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Dietary Chitosan Supplementation Modulates Hematology, Lipid Profile, Rumen Function, Antioxidant Status, and Thyroxin in Zaraibi Goat Bucks Fed on High-Fat Diets

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Abstract: Recently, chitosan gained a great attention due to its unique biological activities as a natural biodegradable polymer derived from chitin with non-antigenic, non-toxic. It has several positive impacts on animal health including potent antioxidant, antimicrobial activities, and anti-immunogenicity. Therefore, it is a natural, bioactive, mucoadhesive, and biocompatible compound used commonly as a safe additive in animal production. This study was conducted to detect the effects of dietary inclusion of chitosan in high-fat diet (HFD) on growth, hematology, lipid profile, rumen function, oxidative stress, and antioxidant status of Zaraibi goat bucks. Total of 18 sexually mature bucks (38.69±0.57 kg BW) were allocated into 3 groups (n= 6); the control group fed the control diet and treatment groups received HFD (the control diet with 3% fat) and the HFD plus 2.5% chitosan for 8 weeks, respectively. Results showed that HFD increase ($P<0.05$) final body weight, total weight gain, white blood cells (WBCs), and serum total cholesterol (TC), triglycerides (TG), VLDL, LDL, and malondialdehyde (MDA) with declined free T4 hormone, and HDL with the exhaustion of GSH, CAT and GPx activities beside reducing ruminal total proteins, glucose, ammonia-N, TVFA, total and L-lactate concentrations. Chitosan dietary inclusion to HFD reversed the aforementioned parameters with a notable enhancement of the antioxidant enzyme activities, suppressed the elevated MDA levels, and restored the depleted T4 level. Therefore, chitosan could be safely utilized as a dietary supplement in buck's diets to improve organ functions, lipid profile, antioxidant defense system, scavenge free radicals, and potentiate Buck's reproductive activities.

Keywords: Chitosan, High Fat Diet, Lipid Metabolism, Antioxidant, Leukocytes, Zaraibi Goat Bucks

1. Introduction

Numerous studies have demonstrated that a diet heavy in fat is unquestionably a contributing factor to weight gain and the global prevalence of obesity, although the causes of obesity are complicated. Additionally, the development of hypercholesterolemia has been linked to rising blood total cholesterol (TC), Low- (LDL) and very low- (VLDL) density lipoprotein [1]. It is well established that lipid homeostasis abnormalities in obese patients are linked to liver and adipose tissue dysfunction. Adipocytes get larger as obesity progresses due to increased triglyceride accumulation [2]. Fatty liver results from an accumulation of triglycerides (TG), which are stored in excess amounts as free fatty acids (FFA) in the liver [3]. Hence, the adipose tissue and the liver have become major targets for anti-obesity foods and food ingredients against human obesity. To date, hypercholesterolemia is treated by pharmacological medicine which has adverse effects. Now, there are many attempts to explore natural products for meeting hypercholesterolemia [4].

Enriching animal diets with natural antioxidant compounds for protecting them against potential oxidative stress and addressing consumer safety concerns [5] and there is a worldwide usage of natural supplements to animal diets that have a positive effect on the human body. In ruminant, several feed additives are incorporated in the diet for improving animal performance and maintaining the metabolic status of health of farm animals.

Chitosan is nontoxic rich natural polysaccharides, the N-deacetylates product of chitin [6] containing β-(1-4)-2-acetamido-D-glucose and β-(1-4)-2-amino D-glucose units and has a higher molecular weight and a polycationic polymer. A large quantity of chitosan is obtained from marine crustaceans and it is found in the exoskeleton of insects, mollusks, crustaceans, and some algae [7]. Chitosan is a new additive and relatively less used in animal production [8]. Chitosan is considered a natural, bioactive, mucoadhesive, and biocompatible compound, and is commonly used as a safe food product [9] to prevent spoilage and to act as a natural antioxidant due to metal ion chelation in lipid-containing meals. Chitosan has also been employed as a food preservative to delay deterioration and act as a natural antioxidant, extending shelf life to metal ion chelation in lipid-containing foods, hence prolonging shelf life [10]. Chitosan also has an antimicrobial effect on bacteria, molds, and yeasts, so it has been widely applied in medicine and food [11]. In recent decades, chitosan gained considerable attention due to its unique biological activities as a natural biodegradable polymer derived from chitin with non-antigenic, non-toxic with several health-beneficial effects including potent antioxidant, antimicrobial activities, and anti-immunogenicity [12]. In addition, chitosan regulates the metabolism of glucose and lipids and improves obesity or diabetes [13]. Moreover, the molecular weight of chitosan and the degree of deacetylation significantly influence its biological activities [14].

To date, there is scarce information on the impact of high-fat diets on the growth of goat bucks and how we can eliminate the harmful effects of these diets on animal productivity. Therefore, the present study aimed to identify the mechanisms of chitosan in regulating growth performance, lipid profile, organ functions, and antioxidant status in Zaraibi goat bucks. We used the high-fat diet to induce a hyperlipidemic state to represent an unbalanced metabolic condition.

2. Materials and Methods

The current study was carried out at Sakha Animal Production Research Station, Animal Production Research Institute (APRI) Agricultural Research Center, Ministry of Agriculture, Egypt, in cooperation with the Faculty of Veterinary Medicine Kafrelsheikh University, Egypt.

2.1. Animals and Feeding System

In this experiment sexually mature Zaraibi goat bucks (n= 18) weighing 38.69±0.57 kg live body weight (LBW) of and aging 18-30 months were used. All animals were clinically normal and housed in a hygienic pen. They were fed on concentrates and corn silage, while water was available all day times. Animal management, handling, and blood and semen collection were achieved with an expert veterinarian's aid during the whole experimental period.

Feeds were offered at 8 a.m. and 4 p.m. and adjusted based on weekly body weight changes and the physiological status of bucks. The initial and final body weights were recorded individually for a feeding period of 8 weeks, and then the total weight gain of bucks was calculated in each group.

Each buck in all groups was fed individually to cover the requirements of goat bucks [15] on concentrate feed mixture (wheat bran, 42%; ground yellow corn, 34%; decorticated cottonseed meal, 18%; cane molasses, 3%; limestone, 2% and common salt, 1%) and corn silage.

2.2. Experimental Design

This experiment included three experimental groups, six animals in each group. The control group was fed on concentrates without any additives and corn silage. Bucks in the 2nd group were fed on the same control concentrate supplemented with 30 g fat/kg (high-fat diet) according to Bernard et al. [16]. Bucks in the 3rd group were fed high-fat concentrate in addition to 2.5 g chitosan/kg according to Chiu et al. [17]. The feeding period lasted for 8 weeks.

Fat supplementation was in form of dry fat (IBELAC, Ibex for Feeds and Fertilizers Production, Giza, Egypt). IBELAC was consisted of crude fat (5%), palm oil (37%), soybean oil (37%), flax oil (11%), calcium oxide (15%), and anti-oxidant (0.15%). Chemical analysis of Ibelac was 84% CF, 7.5% Ca, 4% moisture, 4% non-saponifying substances, and 0.5% free fatty acids. However, chitosan used in this experiment was available in powder form Co. Chitosan Egypt for Manufacturing Chitosan + Products (6

October City, Egypt).

2.3. Blood Sampling, Hematology, and Lipid Profile

At the termination of feeding period of 8 weeks, blood samples were taken from the jugular vein of each animal in all groups. Blood sample of each animal was divided into two parts, the first was placed into a test tube containing EDTA (an anticoagulant) for hematology, including count of red (RCBs) and white (WBCs) blood cells, Hematocrit percentage (Ht%), Concentration of hemoglobin (Hb), and differential counts of WBCs by using automated blood cells counter with an auto hematology analyzer (Sysmex F-800, Japan) according to standard techniques of Feldman et al. [18].

The second was placed into a plain centrifuge tube, left for 2-3 h for clotting and serum was separated after centrifugation at 3000 rpm for 15 min, then serum samples were stored at -20 °C until analyses of serum biochemicals, thyroxin hormone, lipid peroxidation markers, and activity of antioxidant enzymes. Concentration of triglycerides (TG) and total cholesterol (TC) according to Richmond [19], HDL [20], LDL and VLDL [21] were determined in blood serum of bucks using chemical kits (Bio diagnostics, Cairo, Egypt).

2.4. Evaluation of Lipid Peroxidation, Antioxidant Biomarkers, and Hormonal Assays

Lipid peroxidation was evaluated in blood serum by measuring malondialdehyde (MDA) content according to Ohkawa et al. [22]. Reduced glutathione (GSH) activity was evaluated according to the method of Beutler et al. [23]; catalase (CAT) activity was estimated according to Aebi [24], while glutathione peroxidase (GPx) activity was evaluated according to Paglia and Valentine [25]. According to Faix and Miller [26], serum concentration of free thyroxine (FT4) was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (DRG Diagnostics GmbH, Marburg, Germany) following the manufacturer's instructions.

2.5. Ruminal Liquor Parameters

Samples of rumen fluid were collected at the end of the feeding trial from 5 animals in each group 6 h post-feeding. The rumen fluids were collected by aspiration method using a stomach tube. The fluid (20 ml) of the filtrate was collected into plastic containers containing an equal quantity of 0.1 M H_2SO_4 to trap ammonia and lower the bacterial activity. The mixtures were centrifuged at 3000 rpm for 10 minutes. About 20 ml of the supernatant were decanted into plastic bottles and kept in a deep freezer at -196°C until analysis of total volatile fatty acids (TVFA) and ammonia nitrogen (ammonia-N). The TVFA and Ammonia-N in the rumen fluid were determined by the steam distillation method following the procedure of Van-Soest [27]. Ruminal lactate was measured according to Lorenz *et al.* [28], while ruminal glucose and total proteins concentrations were determined using commercial test kits (Beijing Jiuqiang Bio-Technique Co. Ltd., Beijing, China) with an automated biochemistry analyzer (Hitachi 7020; Hitachi Ltd., Tokyo, Japan).

2.6. Statistical Analysis

Shapiro-Wilk's and Levene's tests were used to check the normality and homogeneity of the obtained data in this experiment, respectively. To study the effect of treatment (1-3), data were statistically analyzed by one-way ANOVA. The significant differences between the experimental groups were separated by Tukey's multiple comparison tests. All percentage values were adjusted by arcsine transformation before analysis, then tabulated as percentage values pre-analysis.

3. Results and Discussion

3.1. Live Body Weight

Results of LBW of bucks illustrated in figure 1 revealed that bucks fed high-fat with chitosan diet showed significantly (P<00.05) higher final weights than the controls. However, LBW did not differ significantly in those fed on a high-fat diet as compared to other groups. Total weight gains significantly (P<0.05) increased in bucks fed high-fat with or without chitosan as compared to controls. These results indicated the beneficial effects of chitosan on the growth performance of bucks in terms of increasing final LBW and total weight gain.

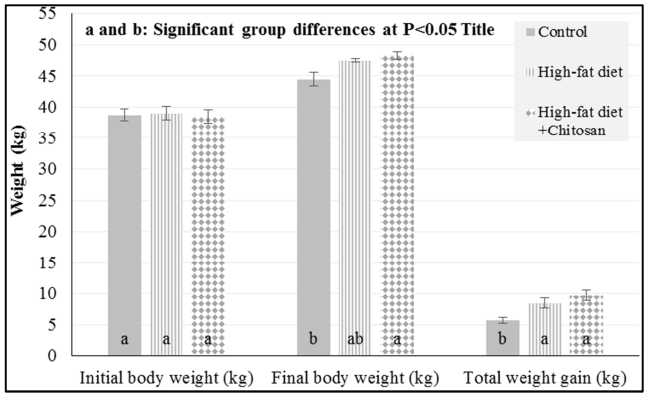

Figure 1. *Live body weight and total gain of bucks in the experimental groups during the experimental period.*

Following the obtained results, several authors stated that rats fed a diet supplied with fat for eight weeks exerted higher weight gain compared with those receiving the normal diet [29]. In our study, chitosan addition maintained the growth performance of bucks as those fed on a high-fat diet but showed significantly the highest LBW and total weight gain. In this respect, Sun et al. [30] found an increased feed gain ratio in an *E. coli*-challenged model in weaned piglets after 28 days of dietary chitosan supplementation. These results are in disagreement with the results of Liu et al. [31], who found that 5% chitosan and 5% Chitosan Oligosaccharide (COS) incorporation into the high-fat diets

of rats significantly reduced body weight. The observed improvement in LBW and gain of bucks fed high-fat diet supplemented with chitosan could be due to the direct enhancement of serum growth hormone levels and improved small intestine morphology [32]. Also, chitosan was reported to enhance the digestion of Ca, P, CP, and DM, increase the levels of amylase in the jejunum [33], and alter the gut microbiota populations [34]. Chitosan improved feed efficiency in cows [35].

3.2. Hematology

Hematological findings of the control and treated bucks are presented in Table 1. Here we found that bucks fed the high-fat diet showed a significant (P<0.05) reduction in RBCs count, Hb concentration, and Ht% which represented microcytic hypochromic anemia. In addition, the leukogram finding showed a marked change in terms of a significant increase in the count of WBCs, as well as numbers and percentages of neutrophils and monocytes, and a significant decrease in lymphocytes, reflecting an inflammatory condition compared with the controls. Interestingly, chitosan supplementation with the high-fat diet was able to modulate the adverse impact of high-fat diets on both erythrogram and leukogram parameters compared with the high-fat diet alone. A chronic low-grade inflammation induced by obesity had negative changes in Fe metabolism, the immune response, and activity of platelets, and other hematological parameter [37, 39].

Table 1. *Hematological findings of bucks in control and treatment groups.*

Hematological parameter	Control	Treatment group		*P-value*
		High-fat diet	High-fat +Chitosan	
RBCs (x10^6/µl)	10.40±0.50^a	8.20±0.47^b	10.16±0.033^a	0.002
Hemoglobin (g/dl)	10.85±0.31^a	8.90±0.57^b	11.05±0.44^a	0. 009
Packed cell volume (%)	29.00±1.52^a	23.33±0.88^b	29.00±0.57^a	0.012
MCV (fl)	2.78±0.30	2.85±1.37	2.85±0.65	0.906
MCH (pg)	1.05±0.76	1.10±1.36	1.08±0.44	0.879
MCHC (%)	3.77±2.92	3.83±3.36	3.80±1.30	0.985
WBCs (x10^3/µl)	9.63±0.35^b	14.33±0.43^a	9.96±0.31^b	0.000
Neutrophils (%)	30.00±2.65^b	52.00±3.05^a	25.33±2.66^b	0.000
Lymphocytes (%)	57.33±3.28^a	34.66±1.20^b	62.33±2.02^a	0.000
Monocytes (%)	9.66±0.33^{bc}	12.33±1.02^a	10.66±1.33^b	0.034
Eosinophils (%)	3.00±0.577	1.00±0.000	1.66±0.330	0.067
Neutrophils (x10^3/µl)	2.87±0.19^b	7.46±0.54^a	2.54±0.33^b	0.000
Lymphocytes (x10^3/µl)	5.53±0.47^{ab}	4.96±0.12^b	6.20±0.19^a	0.003
Monocytes (x10^3/µl)	0.92±0.00^b	1.76±0.28^a	1.05±0.11^b	0.012
Eosinophils (x10^3/µl)	0.28±0.55	0.14±0.004	0.16±0.032	0.140

$^{a \text{ and } b}$: Means within the same row with different superscripts are significantly different (P<0.05).

The observed increase in count of WBCs when bucks were fed high-fat may be an indicator of occurring inflammation and leucocyte activation. Count of WBCs, lymphocytes, neutrophils, and monocytes were affected by obesity [36]. Both IL-6 and IL-1, as adipose-derived cytokines, induces hepcidin expression in the adipose tissue [37] leading to a reduction in absorption of Fe and the efficiency of Fe fortification [38]. Therefore, restoring the control levels of erythrogram and leukogram parameters when bucks were fed on the high-fat diet with chitosan may be attributed to that chitosan improve impaired iron absorption in bucks fed on the high-fat diet.

3.3. Serum Lipid Profile

Serum lipid parameters of the control and treated buck groups are shown in Table 2. In this study, bucks fed the high-fat diet revealed a significant (P<0.05) increase in the concentration of TC, TG, LDL, and VLDL compared with the controls. The addition of chitosan to the high-fat diet showed a significant (P<0.05) marked decline in serum TC, TG, LDL, and VLDL as compared to those fed the high-fat diet alone, but reached values at a non-significant level with the control values.

A strong positive correlation has been demonstrated between body fat and TC and TG levels in blood serum [40]. In rats fed on a high-fat diet, oxidative damage in tissues causes a liver injury by increasing concentration of TG and TC in blood serum, which eventually induce lipo-toxicity development and accumulation of lipid in the liver, consequently developed a fatty liver as compared to controls [4]. The hallmark of dyslipidemia in obesity is the hyper triglyceridemia due in part to increased FFA fluxes to the liver leading to increased blood TC levels [41]. Hypertriglyceridemia is one of the criteria for diagnosis of the metabolic syndrome and seems to be present in Wistar rats fed the high-fat diet. The increase in FFA flux to the liver may cause the overproduction of triglyceride-rich VLDL which results, in turn, in high circulating levels of TG reflecting an insulin-resistant condition [42]. Furthermore, the elevated levels of VLDL, LDL, and TC in serum or plasma have been manifested in the occurrence of hypercholesterolemia [1, 29]. In our study, we detected a significant reduction in serum TC, TG, LDL, and VLDL in bucks-fed chitosan with the high-fat diet to reach their values in the controls.

Table 2. *Lipid profile in blood serum of bucks in control and treatment groups.*

Serum lipid profile	Control	Treatment group		*p-value*
		High-fat diet	High-fat +Chitosan	
Total cholesterol (mg/dl)	96.33±1.56[c]	137.02±3.33[a]	109.91±3.40[b]	0.021
Triglyceride (mg/dl)	114.07±5.27[b]	192.33±2.89[a]	122.26±2.84[b]	0.000
HDL (mg/dl)	48.06±14.94	36.71±4.33	48.10±3.35	0.344
VLDL (mg/dl)	22.81±1.05[b]	38.46±0.58[a]	24.45±0.57[b]	0.000
LDL (mg/dl)	25.45±5.84[c]	61.84±3.48[a]	27.35±0.57[c]	0.015

[a, b, and c]: Significant group differences at P<0.05.

As proved in our study, chitosan showed advantages as a functional additive in preventing or treatment of some chronic diseases. Chitosan has hypocholesterolemic effects [43] and low molecular weight chitosan prevents the accumulation of white adipose tissues in the body and the accumulation of lipids in the liver in mice fed a high-fat diet [44]. Chitosan decreases the level of blood cholesterol by increasing receptor mRNA expression of LDL to clear LDL into the liver [45], leading to an enhancement of the reverse TC transport and decreasing TG and LDL levels in blood plasma [46]. Improvement of the changed lipid homeostasis in rats fed high-fat diet was reversed to the baseline level by addition 5 or 7% chitosan in the diet. Chitosan at a level of 7% resists high-fat diet-induced obesity and hepatic fat accumulation by suppressing the expression of lipogenic transcription factors and lipogenesis-associated genes [17].

In addition, high molecular weight chitosan (5%) has a hypo-cholesterolemic effect in diabetic rats [47]. Chitosan treatment (3%) for 5 months showed a marked effect on modulating the lipid profiles in blood and liver [48] and decreased acetaminophen-induced hepatotoxicity [49].

TNF-α, as an inflammatory marker, plays important role in the lipid metabolism and hepatic inflammation [50]. Chitosan, as immunomodulatory feed additive may use as alternatives to antimicrobial growth promoters in pig production. In this context, the addition of chitosan in the diet of weaned piglets increased the cell-mediated immune reaction by regulation of antibodies and cytokines production [51] and enhancement in concentration of IgG was found in pigs fed diet containing chitosan at a level of 100 mg/kg [52].

3.4. Serum Lipid Peroxidation and Antioxidant Status

The effects of the high-fat diet on lipid peroxidation and antioxidant biomarkers in blood serum are presented in Table 3. In this work, the high-fat diet depleted antioxidant defense via a significant (P<0.05) reduction in the activity of serum antioxidant enzyme (GSH, CAT, and GPx) with a significant (P<0.05) increase in MDA levels in serum of bucks compared with the control diet. Dietary chitosan supplementation with the high-fat diet succeeded to restore the depleted antioxidant defense by a significant reduction in oxidative stress marker MDA compared with the high-fat diet.

The activity of CAT, GSH, and GPX, as natural cellular antioxidants [4] was exhausted due to oxidative stress by feeding bucks on a high-fat diet in our study. In agreement with the present results, GPx and glutathione-s-transferases (GST) activities reduced, while TBARS levels in the liver increased in rats fed the high-fat diet [53]. Intake of a high-fat diet caused oxidative stress in most experimental models and patients [54]. Feeding a high-fat diet elevates MDA and nitric oxide levels, and advanced protein oxidation products, while reduced the activity of SOD and CAT in rats [55]. Improving antioxidant status of bucks fed the high-fat diet supplemented with chitosan may be attributed to that chitosan can decrease TBARS levels in the liver [56]. Also, chitosan improves the activity of SOD and CAT, total antioxidant capacity (TAC), and level of IgG, while reduced serum MDA in pigs [52]. Therefore, chitosan had a property to eliminate oxidative stress and has a potent antioxidant in renal failure [57].

Table 3. *Serum oxidative and antioxidant biomarkers of bucks in control and treatment groups.*

Marker	Control	Treatment group		*P-value*
		High-fat diet	High-fat +Chitosan	
Malondialdehyde (nmol/ml)	14.37±2.16[b]	24.50±1.26[a]	14.39[b]±2.78[b]	0.003
Glutathione reductase (nmol/ml)	6.18±0.24[a]	3.46±1.02[b]	5.59±1.42[a]	0.030
Catalase (U/ml)	8.82±1.91[a]	4.16±0.45[b]	7.48±1.33[a]	0.005
Glutathione peroxidase (pg/ml)	30.67±0.67[a]	19.16±0.28[b]	28.43±0.27[a]	0.005

[a and b]: Significant group differences at P<0.05.

3.5. Ruminal Liquor Characteristics

Rumen liquor parameters of bucks in the experimental groups are presented in Table 4. Ruminal parameters including total protein and glucose concentrations were not affected significantly by the high-fat diet with or without chitosan. However, the concentration of ammonia-N, TVFAs, total lactate, and L-lactate were significantly decreased by the high-fat diet compared with the control diet. Chitosan dietary incorporation with the high-fat diet succeeded to normalize ammonia-N, total lactate, and L-lactate levels and maximized TVFA concentration as in the control in

comparison with the high-fat diet.

Table 4. *Characteristics of ruminal liquor of bucks in control and treatment groups.*

Ruminal parameter	Control	Treatment group		*P-value*
		High-fat diet	High-fat +Chitosan	
Total protein (g/dl)	4.50±0.203	3.80±0.210	4.36±0.266	0.193
Glucose (mg/dl)	51.55±4.65	47.51±1.12	50.74±1.34	0.553
Ammonia-N (mg/dl)	$9.73±0.12^{a}$	$8.07±0.93^{b}$	$9.48±0.40^{a}$	0.001
TVFA (mEq/dL)	$11.70±0.10^{b}$	$9.98±0.04^{c}$	$12.56±0.08^{a}$	0.000
Total lactate (mg/dl)	$6.25±0.07^{a}$	$5.01±0.06^{b}$	$6.41±0.30^{a}$	0.000
L-lactate (mmol/L)	$0.70±0.014^{a}$	$0.55^{c}±0.007^{b}$	$0.71^{b}±0.036^{a}$	0.000

$^{a, b, \text{and } c}$: Significant group differences at P<0.05.

The inclusion of fatty acids has adverse effects on ruminal fermentation [58]. The negative effects of the incorporation of dietary fatty acids on fiber degradation and dry matter intake are associated with the level and source of supplemental fat added to the basal diet [59]. Fatty acids in lipids have an inhibitory effect on some species of microflora in the rumen, consequently affect the ruminal fermentation products, which modifies the ruminal condition and changes microbial diversity. In this way, Jenkins [60] mentioned a high potential of unsaturated fatty acids to positively impact the fermentation products. Yang et al. [61] found that presence high contents of linoleic (polyunsaturated fatty acids) in whole raw soybean had toxic effects on cellulolytic and butyrate-producing bacteria. As in our study, Goiri et al. [62] reported positive effects of chitosan on ruminal fermentation via elevating the concentration of propionate and reducing the ratio of methane and acetate to propionate. These findings increase energy supplementation and reduce disappearance of the ruminal protein. *In-vitro* studies evaluated the effect of chitosan on the fermentation in the rumen of lactating cows; they found that chitosan can reduce *in-vitro* production of methane and increase C3 concentration in ruminants [62, 63], thus improving energy usage [64]. Chitosan increases TVFA and decreases biohydrogenation in the rumen [11, 35].

In addition, chitosan inclusion in the diet reduces the acetic acid levels in the ruminal liquor with increasing level of propionic acid resulted from increasing digestibility of nutrients [63]. Chitosan degradation and the remaining C-skeleton used by certain bacteria within the rumen may alter the fermentation products [65]. Dietary chitosan (5%) in dairy cows increased molar proportions of total lactate, L-lactate, and acetate, decreased the propionate and butyrate proportions, and increased ammonia concentration, 2 h post-feeding [9]. The linkage between gut microbiota and obesity was suggested [66], and gut microbiota can affect inflammation such as atherosclerosis, even in the absence of obesity or high-fat feeding, via several mechanisms that include overwhelming the immune system by triggering an inflammatory response, interfering in the lipid and bile acid metabolism, and the production of harmful microbial metabolites [67].

3.6. Thyroxin Profile

Results of free thyroxin (FT4) in the blood serum of bucks illustrated in Figure 2 revealed that feeding the high-fat diet negatively impacted the thyroid function via significant (P<0.05) reduction in FT4 level as compared to feeding on the control diet. The incorporation of chitosan in the high-fat diet restored this reduction to be similar to that in those fed on the control diet.

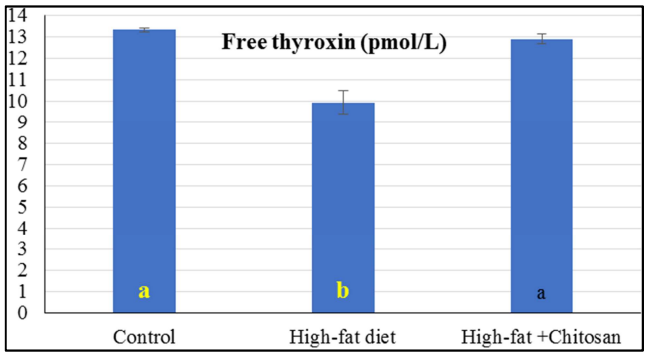

Figure 2. *Serum thyroxin level of bucks in the control, high-fat, and high-fat with chitosan groups ($^{a \text{ and } b}$: significant group differences at P<0.05).*

In agreement with the present results, many authors found that high-fat diets reduced the level of blood FT4 in rats despite the increasing level of thyroid-stimulating hormone (TSH) in the blood due to morphological abnormality in the thyroid gland [68]. The thyroid gland is a crucial endocrine organ that synthesizes and secretes thyroid hormones and plays an important role in the metabolism and the development, differentiation, and maintenance of the central nervous system, skeletal system, and cardiovascular system [69]. Increasing fat in animal diet may be a possible reason for thyroid dysfunction (hypothyroidism), revealing the role of lipids in maintaining the function of the thyroid gland [70. The elevated level of FFA may lead to deleterious lipotoxicity in the thyroid gland. The change in the composition of the unsaturated fatty acids in the diet indicates that dietary fatty acids contribute to fatty acid pooling in the thyroid gland. Lysophospholipid disturbances might indicate that hypothyroidism may be caused by inflammation [68]. In our study, the antioxidant and inflammatory effects of chitosan to protect the thyroid tissues from oxidative stress may lead to amelioration of FT4 concentration in the blood serum of bucks. In this respect, antioxidants scavenge oxidative stress by decreasing caspase-3 activity and DNA damage [71].

4. Conclusion

This study shed a spot of light on the adverse effect of a high-fat diet and pointed to the remarkable positive impacts of dietary chitosan inclusion on growth, hematological parameters, oxidative stress and antioxidant status, and thyroid function of Zaraibi goat bucks fed high-fat diets**.** Thus, chitosan could be safely used to potentiate buck's physiological activities.

Data Availability Statement

The data sets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Conflict of Interest

There are no conflicts of interest.

Animal Welfares Statement

The experimental procedures were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

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