Bulgarian Journal of Agricultural Science, 28 (No 6) 2022, 1131–1139

Impact of castration and sex hormones on some hormonal and biochemical parameters of male rabbits

Sami Ahmed[1*] and Saeb Abdul-Rahman[2]

[1] *University of Duhok, Department of Animal Production, College of Agricultural Engineering Sciences, Kurdistan Region, Iraq*
[2] *University of Mosul, Department of Animal Production, College of Agriculture and Forestry, Mosul, Iraq*
[*]*Corresponding author:* sami.gerges.sg@gmail.com

Abstract

Ahmed, S. & Abdul-Rahman, S. (2022). Impact of castration and sex hormones on some hormonal and biochemical parameters of male rabbits. *Bulg. J. Agric. Sci., 28 (6)*, 1131–1139

The current study aimed to evaluate the effects of castration and sex hormones on hormonal and biochemical traits of male rabbits. 36 mature local rabbits were divided into 6 groups. 1st group: Intact, 2nd group: castrated. 3rd and 5th group: Intact rabbits treated i.m with testosterone 10 mg/kg B.wt and estrogen 0.5 mg/kg B.wt respectively, while 4th and 6th groups: Castrated rabbits treated as in 3rd and 5th groups respectively, treatments continued for 4 weeks. Results revealed that castration increased significantly HDL-C, globulin/albumin and T4 levels, and reduced significantly risk ratio, ALT and cortisol level as compared with intact at $P \leq 0.05$. While testosterone treatment reduce significantly cholesterol, HDL-C, globulin, and increased significantly T4, T3 and cortisol levels. On the other hand, estrogen treatment enhance significantly HDL-C, globulin/albumin, and reduced significantly LDL-C, risk ratio, ALT, glucose, total protein and albumin, T4 and cortisol levels at $P \leq 0.05$. In regard to interaction effects, castration and estrogen treatment reduce the stress as represented by the reduction of risk index, ALT, AST and cortisol levels, also enhances lipid profile, globulin and globulin/albumin. Testosterone and estrogen treatment enhances rabbits final body weight and weight gain in both intact and castrated rabbits. In conclusion, castration and estrogen treatment of castrated and intact male rabbits reduce the stress effects and enhance lipid profile and some immunological parameters.

Keywords: rabbits; castration; testosterone; estrogen; lipid profile
Abbreviations: HDL-C: High-density lipoproteins-cholesterol; LDL-C: Low-density lipoproteins-cholesterol; VLDL-C: Very low-density lipoproteins-cholesterol; TG: Triglyceride; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; T4*:* Thyroxin; T3: Triiodothyronin

Introduction

The primary function of gonadal steroid hormones is to regulate sexual behaviour and prepare the organism for reproduction. Estrogen is a hormone that is essential for female sexual and reproductive development. It is also known as female sex hormone. Estrogen is important not only in female reproduction but also in male reproduction and a variety of other systems in both males and females, including the neuroendocrine, skeletal, and immune systems. Along with its impact on many physiological processes, oestrogen has been linked to a variety of diseases, including obesity, metabolic disorders, cancer, osteoporosis, endometriosis, and fibroids (Burns & Korach, 2012; Deroo & Korach, 2006). Ovarian hormone deficiency is a significant source of oxidative stress (Ha et al., 2006), but the molecular mechanisms by which these steroids regulate oxidant-antioxidant balance in various tissues are unknown. The molecular structure of oestrogens confers certain benefits. For example, all oestrogens have a free phenolic hydroxyl group on the A- ring, which

confers antioxidant properties and is the sole structural determinant for free radical scavenging (Badeau et al., 2005). Because oestrogens are lipophilic, they concentrate in lipid-rich regions of cells such as the cell membrane, where they act in vivo as localised antioxidants (Prokai et al., 2005(.

Testosterone is the most abundant androgen in the body, and it is responsible for spermatogenesis and the development of male secondary sexual characteristics. Many physiological processes are regulated by it, including muscle protein metabolism, erythropoiesis, plasma lipids, and bone metabolism (Wilson, 1988; Bhasin & Bremner, 1997). Testosterone is also an anabolic hormone; it increases metabolic rate, which increases O_2 consumption, which may increase reactive oxygen species (ROS) production (Chainy & Sahoo, 2020). Furthermore, Barp et al. (2002) demonstrated that ovariectomy in rats resulted in a 200 percent increase in thiobarbituric acid reactive substances (TBARs) in myocardium, whereas castration of male rats had no effect on lipid peroxidation (LPO), implying that oestrogen may play an antioxidant role in heart muscle while testosterone does not. Aydilek et al. (2004), on the other hand, reported that testosterone treatment of male New Zealand rabbits decreased glutathione peroxidase (GSH-px) and vit. E activity while increasing malondialdehyde (MDA) in comparison to control rabbits. They concluded that testosterone administration increased oxidative stress significantly. Furthermore, Bellanti et al., (2013) claim that sex hormones control the expression of antioxidant genes.

The current study was aimed to evaluate the effects of castration and sex hormones treatment on some hormonal and biochemical parameters of male rabbits.

Materials and Methods

Location of the experiment

The current study has been conducted at Animal Physiology Laboratory, Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok, from the 1st of July to 12th of September 2021.

Experimental Animals

36 apparently healthy, local mature male rabbits, 8-10 months of age, were used in this experiment. The mean body weight 1560.27 gram. The rabbits were maintained under uniform management and husbandry conditions. Animals were allowed to adapt to the experimental conditions for 14 days prior to the commencement of the study. They were fed standard ration *ad libitum,* green fodder was also used (25% of the ration) and were provided with clean drinking water *ad libitum*. Animals were randomly divided into 6 groups, 6 animals /group. They were housed in a well-ventilated room inside wooden cages. Each group was housed in a separate cage (120 × 100 × 100) cm for length, width and height respectively. Along the experiment period, the room temperature was kept at 20-25°C and 12 hours light/dark cycle with the light on from 6 pm to 6 am.

Materials used in treatments

Estrogen: O.S.T *fort* (10 ml injection solution), composition: Oestradiol cypionate… 5 mg. Exp.qs…1 ml. Vemedim Coporation, Cantho City, Vietnam.

Testosterone: Testosteron Depo (250 mg/ml testosterone. Solution for Injection). 1 ml of solution for injection contains: testosterone enanthate 250 mg. Galenika a.d. Beograd, Belgrad, Republic of Serbia.

Rabbits Castration

After acclimation period (2 weeks), 18 rabbits were castrated surgically as follows:

- Male rabbits starved for at least 12 hours (overnight);
- Rabbits anesthesized by using of Ketamine and Xylazine;
- Rabbits castrated bilaterally;
- Castrated rabbits left for 4 weeks after castration then introduced into the experiment.

Experimental Design

T_1: (Intact) male rabbits, reared on standard ration, mean body weight 1558.33±13.08 gram.

T_2: (Castrated) male rabbits, reared on standard ration, mean body weight 1563.33±9.09 gram.

T_3: (Intact) male rabbits, reared on standard ration, and injected with testosterone 10 mg/kg B.wt. (every other day, i: e: 3 injections/week) for 4 weeks, mean body weight 1560.00±6.70 gram.

T_4: (Castrated) male rabbits, reared on standard ration, and injected with testosterone as in T_3, mean body weight 1565.00±9.45 gram.

T_5: (Intact) male rabbits, reared on standard ration, and injected with estrogen 0.5 mg/kg B.wt. (every other day, i:e: 3 injections/ week) for 4 weeks, mean body weight 1566.67±6.45 gram.

T_6: (Castrated) male rabbits, reared on standard ration, and injected with estrogen as in T_5, mean body weight 1548.33±7.60 gram.

Blood collection

At the end of the experimental period (after 4 weeks), 5 ml of the blood was collected from ear vein of each rabbit for biochemical analysis. The sample was emptied into glass

sterile test tube without EDTA, and left for 2 hrs at the room temperature, then centrifuged (3000 RPM) for 15 minutes and the serum was separated by micropipette and emptied into tubes and stored at -20°C until biochemical and hormonal analysis was carried out.

Determination of biochemical and hormonal parameters

Biochemical and hormonal parameters were determined using kits from Roche Diagnostics Company (Germany), for analysis of Total protein, Albumin, Globulin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Glucose, Cholesterol, Triglyceride (TG), High-density lipoproteins- cholesterol (HDL-C), Low-density lipoproteins-cholesterol (LDL-C), Thyroxin (T4) and Triiodothyronine (T3) by automated method using Biochemical auto analyzer Cobas C 501 in Awny lab in Duhok. Cortisol was determined using kit from Snibe Company (China) by an Automated Chemiluminescence Immunoassay Analyzer (MAGLUMI 800). Globulin was calculated mathematically as the difference between total protein and albumin, also VLDL-C as: VLDL-C = TG/5 and Risk index as: RI = Cholesterol/HDL-C. Body weight was recorded at the end of experiment, weight gain calculated by subtracting final body weight from the initial body weight.

Statistical analysis

The data were analyzed as factorial experiment by two-way analysis of variance using General linear model (SAS, 2013) to study the effect of Castration, hormone treatment and interaction between them on different traits. Duncan multiple tests (1955) within SAS (2013) were used to detect differences among treatments.

Results

Biochemical Parameters

The effects of castration and treatments on lipid profile parameters were summarized in Table 1. Results showed that there was no significant changes in cholesterol, triglyceride, LDL-C, VLDL-C levels between castrated (69.44±2.10, 38.88±0.53, 32.62±2.30, 7.52±0.17 mg/dl) and intact rabbits (65.66±2.76, 38.77±0.84, 36.96±2.55, 7.64±0.19 mg/dl) respectively. While, a significant increase in the level of HDL-C in castrated rabbits (28.66±2.74 mg/dl) compared to intact group (20.72±2.09 mg/dl) was recorded (P≤0.05). Also a significant decrease in risk index (R.I) was observed in castrated (2.77±0.25) compared to intact animals (3.53±0.32). Injection of testosterone caused a significantly decrease in cholesterol level (61.75±1.52 mg/dl) compared to estrogen and control groups (69.83±3.83, 71.08±2.70 mg/dl) respectively. No significant differences in triglyceride level was noticed among estrogen (39.91±1.08 mg/dl), testosterone (38.75±0.59 mg/dl) and control group (37.83±0.77 mg/dl). Estrogen treated rabbits recorded significantly higher value in HDL-C level (35.25±2.25 mg/dl) compared to testosterone and control groups (16.33±0.44, 22.50±2.75 mg/dl) respectively. LDL-C level decreased significantly in estrogen treated group (26.45±2.23 mg/dl) compared to testosterone

Table 1. Effect of castration and sex hormones on serum Cholesterol, Triglyceride, HDL-C, LDL-C, VLDL-C and Risk index of male rabbits (Means ±SE)

Groups	Parameters					
	Cholesterol mg/dl	Triglyceride mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl	Risk index
Effects of castration						
Intact	65.66±2.76 a	38.77±0.84 a	20.72±2.09 b	36.96±2.55 a	7.64±0.19 a	3.53±0.32 a
Castration	69.44±2.10 a	38.88±0.53 a	28.66±2.74 a	32.62±2.30 a	7.52±0.17 a	2.77±0.25 b
Effects of treatments						
Control	71.08±2.70 a	37.83±0.77 a	22.50±2.75 b	40.01±3.45 a	7.56±0.15 a	3.67±0.48 a
Testosterone	61.75±1.52 b	38.75±0.59 a	16.33±0.44 c	37.91±1.46 a	7.58±0.19 a	3.77±0.12 a
Estrogen	69.83±3.83 a	39.91±1.08 a	35.25±2.68 a	26.45±2.23 b	7.60±0.31 a	2.01±0.13 b
Interaction						
Intact	73.50±4.36 a	37.00±1.03 b	16.16±3.01 c	49.60±2.39 a	7.40±0.20 bcd	4.88±0.60 a
Intact-Testosterone	58.33±2.06 b	37.50±0.22 b	16.83±0.74 c	34.16±1.72 bc	7.16±0.24 cd	3.45±0.11 b
Intact-Estrogen	65.16±5.64 ab	41.83±1.83 a	29.16±3.71 b	27.13±2.27 cd	8.36±0.36 a	2.28±0.21 c
Castration	68.66±3.27 ab	38.66±1.14 ab	28.83±2.86 b	30.43±3.16 cd	7.73±0.22 abc	2.46±0.28 c
Castration-Testosterone	65.16±1.13 ab	40.00±0.93 ab	15.83±0.47 c	41.66±0.89 b	8.00±0.18 ab	4.10±0.09 ab
Castration-Estrogen	74.50±4.93 a	38.00±0.57 b	41.33±1.78 a	25.76±4.08 d	6.83±0.24 d	1.75±0.08 c

Means with different letters within each column differ significantly (P ≤ 0.05).

(37.91±1.46 mg/dl) and control groups (40.01±3.45 mg/dl). No significant differences in VLDL-C level was recorded among estrogen (7.60±0.31 mg/dl) testosterone (7.58±0.19 mg/dl) and control groups (7.56±0.15 mg/dl). A significantly lowest value in R.I was recorded in estrogen treated group (2.01±0.13) compared with testosterone (3.77±0.12 mg/dl) and control groups (3.67±0.48).

In regard to interaction effects, animals in intact-testosterone treated group recorded significantly lowest value in cholesterol level (58.33±2.06 mg/dl) compared to other groups (Table 1).

While, significantly higher value in triglycerides level (41.83±1.83 mg/dl) was recorded in intact-estrogen treated group compared to other groups. On the other hand, a significantly highest value in HDL-C level (41.33±1.78 mg/dl) and lowest value in LDL-C level (25.76±4.08 mg/dl) and VLDL-C level (6.83±0.24 mg/dl) and R.I level (1.75±0.08) were recorded in castrated-estrogen treated group compared to other groups.

In the present study results revealed that no significant differences in total protein between intact and castrated groups (6.36±0.14, 6.44±0.09 gm/dl) respectively (Table 2). While, groups injected with testosterone and estrogen were showed a significantly decreased levels of total protein compared with control group (6.11±0.12, 6.27±0.14, 6.81±0.08 gm/dl) respectively. As related to the interaction among groups, the results showed that intact rabbits that injected with estrogen showed a significant decrease in total protein in compared with control group (intact) (5.91±0.13, 6.95±0.11 gm/dl) respectively. Castration not affects the albumin levels compared to intact group (3.46±0.05, 3.53±0.09 gm/dl) respectively. While, a significant difference was observed for control group compared to estrogen and testosterone injected groups. The estrogen and testosterone treatment decreased albumin concentration significantly (3.27±0.05, 3.46±0.09 gm/dl) respectively, compared to control group (3.76±0.06 gm/dl).

Interaction among groups indicated that intact-estrogen treated rabbits had a significant decrease in albumin concentration (3.16±0.06 gm/dl) compared with control group (3.87±0.03 gm/dl). The results showed a non-significant differences between castrated group and intact rabbits in globulin concentration (2.97±0.07, 2.83±0.06 gm/dl) respectively. A significantly decreased globulin concentration of testosterone treated rabbits (2.65±0.03 gm/dl) compared with estrogen and control groups (3.00±0.09, 3.05±0.07 gm/dl) was detected respectively. Among interaction groups, castrated-estrogen treated rabbits had significantly increased globulin concentration compared to castrated-testosterone and intact-testosterone injected groups (3.27±0.07, 2.63±0.02, 2.67±0.07 gm/dl) respectively. Castration significantly increased Globulin/Albumin (0.85±0.02) compared to intact rabbits (0.80±0.01). Estrogen treated group recorded significantly higher level of Globulin/Albumin (0.91±0.01) compared to testosterone treated and control groups (0.76±0.01, 0.80±0.02) respectively. For interaction effects, castrated-estrogen treated group recorded significantly high level

Table 2. Effect of castration and sex hormones on serum Total protein, Albumin, Globulin, Globulin/Albumin, Glucose, ALT and AST of male rabbits (Means ±SE)

Groups	Parameters						
	Total protein gm/dl	Albumin gm/dl	Globulin gm/dl	Globulin/ Albumin	Glucose mg/dl	ALT U/L	AST U/L
Effects of castration							
Intact	6.36 ± 0.14 a	3.53 ± 0.09 a	2.83 ± 0.06 a	0.80 ± 0.01 b	109.38 ± 2.99 a	74.72 ±3.76 a	54.94 ±2.99 a
Castration	6.44±0.09 a	3.46±0.05 a	2.97±0.07 a	0.85±0.02 a	108.44±4.09 a	51.33±3.09 b	51.44±2.82 a
Effects of treatments							
Control	6.81±0.08 a	3.76±0.06 a	3.05±0.07 a	0.80±0.02 b	118.41±4.06 a	67.16±6.29 a	55.08±4.08 a
Testosterone	6.11±0.12 b	3.46±0.09 b	2.65±0.03 b	0.76±0.01 b	106.50±3.47 b	73.33±3.56 a	56.16±3.77 a
Estrogen	6.27±0.14 b	3.27±0.05 b	3.00±0.09 a	0.91±0.01 a	101.83±4.23 b	48.58±3.12 b	48.33±2.44 a
Interaction							
Intact	6.95±0.11 a	3.87±0.03 a	3.08±0.12 a	0.79±0.03 bc	121.16±2.99 a	85.00±5.17 a	65.16±5.52 a
Intact-Testosterone	6.22±0.24 bc	3.55±0.18 b	2.67±0.07 b	0.75±0.02 c	106.83±2.45 ab	81.33±5.12 a	47.50±3.65 b
Intact-Estrogen	5.91±0.13 c	3.16±0.06 c	2.74±0.08 b	0.86±0.01 b	100.16±5.54 b	57.83±2.22 bc	52.16±3.74 b
Castration	6.67±0.09 a	3.65±0.09 ab	3.01±0.10 a	0.82±0.04 bc	115.66±7.79 ab	49.33±4.53 cd	45.00±1.46 b
Castration-Testosterone	6.00±0.08 c	3.37±0.07 bc	2.63±0.02 b	0.77±0.01 bc	106.16±6.86 ab	65.33±2.04 b	64.83±4.40 a
Castration-Estrogen	6.64±0.13 ab	3.37±0.07 bc	3.27±0.07 a	0.96±0.01 a	103.50±6.86 ab	39.33±1.96 d	44.50±2.52 b

Means with different letters within each column differ significantly ($P \leq 0.05$)

in Globulin/Albumin (0.96±0.01) compared to intact-testosterone treated group that recorded significantly lowest level (0.75±0.02).

Serum glucose level of rabbits in castrated group was not differ significantly (108.44±4.9 mg/dl) compared to intact group (109.38±2.99 mg/dl). A significant decline in glucose level was recorded in estrogen (101.83±4.23 mg/dl) and testosterone injected rabbits (106.50±3.47 mg/dl) compared to control group (118.41±4.06 mg/dl). As well as, in regard to interaction effects, glucose level significantly lower (100.16±5.54 mg/dl) in intact-estrogen treated group compared to control group (121.16±2.99 mg/dl).

Castrated rabbits compared to intact rabbits had significantly decreased ALT level (51.33±3.09, 74.72±3.76 U/L), while there was no difference in AST level (51.44±2.82, 54.94±2.99 U/L) respectively. Treatment group with estrogen showed significantly lowest value in ALT level (48.58±3.12 U/L) compared to group treated with testosterone (73.33±3.56 U/L) and control group (67.16±6.29 U/L). AST value showed no difference among groups of estrogen treatment (48.33±2.44 U/L), testosterone treatment (56.16±3.77 U/L) and control (55.08±4.08 U/L). Interaction among groups revealed that castrated-estrogen group recorded significantly lowest value in ALT level compared to intact-testosterone treated group and control group (39.33±1.96, 81.33±5.12, 85.00±5.17 U/L) respectively. Also, a significantly lowest value in AST level was recorded in castrated-estrogen treated group (44.50±2.52 U/L) compared to castrated-testosterone treated group (64.83±4.40 IU/L) and control group (65.16±5.52 U/L).

Hormonal parameters

Effect of castration and treatments on (T4, T3, Cortisol) levels were summarized in Table 3. Results indicate that there was significant increase in T4 level in serum of castrated rabbits (32.14±1.54 nmol/L) compared to intact rabbits (25.80±1.34 nmol/L). While, there was no significant difference in T3 level between castrated rabbits and intact rabbits (1.07±0.04, 0.99±0.05 nmol/L) respectively. A significant decrease in cortisol level was recorded in castrated group (27.03±3.20 nmol/L) compared to intact group (38.44±3.20 nmol/L).

Testosterone injected group recorded significantly higher value in T4 level (33.30±0.81 nmol/L) compared to estrogen injected group (23.30±0.81 nmol/L) and control (29.83±2.66 nmol/L). Also, a significant higher level of T3 was recorded in testosterone injected group compared to estrogen injected group and control (1.15±0.05, 0.95±0.04, 1.00±0.06 nmol/L) respectively. The estrogen injected group showed significantly decreased cortisol level which was (24.45±1.27 nmol/L) compared to testosterone injected group (41.99±1.64 nmol/L) and control (31.78±6.17 nmol/L).

Regarding to interaction effects as shown in Table 3, T4 level recorded significantly higher value in castrated group compared to other treatments. While, T3 level recorded significantly higher value in castrated and testosterone-injected rabbits compared to other treatments. Furthermore, cortisol level showed significantly lowest value in castrated group and intact-estrogen treated group compared to other treatments.

Table 3. Effect of castration and sex hormones on serum T4, T3 and Cortisol levels of male rabbits (Means ±SE)

Groups	Parameters		
	T4 nmol/L	T3 nmol/L	Cortisol nmol/L
Effects of castration			
Intact	25.80±1.34 b	0.99±0.05 a	38.44±3.20 a
Castration	32.14±1.54 a	1.07±0.04 a	27.03±3.20 b
Effects of treatments			
Control	29.83±2.66 b	1.00±0.06 b	31.78±6.17 b
Testosterone	33.30±0.81 a	1.15±0.05 a	41.99±1.64 a
Estrogen	23.78±0.68 c	0.95±0.04 b	24.45±1.27 c
Interaction			
Intact	21.37±0.34 c	0.79±0.02 c	52.20±0.88 a
Intact-Testosterone	32.80±1.19 b	1.21±0.03 a	42.14±1.57 b
Intact-Estrogen	23.23±1.32 c	0.97±0.08 b	20.99±0.56 d
Castration	38.30±1.55 a	1.21±0.04 a	11.36±0.32 e
Castration-Testosterone	33.80±1.17 b	1.08±0.09 ab	41.83±3.07 b
Castration-Estrogen	24.33±0.45 c	0.92±0.02 bc	27.91±1.42 c

Means with different letters within each column differ significantly ($P \leq 0.05$).

Body weight and weight gain

Data regarding body weight and weight gain is summarized in Table 4. Results showed that there was no significant changes in initial weight, final weight and weight gain between castrated (1558.88±5.08, 1735.56±8.40, 176.67±9.11 gram) and intact animals (1561.66±5.33, 1733.89±11.72, 172.22±11.34 gram) respectively. At the same time there was no significant difference in initial weight among animals administrated estrogen (1557.50±5.98 gram), testosterone, (1562.50±5.59 gram) and control group (1560.83±7.63 gram). Furthermore, animals in control group recorded significant decreased in final weight and weight gain (1694.17±8.67, 133.33±4.93 gram) compared to estrogen treated (1765.00±10.92, 207.50±12.25 gram) and testosterone treated group (1745.00±6.74, 182.50±7.65 gram) respectively.

In regard to interaction effects, no significant difference in initial weight was observed among groups. In addition, animals in intact-estrogen treated group recorded the highest final body weight (1780.00±12.90 gram) as compared with other groups, also a significant increase in body weight gain were recorded in intact rabbits treated with testosterone (175.00±14.43 gram) and with estrogen (213.33±17.44 gram) and castrated rabbit treated testosterone (190.00±5.16 gram) and estrogen (201.67±18.51 gram).

Discussion

Biochemical parameters

In accordance to our findings in lipid profile, (Zhao et al., 2013; Aydilek & Aksakal, 2005) noticed that castration significantly increased HDL-C level in rabbits serum. Also, similar results have been reported by Stevenson et al. (2005) when they stated that 17β estradiol increased HDL-C level and decreased LDL-C level. Also, results of current study were supported by Skouby et al. (2005), who stated that contraceptive which contain estradiol decreased LDL-C. On the contrary, Al–Mahmod (2009) found that treatment with 17β estradiol decreased cholesterol, TG and HDL-C and LDL-C increased significantly. Furthermore, Aregeb et al. (2019) showed that testosterone treatment decreased cholesterol level in serum of rabbits. Also, Hassan (2010) reported that when testosterone- administered to the castrated rats it showed a significant decrease in total cholesterol, TG, LDL-C accompanied with no significant change in HDL-C compared with castrated group. Although the effects of estrogens in the liver and, in particular, the involvement of estrogen in reducing plasma LDL-C cholesterol levels have been known for many years, the mechanism by which estrogens reduce LDL-C cholesterol is not well defined, especially at molecular level. Studies using high doses of estrogens have indicated that the up-regulation of hepatic LDL-C receptors is the primary mechanism responsible for the lipid-lowering effect (Kovanen et al., 1979; Chao et al., 1979).

Similar results recorded by Aregeb et al. (2019) who found a decrease in serum albumin concentration of rabbits following treatment with testosterone. Also, Young et al., (1993) reported that testosterone caused a reduction in serum albumin. On the other hand, Hassan (2010) stated that there were no significant changes between control, castrated, testosterone replacement groups in levels of total protein, albumin, globulin. Al–Mahmod (2009), reported that treatment with 17β estradiol increased total protein level. The present results was in accordance with the findings of (Verma et al., 2005), who found that 17B estradiol lower the level of glucose because of raising insulin. While,

Table 4.Effect of castration and sex hormones on body weight and weight gain of intact a castrated male rabbits (Mean ± S.E.)

Groups	Initial weight, g	Final weight, g	Weight gain, g
Effect of castration Intact	1561.66±5.33 a	1733.89±11.72 a	172.22±11.34 a
Castration	1558.88±5.08 a	1735.56±8.40 a	176.67±9.11 a
Effects of treatments			
Control	1560.83±7.63 a	1694.17±8.67 b	133.33±4.93 b
Testosterone	1562.50±5.59 a	1745.00±6.74 a	182.50±7.65 a
Estrogen	1557.50±5.98 a	1765.00±10.92 a	207.50±12.25 a
Interaction			
Intact	1558.33±13.08 a	1686.67±16.26 d	128.33±8.62 c
Intact-Testosterone	1560.00±6.70 a	1735.00±10.00 bc	175.00±14.43 ab
Intact-Estrogen	1566.67±8.13 a	1780.00±12.90 a	213.33±17.44 a
Castration	1563.33±9.09 a	1701.67±6.66 cd	138.33±4.77 bc
Castration-Testosterone	1565.00±9.48 a	1755.00±7.74 ab	190.00±5.16 a
Castration-Estrogen	1548.33±7.60 a	1750.00±16.38 ab	201.67±18.51 a

-Means with different letters within each column differ significantly ($P \leq 0.05$).

another study pointed that estradiol inhibits the insulin and increased glucose level (Nagira et al., 2006). However, recent study showed that glucose level decreased after estrogen therapy (Herrmann et al., 2005). On the contrary, gonadectomy and estradiol did not affect blood glucose in male mice (Iakovleva et al., 2020). On the other hand, Aregeb et al. (2019) noticed that the testosterone treatment significantly reduced glucose level in the serum of treated rabbits. It is well known that testosterone deficiency disturbs insulin sensitivity and impaired glucose tolerance (Wang et al., 2011). As well as, Hassan (2010) found that there was no significant changes between control, castrated, testosterone replacement groups in glucose level. The metabolic rate is depended on the level of the testosterone content of the organism. Also, castration-induced testosterone deficiency primarily affects fasting blood glucose and leads to increased levels of fasting glucose (Xia et al., 2013). Low testosterone levels not only significantly enhance the hepatic gluconeogenesis but also significantly decrease extra-hepatic insulin sensitivity in male rats. Additionally**,** Rebaz et al. (2019) noticed that rabbits injected with high level of testosterone recorded high level of ALT compared to group injected with low level of testosterone and control group.

Hormonal parameters

Regarding to the results of hormonal parameters, also, Soliman et al. (2001) stated that T4 and T3 levels were significantly higher in intact than in castrated rabbits. As well, Taravat et al. (2017) also reported that serum levels of T4 and T3 decreased in ovariectomized rats and significantly increased after estradiol treatment. On the other hand, Lima et al. (2006) noticed that serum T4 did not changed after ovariectomy or when two doses (0.7 *μg/100* g, 0.14 *μg/100* g) of estradiol were administered to ovariectomized, intact adult and pre-pubertal rats. While, ovariectomy significantly decreased serum T3, and both doses of estradiol were able to restore serum T3 levels. Furthermore, they reported that estradiol administration did not change serum T3 in both intact and pre-pubertal rats.

Cortisol is one of the main glucocorticoid hormones that is released in response to stress and low levels of blood glucose. Taravat et al. (2017) showed that castration has not cause significant changes in cortisol level in serum of horses.

Body weight and weight gain

In the present study there were no significant differences in initial weight, final and weight gain between castrated and intact group. While, rabbits treated with testosterone and estrogen had significant increase in final weight and weight gain as compared with untreated rabbits. This result is in agreement with the study of Mohammed et al. (2016), who reported that rabbits treated with boldenone undecylenate (BOL, anabolic steroid) had a significant increase in total body weight and daily gain in comparison to control group. As well, a significant increase in body weight and weight gain was recorded in animal received boldenone undecylenate compared to control group (Neamat-Allah., 2014). Furthermore, Rebaz et al. (2019) noticed that the growth performace improved in treated groups with testosterone enanthate proportional to the control group.

The testosterone also caused significant increase in the weights of treated rabbits (Aregeb et al., 2019). This effect could be attributed to promotion of the body tissue building process by protein synthesis indirectly via stimulation of growth hormone, insulin like growth factor secretion, and animal appetite (Ferreira et al., 1998) or reduction of glucocorticoid receptor levels and sensitivity to endogenous glucocorticoids; therefore, the strong growth promoting potency is based not only on its anabolic activity but also on its antiglucocorticoid effect (Melloni et al., 1997; Thienpont et al., 1998). On contrary, Tawfeek et al. (1994) reported that the growth performance of rabbits was not affected by testosterone injection. Moreover, Hassan (2010) stated that there was no significant difference in body weight among castrated, castrated plus testosterone and untreated rats. Also, Zhang et al. (2013) reported that Estrogen and dihydrotestosterne did not increase significantly total body weight compared with intact male rats. In another study, administration of 17β-estradiol to rabbits did not have significant effect on body weight compared with the control group (Al-Mahmod, 2009).

Conclusion

From the obtained results it could be concluded that, castration and estrogen treatment of castrated and intact male rabbits reduce the stress effects and enhance lipid profile and some immunological parameters, and its generally better than that obtained by testosterone treatment.

Acknowledgments

The authors gratefully thank the University of Duhok, College of Agricultural Engineering Sciences and University of Mosul, College of Agriculture and Forestry, for their assistance, supporting, providing facilities and allocating a place to conduct this study.

References

AL–Mahmod, W., D. (2009). The effect of hcg, 17 β-estradiol and hydrocortisone on blood cholesterol, hdl, ldl tg, total protein, glucose, ph and body weight in female rabbits. *Basrah Journal*

of Veterinary Research, 8(1), 11-18.

Aregeb S. A., Abdraba, I. O., Moftah, S. A., Mohamed, A. A., Eldurssi, I. S. & Bolhaj, A. M. (2019). The effects of testosterone on body weight and blood picture in male rabbits. *Journal of Benghazi Modern University of Sciences and Humanities, 4,* 4-17.

Aydilek, N. & Aksakal, M. (2005). Effects of testosterone on lipid peroxidation, lipid profiles and some coagulation parameters in rabbits. *Journal of Veterinary Medicine Series A*, *52(9),* 436-439.

Aydilek, N., Aksakal, M. & Karakılçık, A. Z. (2004). Effects of testosterone and vitamin E on the antioxidant system in rabbit testis. *Andrologia*, *36(5),* 277-281.

Badeau, M., Adlercreutz, H., Kaihovaara, P. & Tikkanen, M. J. (2005). Estrogen A-ring structure and antioxidative effect on lipoproteins. *The Journal of Steroid Biochemistry and Molecular Biology*, *96(3-4),* 271-278.

Barp, J., Araújo, A. S. D. R., Fernandes, T. R. G., Rigatto, K. V., Llesuy, S., Belló-Klein, A. & Singal, P. (2002). Myocardial antioxidant and oxidative stress changes due to sex hormones. *Brazilian Journal of Medical and Biological Research*, *35(9),* 1075-1081.

Bellanti, F., Matteo, M., Rollo, T., De Rosario, F., Greco, P., Vendemiale, G. & Serviddio, G. (2013). Sex hormones modulate circulating antioxidant enzymes: impact of estrogen therapy. *Redox Biology*, *1(1),* 340-346.

Bhasin, S. & Bremner, W. J. (1997). Emerging issues in androgen replacement therapy. *The Journal of Clinical Endocrinology & Metabolism*, *82(1),* 3-8.

Burns, K. A. & Korach, K. S. (2012). Estrogen receptors and human disease: an update. *Archives of Toxicology*, *86(10*), 1491-1504.

Chainy, G. B. & Sahoo, D. K. (2020). Hormones and oxidative stress: an overview. *Free Radical Research*, *54(1),* 1-26.

Chao, Y. S., Windler, E. E., Chen, G. C. & Havel, R. J. (1979). Hepatic catabolism of rat and human lipoproteins in rats treated with 17 alpha-ethinyl estradiol. *The Journal of Biological Chemistry*, *254(22),* 11360-11366.

Deroo, B. J. & Korach, K. S. (2006). Estrogen receptors and human disease. *The Journal of Clinical Investigation*, *116(3),* 561-570.

Ferreira, I. M., Verreschi, I. T., Nery, L. E., Goldstein, R. S., Zamel, N., Brooks, D. & Jardim, J. R. (1998). The influence of 6 months of oral anabolic steroids on body mass and respiratory muscles in undernourished COPD patients. *Chest*, *114(1),* 19-28.

Ha, B. J., Lee, S. H., Kim, H. J. & Lee, J. Y. (2006). The role of Salicornia herbacea in ovariectomy-induced oxidative stress. *Biological and Pharmaceutical Bulletin*, *29(7),* 1305-1309.

Hassan, A. A. (2010). Effect of castration on some physiological aspect in rats: Effect of testosterone hormone. *Journal of Education and Science*, *23(3),* 28-39.

Herrmann, B. L., Janssen, O. E., Hahn, S., Broecker-Preuss, M. & Mann, K. (2005). Effects of estrogen replacement therapy on bone and glucose metabolism in a male with congenital aromatase deficiency. *Hormone and Metabolic Research*, *37(03),* 178-183.

Iakovleva, T. V., Kostina, N. E., Makarova, E. N. & Bazhan, N. M. (2020). Effect of gonadectomy and estradiol on the expression of insulin signaling cascade genes in female and male mice. *Vavilov Journal of Genetics and Breeding*, *24(4),* 427.

Kovanen, P. T., Brown, M. S. & Goldstein, J. L. (1979). Increased binding of low density lipoprotein to liver membranes from rats treated with 17α-ethinyl estradiol. *Journal of Biological Chemistry*, *254(22),* 11367-11373.

Lima, L. P., Barros, I. A., Lisbôa, P. C., Araújo, R. L., Silva, A. C., Rosenthal, D., Ferreira, A. C. & Carvalho, D. P. (2006). Estrogen effects on thyroid iodide uptake and thyroperoxidase activity in normal and ovariectomized rats. *Steroids*, *71(8),* 653-659.

Melloni Jr, R. H., Connor, D. F., Hang, P. T. X., Harrison, R. J. & Ferris, C. F. (1997). Anabolic-androgenic steroid exposure during adolescence and aggressive behavior in golden hamsters. *Physiology & Behavior*, *61(3),* 359-364.

Mohammed, H. H., Badawi, M. E., El-Tarabany, M. S. & Rania, M. (2016). Effects of boldenone undecylenate on growth performance, maintenance behaviour, reproductive hormones and carcass traits of growing rabbits. *Polish Journal of Veterinary Sciences*, *19(2).*

Nagira, K., Sasaoka, T., Wada, T., Fukui, K., Ikubo, M., Hori, S., Hiroshi, T., Saito, S. & Kobayashi, M. (2006). Altered subcellular distribution of estrogen receptor α is implicated in estradiol-induced dual regulation of insulin signaling in 3T3-L1 adipocytes. *Endocrinology*, *147(2),* 1020-1028.

Neamat-Allah, A. N. (2014). Effect of Boldenone undecylenate on haematological and biochemical parameters in veal calves. *Global Veterinaria*, *13(6),* 1092-1096.

Prokai, L., Prokai-Tatrai, K., Perjési, P., & Simpkins, J. W. (2005). Mechanistic insights into the direct antioxidant effects of estrogens. *Drug Development Research*, *66(2),* 118-125.

Rebaz, R. A., Chowman, A. O., & Bahzad, H. M. (2019). Effect of Testosterone Enanthate hormones on some production, physiological traits and their residue in the meat of local rabbits in Sulaymaniyah, Iraq. *Applied Ecology and Environmental Research*, *17(6),* 15277-15287.

Soliman, E. B., Zahran, W. M. & El-Bakry, H. A. (2001). Histological structure and hormonal profile of pituitary and thyroid glands affected by castration and iodine supplementation in male rabbits. In: *Proceedings of the First International Conference (Egyptian British Biological Society)* (Vol. 3, 38-47).

Taravat, S., Tabatabei-Naeini, A., Nazifi, S. & Rahnama, S. P. (2017). The effect of castration on cortisol changes and blood glucose in horses. *Comparative Clinical Pathology*, *26(3),* 617-620.

Tawfeek, M. I., El-Gaafary, M. N. & Abdel-Hamid, M. Y. (1994). Effect of testosterone injection on pre-and post-sexual maturity in male rabbits. *Cahiers Options Mediterraneennes (CIHEAM)* (abstract English).

Thienpont, L. M. & De Leenheer, A. P. (1998). Efforts by industry toward standardization of serum estradiol-17β measurements. *Clinical Chemistry*, *44(3),* 671-674.

Verma, S., Srivastava, R. K., Sood, S. & Sharma, S. (2005). Effect of estrogen on hypoglycemia-induced neurological impairment in ovariectomized rats. *Methods and Findings in Experi-*

mental and Clinical Pharmacology, *27(6),* 405-410.

Wang, C., Jackson, G., Jones, T. H., Matsumoto, A. M., Nehra, A., Perelman, M. A., Swerdloff, R. S., Traish, A., Zizmann, M. & Cunningham, G. (2011). Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with type 2 diabetes. *Diabetes Care*, *34(7),* 1669-1675.

Wilson, J. D. (1988). Androgen abuse by athletes. *Endocrine Reviews*, *9(2),* 181-199.

Xia, F., Xu, X., Zhai, H., Meng, Y., Zhang, H., Du, S., Xu, H., Wu, H. & Lu, Y. (2013). Castration-induced testosterone deficiency increases fasting glucose associated with hepatic and extra-hepatic insulin resistance in adult male rats. *Reproductive Biology and Endocrinology*, *11(1),* 1-11.

Young, N. R., Baker, H. W., Liu, G. & Seeman, E. (1993). Body composition and muscle strength in healthy men receiving testosterone enanthate for contraception. *The Journal of Clinical Endocrinology & Metabolism*, *77(4),* 1028-1032.

Zhang, H., Liu, Y., Wang, L., Li, Z., Zhang, H., Wu, J., Rahman, N., Guo, Y., Li, D., Li, N., Huhtaniemi, I., Tsang, S., Gao, G. & Li, X. (2013). Differential effects of estrogen/androgen on the prevention of nonalcoholic fatty liver disease in the male rat [S]. *Journal of Lipid Research*, *54(2),* 345-357.

Zhao, C., Moon, D. G. & Park, J. K. (2013). Effect of testosterone undecanoate on hematological profiles, blood lipid and viscosity and plasma testosterone level in castrated rabbits. *Canadian Urological Association Journal*, *7(3-4),* E221.

*Received***: August, 04, 2022**; *Accepted***: November, 07, 2022**; *Published*: **December, 2022**