with oxidative stress caused by high-fat diet**(Vigueras-Villasenor et al., 2011; Chen et al., 2013**), so improving viability and reducing sperm necrosis in CTZ with or without fat imply the beneficial effects of chitosan as antioxidant and anti-inflammatory agent **(Xia et al., 2011;Anitha et al., 2014; Kerch,2015)**.

Table 3: Flow cytometeric parameters in buck semen of the experimental groups during semen collection period.

Item	CG	CTZ	CTZ + FAT	*P*-value
Viability(%)	81.5±2.48[b]	86.1±1.03[a]	87.4±2.89[a]	0.042
Early apoptosis (%)	4.35±1.06	2.22±0.36	4.10±0.98	0.510
Apoptosis (%)	5.55±0.93	6.42±0.49	4.12±1.19	0.345
Necrosis (%)	8.67±0.42[a]	5.28±0.54[b]	4.50±0.53[b]	0.042

Groups with different superscripts are statistically significant (P<0.05).

Sperm DNA fragmentation

Parameters of sperm DNA fragmentation of the control and treated bucks are shown in Table 4. Results showed that the percentage of sub G1 was significantly (P<0.05) decreased while haploid percentage significantly (P<0.05) increased by chitosan alone or in high-fat group compared with the control group. This improved spermatic cell cycle in CTZ and CTZ+FAT groups but the differences were not significant. However, the diploid and spermatid percentages were not affected by chitosan treatment. Sperm ROS are generated by both endogenous and exogenous sources. Feeding high-fat diet increases the production of endogenous ROS (**Torres-Arce etal.,2021)**. The consumption of a high-fat and high-energy diets is considered a major cause of the development of obesity complications. Other studies have shown that obesity leads to higher proportion of sperm with DNA fragmentation (**La Vignera et al., 2012).**Sperm DNA fragmentation leads to reduced fertility **(Bieniek et al., 2016)**.

Table 4: Parameters of sperm DNA fragmentation of the control and treated bucks **using Flow cytometer .**

Item	CG	CTZ	CTZ + FAT	*P*-value
Sub G1 (%)	13.23±2.37[a]	7.70±2.04[b]	6.52±1.45[b]	0.050
Haploid (%)	76.18±2.19[b]	83.57±1.15[a]	83.87±0.69[a]	0.021
Diploid (%)	4.93±1.58	2.73±0.76	3.67±0.64	0.410
Spermatid (%)	5.64±1.64	7.50±2.14	5.92±1.32	0.753
Spermatic cell cycle (%)	86.77±2.37	92.38±1.96	93.38±1.96	0.074

Groups with different superscripts arestatistically significant (P<0.05).

Oxidative stress causes changesin sperm concentration, livability, motility and DNA integrity **(Vigueras-Villasenor et al., 2011; Chen et al., 2013**).In our study, feeding CTZ or CTZ+FAT diet improved sperm DNA damage in developing sperm cells due to increasing oxidative stress products and/or an abnormal chromatin condensation (**Fariello et al. 2012**). CTZ, as an antioxidant, reduced ROS in sperm cells and the seminal plasma by modulating spermatogenesis, and resetting the normal physiological status**(Smits et al., 2019).**Interestingly, in the current study chitosan supplementation decreased levels of lipid peroxidation marker MDA in the seminal plasma with restoring the cellular antioxidant.

Lipid peroxidation and antioxidant status in the seminal plasma

Antioxidant biomarkers in the seminal plasma of bucks in the experimental groups are presented in Table 5. In this work, high-fat diet depleted antioxidant defense via the significant increase in the activity of antioxidant enzymes (GSH, CAT, and GPx), with significant (P<0.05) reduction in MDA levels in the seminal plasma of bucks compared with the controls. Peroxidation marker (MDA) significantly (P<0.05) reduced while antioxidant enzyme activity of GSH, CAT, and GPx significantly (P<0.05) increased in CTZ than in CG and CTZ+FAT. Also, CTZ addition tohigh-fat diet significantly (P<0.05) restore the depleted levels of antioxidant enzyme and MDA to their values in CG.

Antioxidant enzymes such as GSH, CAT, and GPX play an important role in scavenging oxidative stress (**Chiu et al., 2019).** The positive effect of CTZ is proved by the results reported on rats fed high-fat diet in terms of decreased the activities of GPx and glutathione-s-transferases (GST) and increased level of TBARS in the liver **(Noeman et al., 2011).** This was evidenced in by **Yadav et al. (2008)** and **Lasker et al., 2019)**.Generally, CTZ reported to improve the activity of SOD and CAT, total antioxidant capacity (TAC) and IgG concentration and reduced MDA level in pigs (**Wan et al., 2017)** and even decreased hepatic TBARS level (**Wang et al., 2017)**. Therefore, CTZ reported to eliminate harmful

effects of oxidative stress, as a potent antioxidant (**Anraku et al., 2009)**.

Testosterone profile in serum and seminal plasma

The effect of treatment was found to be significant on testosterone concentration in blood serum and seminal plasma of bucks as illustrated in Fig. 4. Despite this effect, serum testosterone level did not differ significantly in both treatment groups (CTZ and CTZ+FAT), as compared to control, but testosterone level was significantly (P<0.05) higher in CTZ than in CTZ+FAT. In the seminal plasma, a pronounced improvement in testosterone concentration as affected by chitosan treatment in terms of significantly (P<0.05) higher values in CTZ group than in CTZ+FAT and CG groups.

Table 5: Oxidative and antioxidant biomarkers in blood serum and seminal plasma of bucks in different experiment at the end of the experimental period.

Item	CG	CTZ	CTZ + FAT	*P*-value
Malondialdehyde (nmol/ml)	4.10±0.16[a]	3.44±0.10[b]	4.96±0.17[a]	0.042
Glutathione reductase (nmol/ml)	7.18±0.37[b]	10.43±0.74[a]	6.19±0.44[b]	0.009
Catalase (U/ml)	10.18±0.37[a]	10.59±1.18[a]	7.59±0.71[b]	0.014
Glutathione peroxidase (pg/ml)	40.83±0.39[b]	48.82±0.21[a]	38.66±0.34[c]	0.001

Groups with different superscripts are statistically significant (P<0.05).

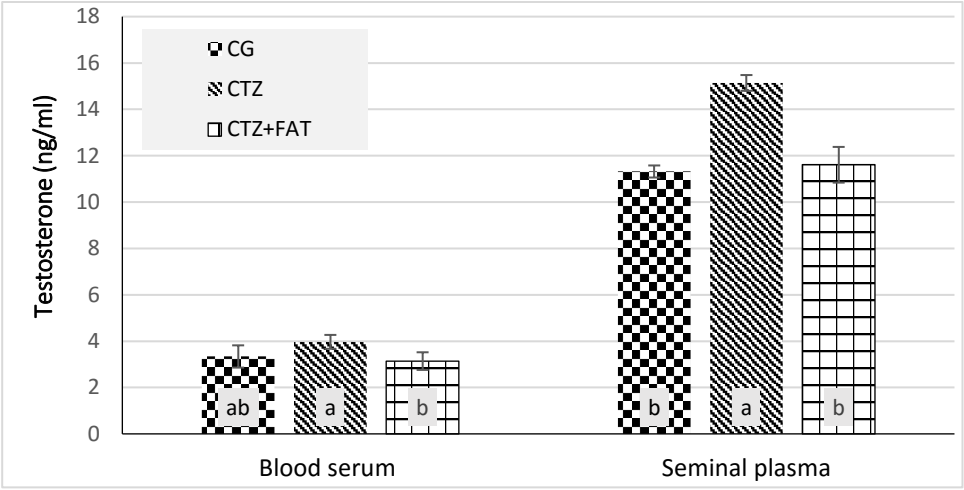


Figure 4: Testosterone concentration in blood serum and seminal plasma of bucks in the experimental groups at the end of the semen collection period.

It is known that blood testosterone level at puberty has a vital role in the sexual development and spermatogenesis of males **(Johnson et al., 2015; Mortazavi et al., 2014**). In male rats feeding high-fat diet at a level of 35% fat for 68 days (**Cani et al., 2008)** or 21% fat for 10 weeks (**Palmer et al., 2012**) resulted in reduction of plasma testosterone level as compared to control leading to reproductive dysfunction**(Ibáñez etal.,2017**). Moreover, the decrease of testosterone level may be due to increasing the adipose tissue through increasing level of leptin and estrogen. Similar results were reported on mouse **(Fuietal.,2014).** Specifically, aromatase in adipose tissue converts testosterone into estradiol, which results in elevated estrogen levels in obese men **(Davidson et al., 2015;Bieniek et al., 2016)**. Some studies reported that long-term treatment of leptin at a high level reduced production of testosterone **(Rao et al., 2013; Davidson et al., 2015)** beside that the adipose tissue produces leptin hormone which is able to inhibit the androgen secretion from *Leydig* cells in the testis (**Davidson etal.,2015;Wang etal.,2018).** Moreover, increasing estrogen level may affect the hypothalamic–pituitary–gonadal (HPG) axis to reduce the level of testosterone **(Raoetal.,2013)**. In our study, no harmful effects were found on testosterone concentration in serum or the seminal plasma of bucks in CTZ+FAT group. Thismay be attributed to that bucks fed 3% fat, being lower than do in the later reports or to the beneficial impacts of chitosan. We suggest that chitosan may increase FSH which led to elevate ABP (Androgen Binding Protein) that can bind to testosterone in the seminiferous tubules of the testes. This causes a normality or somewhat improvement in the growth and development of germ cells.

Serum biochemical parameters

Serum biochemical parameters of the control and treated buck groups are shown in Table 6. In this study, treatment revealed insignificant differences in serum total protein, albumin, globulin, and urea concentrations. While serum activity of AST and ALT were significantly (P<0.05) increased by CTZ+FAT treatment as compared to CG. Feeding high-fat diet induces a significant increase in both serum AST and

ALT activities induced by the obesity and fatty liver **(Stern et al., 2016; Lee, et al., 2021).**In rats, oxidative damage in the body tissues resulted in hepatocyte damage, as it might liberate of serum ALT and AST activities via consumption of high-fat diet. Incidence of liver injury by increasing the levels of triglycerides and total cholesterol may lead to lipo- toxicity development and fatty liver compared to the control rats (**Chiu et al., 2019**

Table6: Serum biochemicals of bucks in different experimental group at the end of the experimental period.

Item	CG	CTZ	CTZ + FAT	*P*-value
Total protein (g/dl)	4.98±0.399	5.07±1.23	4.69±0.09	0.669
Albumin (g/dl)	2.33±0.136	2.60±0.347	1.88±0.852	0.352
Globulins (g/dl)	2.65±0.47	2.47±1.05	3.50±0.82	0.397
AST (U/ml)	51.00 ± 2.51^{b}	53.00 ± 2.35^{b}	61.33 ± 2.45^{a}	0.044
ALT (U/ml)	13.00 ± 0.58^{b}	13.00 ± 1.00^{b}	17.33 ± 0.88^{a}	0.027
Urea (mg/dl)	41.29±2.14	41.20±0.50	43.16±3.83	0.163

Groups with different superscripts are statistically significant (P<0.05).

The detected non-significant reduction in serum albumin and globulin concentrations in our study contrasted the results of **Marques et al., (2015)**, who reported reduced serum albumin after feeding on high-fat diet in Wistar and Sprague-Dawleyrat strains**.** Albumin is considered a negative acute phase protein and might be decreased during inflammatory conditions, such as obesity **(Hubner and Voss,1978; Schreiber etal.,1986)**.Based on the foregoing results, our study indicated that CTZ had many advantages duo to its roles against bacteria, lipid peroxidation, cancer, inflammation, and **(Xia etal.,2011;Anitha etal.,2014; Kerch,2015**), andCTZ treatment **(3%) also** significantly reduced acetaminophen-induced hepatotoxicity **(Yao et al., 2015)**.

CONCLUSION

The dietary chitosan supplementationat a level of 2.5 g/kg for 8 week-interval pre-semen collection has a vital role on scavenging the negative effects of oxidative stress in the seminal plasma of bucks fed control or high-fat diets. Thus, chitosan could safely use in Zarai bibuck's diets to improve semen quality and antioxidant defense system and potentiates the reproductive activity of Zaraibi bucks

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الملخص العربى

جودة السائل المنوى، بيروكسيد الدهون في البلازما المنوية ومستوى التستوستيرون في الماعز الزرايبي المغذىعلى الشيتوزان في العلائق العادية أو عالية الدهون

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تهدف هذه الدراسة إلى تحديد تأثير الأضافة الغذائية للشيتوزانعلى متغيرات السائل المنوي، وموت الخلايا المبرمج للحيوانات المنوية، وتكسر الحمض النووي، وحالة مضادات الأكسدة في البلازما المنوية لذكور الماعز الزرايبي التي تغذت على نظام غذائي طبيعى أو غني بالدهون.تم تقسيم إجمالي ١٥ تيس ناضجً جنسيا (٣٩,٤٢ ± ٠,٤٨ كجم LBW و ١٨-٣٠ شهرًا) إلى ٣ مجموعات (ن = ٥/مجموعه) ؛مجموعة الكنترول ومجموعتين غذيت على عليقة الكونترول مع ٢,٥ جم شيتوزان /كجم علف مركزأو ٢,٥ جمشيتوزان + 30 جم دهن جاف / كجم علف مركز، على التوالي لمدة ٨ أسابيع.وأظهرت النتائج تحسنا (P <0.05) فىحجم السائل المنوي ، ونسب الحركة التقدمية ، والحيوية ، ونسب الشذوذ فى الحيوانات المنوية ، وتركيز الحيوانات المنوية ، وكذلك عدد الحيوانات المنوية (الكلي ، والمتحرك والحي ، والطبيعي والوظيفي) لكل قذفهمن خلال اضافة الشيتوزان.كما أدتإضافةالشيتوزانمع الدهون الى تحسن جميع الصفات السابقة ، ما عدا نسبة الشذوذ في الحيوانات المنوية. حيث كانت نسبة الحيوانات المنوية الحيوية أعلى (P <0.05) ، بينما كانت نسبة الموت المبرمج فى الحيوانات المنوية أقل (P <0.05) معالشيتوزان أو الشيتوزان + الدهون. ولم تتأثر نسبة الموت المبكر المبرمج و الموت المبرمج للحيوانات المنوية بشكل معنوي بالمعاملتين.وقد انخفضت النسبة المئوية لتكسر DNA(P <0.05). بينما زادت النسبة المئوية للحيوانات المنوية فردية الصبغيات (P <0.05) بواسطة الشيتوزان أو الشيتوزان + الدهون ولم تتأثر النسب المئوية الحيوانات المنوية ثنائية الصبغيات. وأيضا انخفض مستوى(P <0.05) MDA. بينما تم زيادة نشاط GSH و CAT وGPxبواسطةالشيتوزان فقط.كما تم استعادة المستويات المستنفدة من إنزيمات مضادات الأكسدة و MDA إلى قيمتها فيمجموعة الكنترول. ولم يختلف مستوى التستوستيرون في الدم ، لكن التستستيرون كان أعلى (P< 0.05) بواسطة الشيتوزانعنه بواسطة الشيتوزان + الدهون في البلازما المنوية.وقد إتضح أن الفروق في تركيز البروتين الكلي ، الألبيومين ، الجلوبيولين ، واليوريا في الدم كانت غير معنوية ، بينما زاد نشاط AST و ALT معنوياً(P <0.05) فى البلازما المنويه بواسطة الشيتوزان أو بواسطة الشيتوزان + الدهون مقارنة مع مجموعة الكنترول.

ولذلك نستخلص من هذه الدراسة أن إضافة الشيتوزان الغذائية بمستوى ٢,٥ جم / كجم علف مركز قبل جمع السائل المنوي لمدة ٨ أسابيع لها دورا حيويا في التخلص من الآثار السلبية للإجهاد التأكسدي في البلازما المنوية للتيوس التي تتغذى على علائق عاديه أو عالية الدهون. وبالتالي ، يمكن استخدام الشيتوزان بأمان في علائق الماعز الزرايبي لتحسين جودة السائل المنوي ونظام الدفاع المضاد للأكسدة وتحسين التناسل فى ذكور الماعز .