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EFFECT OF DIFFERENT DIETARY PROTEIN LEVELS ON TESTOSTERONE AND TESTICULAR PARAMETERS IN AWASSI LAMBS

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ABSTRACT

This study aimed to investigate the effect of three different dietary protein levels on testosterone concentrations and testicular parameters in Awassi male lambs. Fifteen weaned Awassi ram lambs were randomly divided into three different dietary protein levels, 13% crude protein (T1), 14% crude protein (T2), and 15% crude protein (T3), with an equal energy level (12.1 MJ/kg/DM) for all treatments. The lambs were fed daily on concentrate *ad libitum* with access to fresh water. During the experimental period (75 days), all lambs were weighed weekly. Live weight and testicular parameters, as well as the serum testosterone concentrations were measured. The results showed that the dietary protein levels significantly increased (P < 0.001) lambs body weight in both T3 and T2 compared to T1. Although there were no differences found in relative weight of the testicle to lambs live weight, the dietary protein significantly increased (P < 0.05) testicular weight (110.83g ± 5.05, 135.32g ± 11.10 and 159.04g± 17.94) for T1, T2 and T3 respectively. There were high significant (P < 0.001) effects of protein diet noticed on the serum testosterone concentrations (0.46 ± 0.05, 4.70 ± 1.21 ± 1.03 and 4.24 ng/ml) for T1, T2 and T3 respectively. There were significant differences observed in right (P < 0.05) and left (P < 0.05) testicular parameters, whilst there were no effects of protein diet observed in both right and left epididymis between groups. The results indicated that the dietary protein levels positively affect in live body weight and reproductive activity of growing awassi lambs.

KEYWORDS: Nutrition, supplement, male lambs, reproductive hormone.

INTRODUCTION

Nutrition considers one of the most important factors that acts as a main role in performing sexual efficiency in all animals, as well as energy and protein are the very curtail nutrients that required to be available in the adequate amounts in order to get optimize reproduction in farm animals (Yugal Raj et al., 2013). The relationship between nutrition and reproduction is one of the important matters that those concerned should pay attention to animal production projects (Williams, 2018). Spermatogenesis and testosterone secretion are the two main functions of the testis (Shalet, 2009). Testosterone plays a key role in the correct development of male reproductive tissues and in the maintenance of many male characteristics such as libido, potency, spermatogenesis, muscle mass, strength and bone mass (Winters, 1999). Testosterone levels reduce progressively with age, and deficiency of testosterone can cause big morbidity and essential reduction in life qualities, and can be dangerous if not treated due to the association of the functions above (Winters, 1999).

Understanding the impact of dietary protein on reproduction is sophisticated (Surai et al., 2019). Inadequate protein intake for long time has been reported to decrease reproductive efficiency. Moreover, it has been pointed that protein fed in amounts that greatly exceed the cow's requirements and will affect reproductive activity (Yugal Raj et al., 2013). Therefore, overfeeding protein without adequate of providing energy during the breeding season and early gestation, may be associated with decreased fertility in livestock (Dunn and Moss, 1992).

The reproductive potential of young lamb males may also be impaired by over feeding (Elmaz et al., 2007). However, it is worthy to mention that there is some disagreement about the effects of extra nutritive supply higher than maintenance requirements during the pre-

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pubertal period on testicular growth and development (Elmaz et al., 2007).

It is obvious that nutrition is directly connected to reproduction, as it has been proven that feeding animals either in deficient amount or in higher amount able to alter reproduction (Thatcher, 1985). The main problem is that the grade of the excess, deficiency or imbalance is still unclear to alter reproduction (Thatcher, 1985).

The aim of this work was to determine the effect of three different dietary protein levels with the same energy levels on growth performance, testosterone hormone concentration and testicular parameters in awassi male lambs.

MATERIALS AND METHODS

This research was conducted in the Animal Farm Project, Department of Animal Production, College of Agriculture Engineering Sciences at the University of Duhok. In this experiment, fifteen weaned Awassi ram lambs at the age of 105 days (3.5 months), with an average of initial body weights (23 Kg) were used. Ram lambs were randomly divided into three different dietary protein levels groups (n=5). The experimental groups were divided according to the dietary crude protein percentage including 13% C.P (T1), 14% C.P (T2), and 15% C.P (T3) with an equal energy level (12.1 MJ/kg/DM) for all treatments during the experiment period. The experimental animals were fed daily on diets twice at 8 a.m. and 5 p.m. *ad libitum* with free access to fresh water until they reached six months of age. During the experimental period (75 days), the weight of the lambs was recorded weekly in the morning before feeding. Measurements of live weight and testicular parameters (testicular weight, testicular fat, testicular diameter, testicular length, and testis volume, as well as the epididymis weight, epididymis diameter, epididymis length, and epididymis volume for both right and left testicles) after slaughter, and in the end of experiment (six months age), blood samples for serum testosterone concentrations were collected. The testes and epididymis were separated free of connective tissues and fats. The right and left testes and epididymis were measured separately and their weight recorded using a sensitive balance. Testicular fat also was recorded using a sensitive balance (KERN & Sohn GmbH, Germany). While an electronic calliper has been used for measuring testicular and epididymis diameter and length on the left and right testicles. The volume of testes and epididymis was measured using the method of water displacement (the Archimedes principles of water displacement in a measuring cylinder) (Hughes, 2005). Blood samples from each ram lambs were collected by jugular venepuncture, at the end of the experiment before slaughtering. Collection of blood samples were completed less than one hour. After collection, blood samples were centrifuged at 1200 × g for 20 minutes, and serum samples were stored at -20°C until analysis. After that, serum samples have been sent to medical laboratories to measure serum testosterone concentrations by immunoassay analyzers, Cobas 6000. The statistical analysis used in this study was the SPSS program (SPPP, 2019). Duncan multiple range was used to observe whether any differences existed between treatments (Duncan, 1955).

RESULTS AND DISCUSSION

The dietary protein changes affected live weight during the experimental period. Nutritional status positively affected on live weight, testicular growth and serum testosterone concentration as well as the reproductive activity of growing Sanjabi ram lambs (Ghorbankhani et al., 2015).

In this study there were high significant differences ($P < 0.001$) of protein levels in body weight of lambs in both treatments (T3) and (T2) compared to T1, but there were no significant differences observed between (T3) and (T2) (Table 1). Although no significant differences found in relative weight of the testicle to live weight, but there was a significant difference ($P < 0.05$) in testicular weight among treatments (110.83g, 135.32g and 159.04g) for T1, T2 and T3 respectively (Table 1). Perhaps the reason for the compatibility of the relative weight of the testis with body weight is due to the use of similar management systems in raising local sheep, represented by feeding and grazing systems. In a similar study by Mukasa-Mugerwa and Ezaz (1992) observed the significant effect of nutrition on live weight and testicular growth in Menz ram.

Martin et al. (1987) recorded that protein supplement increased body weight in Merinos and Booroolas lambs.

Andrew and Ørskov (1970) reported that the optimum dietary crude protein percentage for

growth the early weaned lamb was about 17, 15 and 11% for body weight between 16 and 40 kg, while the mean digestible energy was 3·0, 2·6 and 2·1 Mcal/day respectively.

In a study carried out by Martin et al. (1987) in the impacts of nutritional supplements on testicular volume and the secretion testosterone and LH hormones in Merino and Booroola rams found that supplemented protein significantly increased testicular growth in both rams, but no differences observed in LH and testosterone levels which may be related to differences in their fertility.

Undernutrition decreases the testicular volume in bucks exposed to long days, but still fit to motivate reproductive efficacy in seasonally anestrous goats (Delgadillo et al., 2020).

Increased protein in dietary significantly ($P < 0.001$) increased testicular fat in both T3 and T2 compared to T1 (Table 1). This may be due to the increase in the weight of the testicles and the large feed intake by the animals in these groups.

Mean concentrations of serum testosterone in awassi ram lambs are presented in Fig. 1. There was high significant ($P < 0.001$) effect of protein diet on concentration of this hormone in both T3 and T2 compared to T1 (0.46, 4.70 and 4.24 ng/ml) for T1, T2 and T3 respectively, and this may be due to increase in body weight and testicle weight as well as individual differences between lambs.

Table (1): Mean (± S.E.) value of live weight and testicular characteristics and in awassi ram lambs at 180 days

Parameters/ Groups	T1	T2	T3	Significant
Live Weight (kg)	32.12 ± 0.27 c	36.70 ± 0.75 b	38.840 ± 0.67 a	**
Testicular Weight (g)	110.83 ± 5.05 b	135.32 ± 11.10 ab	159.04 ± 17.94 a	*
Relative Weight of the Testicle to Live Weight (%)	0.35 ± 0.02	0.37 ± 0.03	0.41 ± 0.04	NS
Testicular fat (g)	40.57 ± 10.20 b	76.52 ± 7.60 a	73.39 ± 7.42 a	**

NS means non-significant. Star mean there was a significant difference between treatments ($P < 0.05$ or $P < 0.001$), (a, b, c) letters in the same row mean there was a significant difference between treatments ($P < 0.05$ or $P < 0.001$).

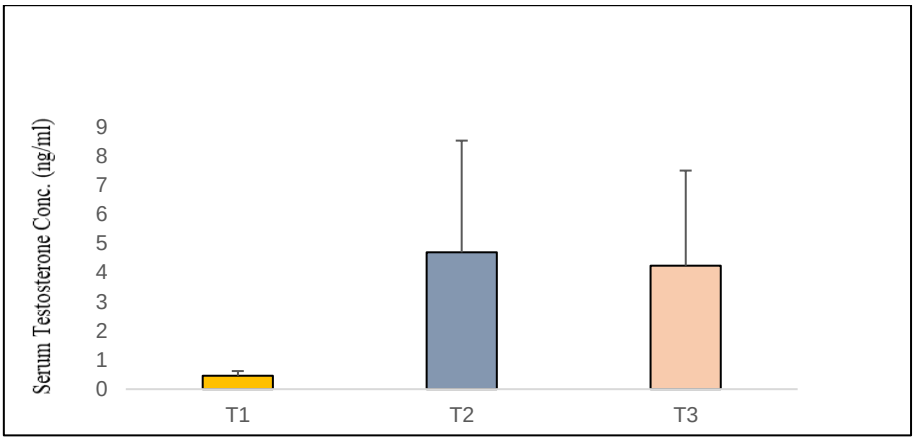

Fig. 1. Serum testosterone concentrations in awassi ram lambs at 180 days

Testosterone concentration in plasma and reproductive activities not impacted by protein intake, but there was significant effect in both testicular volume and spermatogenesis among different diets and suggested that undegradable protein should be provided during the mating season to Assaf male lambs to improve their sexual activity (Fernández et al., 2004).

Although increased energy levels boosted scrotal circumference of Simmentals breed, but there was no quick stimulation for sexual development as measured by serum testosterone, age at first mating or age at puberty in both Simmental and Hereford breeds (Pruitt et al., 1986).

In this study there were significantly increased in right testicular (weight without epididymis, length, diameter and volume) especially for T3 compared to T1 (Table 2). As well as, there were differences observed in left testicular parameters (Table 3).

No significant differences were noticed between measurements of the left and right testis. The top growing in testicular parameters appeared between 7 and 10 months of age at 34.6 kg live body weight. They growth gradually and were associated with body weight more than with age. However parental size, age and body weight influenced testicular development in growing Awassi ram lambs (Salhab et al., 2001).

No significant differences found in both right and left epididymis weight, length and volume, except the high significant observed only in the right epididymis length in both T3 and T2 compared to T1 (Table 2 and Table 3). It has been reported by Abdullahi Mahmud (2015) that several likenesses in epididymis and form differences in ruminant species contrasted to the majority of mammals and they decided that these characteristics are essential in the future researches.

Table (2): Mean (± S.E.) value of right testicular characteristics in awassi ram lambs at 180 days

Groups	T1	T2	T3	Significant
Right Testis wt without Epididymis (g)	47.16 ± 2.94 b	59.77 ± 5.42 ab	71.70 ± 7.87 a	*
R. Testicle Length (mm)	52.25 ± 3.75 b	62.26 ± 2.85 a	66.51 ± 2.30 a	**
R. Testicle Diameter (mm)	36.25 ± 3.77 b	44.60 ± 1.25 a	48.04 ± 1.41 a	**
R. Testicle Volume (Cm^3)	43.50 ± 4.41 b	54.90 ± 5.67 ab	67.50 ± 8.16 a	*
R. Epididymis wt (g)	7.86± 0.24	8.80± 0.51	9.64± 0.97	NS
R. Epididymis Length (mm)	106.22 ±3.16 b	119.13 ±3.78 a	126.61 ±3.83 a	**
R. Epididymis Volume (Cm^3)	8.36±0.66	8.20±0.74	8.20±1.00	NS

NS means non-significant. Star mean there was a significant difference between treatments ($P < 0.05$ or $P < 0.001$), (a, b, c) letters in the same row mean there was a significant difference between treatments ($P < 0.05$ or $P < 0.001$).

Table (3): Mean (± S.E.) value of left testicular characteristics in awassi ram lambs at 180 days

Groups	T1	T2	T3	Significant
Left Testis wt without Epididymis (g)	48.02 ± 2.15 b	58.11 ± 5.52 ab	68.12 ± 8.68 a	*
L. Testicle Length (mm)	58.65 ± 0.54	61.51 ± 2.36	64.95 ± 3.32	NS
L. Testicle Diameter (mm)	41.00 ± 0.51 b	43.86 ± 1.48 ab	46.63 ± 1.74 a	*
L. Testicle Volume (Cm^3)	47.20 ± 3.11	54.00 ± 5.68	65.60 ± 8.61	NS
L. Epididymis wt (g)	7.80 ± 0.33	8.64 ± 0.42	9.58 ± 0.99	NS
L. Epididymis Length (mm)	116.73 ± 4.13	120.94 ± 4.34	124.12 ± 4.20	NS
L. Epididymis Volume (Cm^3)	7.68 ± 0.71	7.10 ± 0.52	7.40 ± 0.92	NS

NS means non-significant. Star mean there was a significant difference between treatments ($P < 0.05$ or $P < 0.001$), (a, b, c) letters in the same row mean there was a significant difference between treatments ($P < 0.05$ or $P < 0.001$).

While in a study carried out by Ibrahim et al. (2012) on three different lambs breeds in Nigeria, observed significant differences in epididymis weight and length.

There were differences between the epididymis of the right and left testis among many different studies. The reason for the difference may be due to the difference of the studied animals and the different laboratory, administrative, nutritional, health and environmental conditions of the experiment, and the different composition of the studied descents, the physiological structure and its characteristics of each descent that affect the anatomical measurements of the growth and development of the epididymis.

In conclusion, the results indicated that the dietary protein levels affect live body weight and reproductive activity of growing awassi ram lambs, however it has been recommended that it will be valuable if using large number of lambs with different species and ages, as well as the use of protein in high concentrations between groups in the future researches.

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تأثير مستويات مختلفة من البروتين الغذائي على هرمون التستوستيرون والخصية في الحملان العواسية

الخلاصة

الهدف من هذه الدراسة كانت لمعرفة تأثير ثلاث مستويات مختلفة من البروتين الغذائي على تراكيز هرمون التستوستيرون ومقاييس الخصية في ذكور الحملان العواسية .تم تقسيم خمسة عشر حملا مفطومًا بشكل عشوائي إلى ثلاث مستويات مختلفة من البروتين الغذائي ، 13٪ بروتين خام (T1)، 14٪ بروتين خام (T2) و 15٪ بروتين خام (T3) ، بمستوى طاقة متساوٍ (12.1 ميجا جول / كجم/ DM) لجميع المعاملات. تم تغذية الحملان يوميًا على العلف المركز مع إمكانية الوصول إلى المياه العذبة للشرب. تم وزن الحملان كل أسبوع خلال فترة التجربة (75 يوم). تم قياس الوزن الحي ومقاييس الخصية وكذلك تراكيز هرمون التستوستيرون في الدم. أظهرت النتائج أن مستويات البروتين الغذائي ازدادت معنويا (P <0.001) من وزن الحملان في كل من T3 و T2 مقارنة مع T1. بالرغم من عدم وجود فروقات معنوية في الوزن النسبي للخصية بالنسبة للوزن الحي للحملان ، إلا أن البروتين الغذائي ادت الى زيادة معنوية (P <0.05) في وزن الخصية (110.83 جم ± 5.05 ، 135.32 جم ± 11.10 و 159.04 جم ± 17.94) في كل من T1 و T2 و T3 على التوالي. لوحظ وجود تأثيرات معنوية عالية (P <0.001) للنظام الغذائي البروتيني على تراكيز هرمون التستوستيرون في الدم (0.46 ± 0.05 ، 4.70 ± 1.21 ± 1.03 و 4.24 نانوغرام / مل) في T1 و T2 و T3 على التوالي. اظهرت النتائج وجود فروقات في قياسات الخصية اليمنى (P <0.05) واليسرى (P <0.05) ، بينما لم يلاحظ أي تأثير للنظام الغذائي البروتيني في كل من البربخ الأيمن والأيسر بين المجاميع. أشارت النتائج إلى أن مستويات البروتين الغذائي لها تأثير إيجابي على وزن الجسم الحي والنشاط التناسلي لنمو الحملان العواسية.

الكلمات المفتاحية: التغذية ، المكملات ، حملان الذكور، الهرمون التناسلي.