EFFECT OF REDUCING SUNFLOWER CAKE AND SEEDS DEGRADABILITY ON PUBERTY AND TESTICULAR MEASUREMENTS Of AWASSI LAMBS

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ABSTRACT

This study was conducted to elucidate the effect of lowering sunflower cake degradability or seeds on puberty, testicular and blood measurements of Awassi lambs. Thirty Awassi lambs with an average body weight of 25.93 ± 0.42 kg and 3- 4 months old. Lambs were divided into three groups (10 lambs in each group) the first group was fed a diet contained 11% of sunflower cake (T1), the second group was fed a diet contained 11% of low degradable sunflower cake (T2), while the third group fed a diet that contained 11% of low degradable sunflower seeds (T3). The results indicated that reduce sunflower seeds degradability in (T3) led to a significant increase (p≤ 0.05) in the length of right and left testes (8.35 and 8.30 cm) respectively as compared to the T1 (7.84 and 7.77 cm) respectively and T2 (7.88 and 7.63 cm) respectively, scrotum volume increased significantly (p≤ 0.05) in the T3 165.72 cm^3 as compared to the T2 151.86 cm^3, Testosterone hormone concentration increased significantly (p≤ 0.01) in T3 2.81 ng/ml as compared to the T1 and T2 (1.455 and 1.75 ng/ml) respectively . Also, feeding lambs with low degradable sunflower cake (T2) and seeds (T3) enhance significantly (p≤ 0.05) seminiferous tubule diameter, germinal epithelium height, and Sertoli cells count as compared to lambs that fed sunflower cake (T1). The results indicate that reducing degradability of protein and fat sources rich in unsaturated fatty acids in the diets of lambs before puberty had a positive role in testicular development and testosterone hormone secretion at puberty.

keywords: protected protein, protected fat, puberty, testicular measurements.

تأثير خفض تحلل كسبة وبذور زهرة الشمس في البلوغ وبعض قياسات الخصية والدم في الحملان العواسية

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المستخلص

أجريت الدراسة لتوضيح تأثير البروتين منخفض التحلل لكسبة وبذور زهرة الشمس في البلوغ الجنسي وقياسات الخصية والدم في الحملان العواسية، استخدمت ثلاثون حمل عواسي معدل اوزانها 25.93 ± 0.42 كغم واعمارها 3–4 شهر وقسمت الحملان الى ثلاث مجاميع (10 حملان في كل مجموعة). وغذيت المجموعة الأولى على عليقة احتوت 11% كسبة زهرة الشمس، المجموعة الثانية غذيت على عليقة احتوت 11% كسبة زهرة الشمس منخفضة التحلل، في حين غذيت المجموعة الثالثة غذيت على عليقة احتوت 11% بذور زهرة الشمس منخفضة التحلل. اشارت النتائج الى ان خفض تحلل بذور زهرة الشمس أدى الى زيادة معنوية (أ≤ 0.05) في طول الخصيتين اليمنى واليسرى (8.35 و8.30 سم) على التوالي مقارنة بالمعاملتين الأولى (7.84 و7.77 سم) على التوالي والثانية (7.88 و7.63 سم) على التوالي، كما زاد حجم كيس الصفن معنويا (أ≤ 0.05) في المعاملة الثالثة (165.72 سم^3) مقارنة بالمعاملة الثانية (151.86 سم^3). كما ازداد معنويا (أ≤ 0.01) هرمون التستوستيرون في المعاملة التي غذيت على بذور زهرة الشمس منخفضة التحلل (2.81) نانو غرام/مل مقارنة بالمعاملتين الأولى والثانية بلغ (1.45 و1.75) نانو غرام/مل على التوالي. ولوحظ أيضا ان تغذية الحملان على كسبة وبذور زهرة الشمس منخفضة التحلل في المعاملتين الثانية والثالثة أظهرت تفوقا معنويا (أ≤0.05) في قطر الانيبيبات المنوية وارتفاع الخلايا الجرثومية وعدد خلايا سرتولي مقارنة مع الحملان في المعاملة الأولى والمغذاة الحملان فيها على كسبة زهرة الشمس. تبين النتائج ان خفض تحلل البروتين ومصادر الدهون الغنية بالأحماض الدهنية غير المشبعة في علائق الحملان قبل البلوغ كان لها دور إيجابي في تطور الخصية وافراز هرمون التستوستيرون عند البلوغ.

الكلمات المفتاحية: بروتين محمي، دهن محمي، بلوغ، قياسات خصية.

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INTRODUCTION

Nutrition affects reproductive performance in two directions, the first by the hypothalamus-pituitary gland, the second by the development of the reproductive endocrine glands (5). This effect of nutrition is associated with increased body weight that determine puberty and maturity which is usually 50-70% of body weight (5,26,36). The balance of diet in its protein and energy content has a critical role in growth after weaning and weight gain (8,19), which leads to an increase in the growth testicular and testosterone secretion for the development of sexual characteristics and behavior and the production of sperm as the hormone secretion increases with the increase in growth until the age of puberty and sexual maturity (27). Moreover, the source of energy and protein affect reproductive performance, Kaur and Arora (18) and Scaramuzzi et al. (29) noticed that an increase in degradable protein feeding leads to increasing urea in the seminal plasma and negatively affects reproductive performance. Therefore, the use of the degradable protein within limits recommended NRC (23) necessary to reduce the negative impact on reproductive performance, both in males and females, (34). It was found that adding polyunsaturated fatty acids to diet subjected to processes of elongation and desaturation to produce fatty acid DHA (C22:6-3), increase phospholipids in seminal plasma, gonadotropin receptors and testosterone production (12,34). In addition, to improving semen quality because phospholipids are comprised 70% of the sperm plasma membrane composition, they can also be used as an energy source in the event of fructose deficiency, polyunsaturated fatty acids feeding also contribute to building insulin-like growth hormone (IGF-I) and increasing Ledig cell generators (5). polyunsaturated faty acids effect on reproductive Performance is more significant when using low degradable sources like fish oil than oilseeds. This study was suggested to exam the role effect of feeding lambs with low degradable protein or fat rich in poly unsaturated fatty acid after weaning in testis development and testosterone hormone secretion at puberty.

MATEERIALS AND METHODS

Thirty Awassi lambs with an average body weight 25.93 ± 0.42 kg and 3-4 months old were used in this study to clarify the effect of feeding low degradable sunflower seeds and cake in puberty, some blood parameters and histological structure of the testis lambs were allocated into three groups. the first group (T1) fed on a diet contained sunflower cake at 11%, the second group (T2) fed on a diet contained 11% low degradable sunflower cake, and the third group was fed a diet contained 11% of low degradable sunflower seeds (Table 1) lambs were fed using ad-libitum system in all groups. The degradability of sunflower cake and seeds was reduced by treatment with formaldehyde (17). Also, fresh water was provided freely during the period of study, the semen was collected from the lambs biweekly using the ejaculator method, and the age and weight at puberty were determined when the first sperm appeared in the semen. At puberty samples of blood was collected from the jugular vein, which was divided into two parts after separating the serum to estimate some blood biochemical measurements using commercial agent kits (Biolabo, French). testosterone hormone concentration was estimated using the Tosoh AiA 360, analysis technique. Testicular measurements were evaluated using a tap measurement, the circumference of the scrotum, according to Goijam et al. (15), the length and depth of the testicles using Vernia as reported Toe et al. (33), Schoeman and Combrink (30), and scrotum size according to as reported Elmaz et al. (13) using the equation. Testes volume (cm^3) = 0.0396× (average testis length) × (scrotal circumference)^2. Histological sections were prepared, as described by Luna (20). The results were analyzed statistically by using the complete random design (CRD) by the computer using the program (SAS 28), and the significance between mean was determined by the analysis of polynomial variance Duncan (9).

$Yij = \mu + ti + eij$

Yij= observation from treatment.

Ti= any treatment (diet).

Eij= value of standard error.

Table 1. components and Chemical composition of the experimental diets.

Ingredients	Experimental diets		
	T1	T2	T3
Crushed barley	65	65	64.8
Wheat bran	17	17	18
Sunflower cake	11	---	---
low degradable sunflower cake	---	11	---
low degradable sunflower seeds	---	---	11
Wheat straw	5.35	5.35	4
Urea	0.15	0.15	0.70
salt	0.5	0.5	0.5
Sodium chloride	0.5	0.5	0.5
Calcium carbonate	0.5	0.5	0.5
Chemical composition %			
Dry matter	91.62	91.62	92.43
Organic matter	96.01	96.01	95.91
Crude protein	13.62	13.62	13.47
Crude fiber	2.41	2.41	7.40
Ether extract	11.50	11.50	9.75
Nitrogen-free extract	72.47	72.47	69.40
Metabolism energy, MJ / kg	10.15	10.15	11.32

Chemical composition was laboratory determined according to AOAC (3), the energy was calculated from the tables of the chemical composition of Iraqi feed materials Al-Khawaja et al. (2).

RESULTS AND DISCUSSION

Results in table (2) revealed non-significant differences in the mean of initial weight between the groups being 26.15, 25.63, and 26.05 kg respectively, also, the differences were non-significant in age at puberty 179.78, 175.91, 180.00 days and weight at puberty 33.33, 33.20, and 32.81 kg respectively. The scrotal circumference was not significantly affected by treatments being 22.38, 21.90, and 22.25 cm, respectively. The length of the right and left testes increased significantly ($p \leq 0.05$) in the third treatment (8.35 and 8.30 cm) respectively, compared to the first treatment (7.84 and 7.77 cm) respectively, and the second, (7.88 and 7.63 cm), respectively. The depth of the testicles was approximately similar between the treatments being (3.97, 3.86, 4.05 cm) in the right testis and (4.10, 4.01,4.07 cm) on the left, respectively. Scrotum volume was significantly decreased ($p \leq 0.05$) in the second treatment fed on the low degradable cake (151.86 cm^3) compared to those fed on low-degradable seeds in the third treatment (165.73 cm^3). The increase of testes length and scrotum size in the third treatment may be due to the increase in energy intake from sunflower seeds (10.75 Mj/day) as compared to other treatments (9.95, 9.96 Mj/day), respectively. Moghaddam et al. (21) indicated that feeding lambs at a high level of energy led to an increase in the size of the seminiferous tubules and an increase in the proliferation of germ cells in the testis as a result to the increase in testosterone hormone concentration.

Table 2. Effect of treatments on puberty and some testis measurements.

Traits	T1	T2	T3
Initial weight Kg	26.1 ± 0.78	25.6 ± 0.80	26.0 ± 0.67
Dry matter intake kg/day	0.980	0.981	0.950
Age at puberty/day	179.78 ± 3.83	175.91 ± 2.46	180.00 ± 3.25
Weight at puberty/kg	33.33 ± 0.62	33.20 ± 0.75	32.81 ± 0.76
Scrotum circumference / cm	22.38 ± 0.73	21.90 ± 0.43	22.25 ± 0.71
Right testis length / cm *	7.84 ± 0.20 b	7.88 ± 0.15 b	8.35 ± 0.13 a
Left testis length / cm *	7.77 ± 0.09 b	7.63 ± 0.21 b	8.30 ± 0.12 a
Right testis depth / cm	3.97 ± 0.13	3.86 ± 0.06	4.05 ± 0.08
Left testis depth / cm	4.10 ± 0.16	4.01 ± 0.09	4.07 ± 0.08
Scrotum volume / cm3 *	163.00 ± 5.25 ab	151.86 ± 2.92 b	165.73 ± 4.22 a

*** a,b, Mean values within a row with different superscripts differed ($P \leq 0.05$).**

This was confirmed from the results in a Table (4). Which indicates that the best development of the testis in the third treatment, which was fed on fat. Boulanouer et al. (7), EL-Madawy et al. (12), Saaed and Zain (27) stated that energy has an essential role in sexual puberty and testis development in sheep. The effect of unsaturated fatty acids intake increment is related to regulating hypothalamus secretion (GnRH) of LH hormones, insulin, and leptin, which enhance testes development. (32). this result was consistent with those reported by El-Badawy et al. (11) and El-Madawy et al., (12) that feeding on oils high in unsaturated fatty acids led to a significant increase in the

size of the testicle and scrotum circumference. It also agreed with El-Zelaky et al. (10) that feeding lambs on a low degradable jatropha cake led to a significant increase in scrotum size, while Dantos et al. (8) observed that the age or weight at puberty was not affected by the increased protein and energy intake (1). Results of blood biochemical measurements in Table (3) showed that the reduction in the degradation of the sunflower cake and seeds did not lead to significant differences in total protein concentrations (6.82, 6.61, 6.89 g/dl), albumin (3.50, 3.57, 3.72 g/dl), and globulin (3.31, 3.03, 3.16 g/dl) and blood glucose concentration (66.26, 65.45, 66.64 mg/dl) respectively, urea concentration decreased significantly ($p \leq 0.05$) in the second treatment (24.94 mg/dl) compared with the highest concentration in third treatment, (29.97 mg/dl). Lowering degradability of sunflower seeds led to a significant increase ($p \leq 0.01$) in cholesterol concentration (89.39 mg/100ml) compared to the first 80.68 mg /dl and second treatments (62.18 mg /dl), also significant differences were recorded between the first and second treatments, a significant ($p \leq 0.01$) increased was noted in blood triglyceride in the third treatment (59.94 mg/dl) compared to the first and second treatments (44.60 and 48.11 mg/dl) respectively, testosterone hormone concentration in the third treatment was highest ($p \leq 0.01$) as compared to the first and second treatments. It is well known that feeding undegradable protein is associated with a decrease in blood urea (22), this may relate to the improved efficiency of protein utilization (25). On the other hand, it is noticed that lower sunflower seeds degradability caused an increase in blood urea, the reason for that may correlate with increasing urea percentage in the diet components of the third treatment (Table 1) Yixuan et al.(37) explained that increase urea in the diet causes an increase in blood urea, as well Umar et al. (35) explained that an increase in energy or fat in the diet leads to an increase in blood urea, which is consistent with this study.

Table 3. Effect of treatments on some blood parameters.

parameter	T1	T2	T3
Total protein (g/dl)	6.8 ± 0.18	6.61 ± 0.07	6.89 ± 0.29
Albumin (g/dl)	3.50 ± 0.15	3.57 ± 0.05	3.72 ± 0.05
Globulin (g/dl)	3.31 ± 0.08	3.03 ± 0.12	3.16 ± 0.24
Glucose (mg/dl)	66.2 ± 2.55	65.4 ± 1.56	66.6 ± 0.21
Urea (mg / dl) *	27.4 ± 1.03 ab	24.9 ± 0.97 b	29.9 ± 0.58 a
Cholesterol (mg /dl **)	80.6 ± 1.20 b	62.1 ± 2.21 c	89.3 ± 1.10 a
Triglycerides (mg/dl **)	44.6 ± 1.34 b	48.1 ± 1.03 b	59.9 ± 1.42 a
Testosterone concentration (ng / ml **)	1.45 ± 0.06 b	1.74 ± 0.03 b	2.81 ± 0.26 a

*** a,b, Mean values within a row with different superscripts differed ($P \leq 0.05$).**

*** a,b,c Mean values within a row with different superscripts differed ($P \leq 0.01$).**

In general, despite the differences between the treatments in the concentration of urea but it is close was close to the normal range in the blood 26.6-45.6 mg/100 ml. Nudda et al. (24) found that low degradable flaxseed did not lead to significant differences in blood urea and proteins. El-Badawy et al. (11) mentioned that the different levels of rocket oil rich in linolenic acid in lambs diets caused a significant increase in blood proteins. In our study, the increased intake of polyunsaturated fatty acids from sunflower seed was associated with increased blood cholesterol, triglycerides, and enhancing blood testosterone secretion in the third treatment, this led to an increase in a ledge cell number and the development of the size of the testicles (6,36). The results are agreed with what obtained by (4,12,14) they indicated that adding oil to the diet led to an increase in blood cholesterol and triglycerides. Esmaeili et al. (14), Behery et al. (4), El-Madawy et al. (12) found that low degradable oils such as fish oil led to a significant increase in the testosterone hormone concentration. El-Zelaky et al. (10) noticed that feeding lambs a low degradable jatropha cake led to a significant increase in testosterone concentration during 115-275 days of old, this

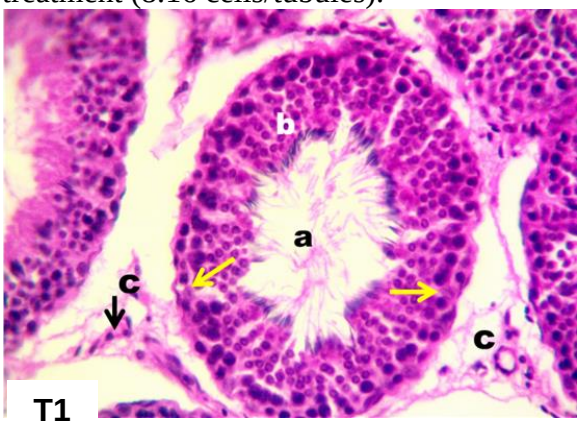
may be due to changes in testicular size resulting from an increased protein supply, which is positively correlated with changes in gonadotrophin and testosterone secretion (31). On other hand the results did not agree with the result of El-Badawy et al. (11), Behery et al., (4) mentioned that increasing the percentage of low degradable fat did not have a significant effect on cholesterol and triglycerides in the blood.

Table 4. Effect of treatments on histological structures of testis.

properties	T1	T2	T3
Seminiferous Tubule diameter (µm*)	172.78 ± 2.19 b	189.98 ± 3.69 a	198.50 ±3.78 a
Germinal epithelium height (µm*)	38.8 ±1.51b	47.4 ±1.40 a	52.7 ±2.36 a
No. of Sertoli cells/ tubule*	8.16 ±0.47 b	9.16 ±0.65 ab	10.1 ± 0.47 a

*** a,b, Mean values within a row with different superscripts differed (P ≤ 0.05).**

Table 4 and the figures (1,2,3) illustrates that reduce the degradability of sunflower cake and seed in the second and third treatments increased significantly (p≤ 0.05) the diameter of seminiferous tubule (189.98 and 198.50 µm) compared with the first treatment (172.78 µm). similar effect was noted in the high germ cells (47.45 and 52.78 µm) compared to (38.83 µm) for the first treatment. Similarly, a significant increase (p≤ 0.05) was observed in Sertoli cells count for the third treatment (10.16 cells/tubule) compared to the first treatment (8.16 cells/tubules).



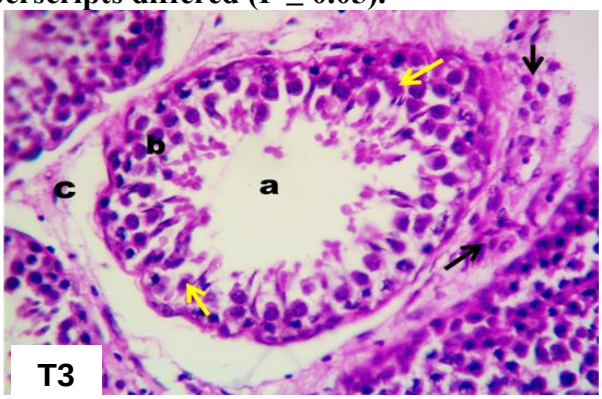


Figure.1: Cross section of testis of Awassi lambs.

T1. Fed Sunflower cake
T2. Sunflower cake low degradable
T3. Fed Sunflower seeds low degradable

a. **Lumen of the Seminiferous Tubule**
b. **Germinal epithelium height**
c. **Interstitial tissue**

Yellow arrow. sertoli cells (400x)
Black arrow. Leyding cells (400x)

The results were agreed with Moghaddam et al. (21) who showed that feeding lambs on a diet high in polyunsaturated fatty acids stimulate the growth and development of the seminiferous tubules, increases germ cell proliferation, and in Sertoli cells count. Contrary to what Ahmad and others (1) stated that feeding lambs on low degradable palm oil did not lead to significant differences in the diameter of the seminiferous tubules.

Conclusion

The results show that feeding low degradable sunflower cake and seeds enrich in polyunsaturated fatty acids (PUFA) in the diet of lambs before puberty led to an increase in testes development as a result of the increase in the seminiferous tubule diameter and sertoli cells count in addition to increase testosterone hormone secretion.

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REFERENCES

1. Ahmad, M. H., L. T. Chen, M. S. Maidin, and A. A. Samsudin. 2018. Effect of different rumen-protected fat from palm oil on testosterone level and testicular traits in Malin rams. Mal. J. Anim. Sci. 21(2): 27-38.

2. Al-Khawaja, A. K, E. Abdullah and S. Abdul-ahad. 1978. The chemical composition and nutritional value of Iraqi feed materials. A bulletin issued by the Nutrition Department of the General Livestock Directorate. Ministry of Agriculture and Agrarian Reform. The Republic of Iraq.

3. AOAC, 2000 Official Methods of Analysis, Association of Official Analytical Chemists, Arlington, Va, USA, 17th edition.

4. Behery, H. R, E. I. Khalifa, and A. A. Mahrous. 2014. Influence of feeding non-conventional energy sources on pubertal phases, blood metabolites and fattening of zaraibi male kids. J. Anim. And poultry prod. Mansoura. Univ. 5 (3): 127-141.

5. Blache, D., S. Zhang, and G. B. Martin, 2006. Dynamic and integrative aspects of the regulation of reproduction by metabolic status in male sheep. Reproduction Nutrition Development, 46(4), 379-390.

6. Bollwein, H., F. Janett, and M. Kaske, 2017. Effects of nutrition on sexual development of bulls. Animal Reproduction (AR), 14(3), 607-613.

7. Boulanouar, B. M. Ahmed, T. Klopfenstine, D. Brink, and J. Kinder. 1995. Dietary protein or energy restrictions influences age and weight at puberty in ewe lambs. Animal Reproduction Science. 40 :229-238 93.

8. Dantas, A., E. R. Siqueira, S. Fernandes, E. Oba, A. M. Castilho, P. R. L. Meirelles, and P. T. R. Santos. 2016. Influence of feeding differentiation on the age at onset of puberty in Brazilian Bergamasca dairy ewe lambs. Arquivo Brasileiro De Medicina Veterinaria E Zootecnia, 68(1), 22-28.

9. Duncan, D. B. 1955. Multiple ranges and Multiple F. test. Biometrics. 11:1.

10. El- Zelaky, O.A, E. I. Khalifa, A. H. Mohamed, B. K. Mohamed and A. M. Hussein. 2011. Productive and reproductive performance of Rahmani male lambs fed rations containing Jatropha cake. Egyptian J. of Sheep and Goats Sciences, 6(2), 1-10.

11. El-Badawy, M., H. Youssef, Y. Hafez, H. El-Sanafawy, and M. El-Maghraby. 2018. Effect of rocket oil addition on productive and reproductive performance of growing ram lambs under hot climate condition. Journal of Animal and Poultry Production, 9(1), 9-15.

12. El-Madawy, A, M. El-Sharawy, M. Ali, Y. Hafez, N. Yamauchi, and E. S. Metwally. 2019. Effect of unsaturated fatty acids supplementation on productive and reproductive performance of ram lambs. kyushu University Institutional Repository, 64(2), 247-255.

13. Elmaz O., U. Cirit, O. Keser, K. Gurbulak, K. Guvenc and C. Kutay. 2007. Effect of two dietary protein levels on testosterone, testicular parameters and semen quality in ram lambs during pubertal development. Medycna Vet. 63(10): 1177-1180.

14. Esmaeili, V., A. H. Shahverdi, A. R. Alizadeh, H. Alipour, and M. Chehrazi. 2014. Saturated, omega-6 and omega-3 dietary fatty acid effects on the characteristics of fresh, frozen-thawed semen and blood parameters in rams. Andrologia 46:42-49.

15. Goijam, Y., S. Gizaw, S. Abegaz, and C. J. Thwaites. 1995. Reltionship between body weight and scrotal characteristics and between environment effects and fertility in ethiopian Horro rams. J. Agric. Sci. Cambridge. 124:297-299.

16. Hungate, E. Ropert .1966. The rumen and its microbes. Academic press. New York and London.

17. Kassem, M. M. 1986. Feed intake and Milk Production in Dairy Cow With Special reference to diets containing grass and Lucerne silage with barley supplement. Ph.D. Thesis. Hanna Research Institute. Ayr. Scotland.

18. Kaur, H., and S. P. Arora. 1995. Dietary effects on ruminant livestock reproduction with particular reference to protein. Nutri. research reviews, 8(1),121-136.

19. Khalifa E.I., M.E. Ahmed, Y.H. Hafez, O.A. El-Zolaky, K.M. Bahera, and A.A. Abido. 2013. Age at puberty and fertility of

Rahmani sheep fed on biological culated corn silage. Annals Agric. Sci. 58 (2): 163–172.
20. Luna, L. G., 1968. Manual of histological staining methods of the armed forces institute of pathology. Mcgraw Hill Book Company, New York, pp. 38-39.
21. Moghaddam, A., M. Panah, and M. Souri. 2019. Improved early postnatal nutrition and its effect on histomorphological parameters in the testes of Sanjabi ram lambs. Tropical animal health and production, 51(6), 1539-1544.
22. National Research Council, (2001). Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
23. National Research Council, Committee on the Nutrient Requirements of Small Ruminants, Board on Agriculture, Division on Earth, and Life Studies. 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids.
24. Nudda, A., G. Battacone, A. S. Atzori, C. Dimauro, S. P. G. Rassu, P. Nicolussi, and G. Pulina, 2013. Effect of extruded linseed supplementation on blood metabolic profile and milk performance of Saanen goats. Animal, 7(9), 1464-1471.
25. Paengkoum, P., J. B. Liang, Z. A. Jelan, and M. Basery. 2004. Effects of ruminally undegradable protein levels on nitrogen and phosphorus balance and their excretion in Saanen goats fed oil palm fronds. Songklanakarin J. Sci. Tehnol, 26,15-22.
26. Polkowska, J. 1989. Effect of protein deficiency on some hypothalamic and pituitary hormones in growing male lambs. An immunohistochemical study. Reproduction Nutrition Development, 29(3), 347-356.
27. Saeed, F. F., and N. W. Zaid. 2019. Serum and testicular testosterone levels of ram lamb during puberty. Adv. Anim. Vet. Sci, 7(2), 92-95.
28. SAS. 2005. Statistical Analysis System. User's Guide for Personal Computer Release 8.2, SAS Institute, Inc., Cary, Nc, USA.
29. Scaramuzzi, R. J., B. K. Campbell, J. A. Downing, N. R. Kendall, M. Khalid, M. Muñoz-Gutiérrez, and A. Somchit. 2006. A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Reproduction Nutrition Development, 46(4), 339-354.
30. Schoeman, S. J., H. C. Els and G. C. Combrink 1987. A preliminary investigation into the use of testis size in crossbred ram as a selection index for ovulation rate in female relatives. S. Afri. J. Anim. Sci. 17:144-147.
31. Tjondronegoro, S., G. B. Martin, S. R. Sutherland, and R. Boukhliq. 1996. Interactions between nutrition, testosterone and inhibin in the control of gonadotrophin secretion in mature rams. Repro. Ferti. 8(5), 855-862.
32. Tegegne, A., K. W. Entwistle, and E. Mukasawa-Mugerwa, 1992. Nutritional influences on growth and onset of puberty in Boran and Boran **x** Friesian bulls in Ethiopia. Theriogenology 37**,** 1005-1016.
33. Toe, F. J., E. O. Rege, E. Mukasa-Mugerwa, S. Tembely, D. Anindo, R. L. Baker and A. Lahlou-Kassi. 2000. Reproductive characteristics of Ethiopian highland sheep 1. Genetic parameters of testicular measurements. I ram lambs and relationship with age at puberty in ewe lambs. Small Rumin. Res. 36:227-240.
34. Tran, L. V., B. A. Malla, A. N. Sharma, S. Kumar, N. Tyagi, and A. K. Tyagi. 2016. Effect of omega-3 and omega-6 polyunsaturated fatty acid enriched diet on plasma IGF-1 and testosterone concentration, puberty and semen quality in male buffalo. Anim. Reprod. Sci. 173: 63–72.
35. Umar, M., B. Kurnadi, E. Rianto, E. Pangestu, and A. Purnomoadi. 2015. The effect of energy level of feeding on daily gain, blood glucose and urea on Madura cattle. J. of the Indonesian Trop. Anim. Agric, 40(3), 159-166.
36. Waddad, **S.** A. and E. **S.** E. Gaili, (1985). Effect of nutrition on sexual development of Western Sudan Baggara bull calves. Acta Veteri. Beograd 35, 299-304.
37. Xu, Y., Z. Li, L. E. Moraes, J. Shen, Z. Yu, and W. Zhu, 2019. Effects of incremental urea supplementation on rumen fermentation, nutrient digestion, plasma metabolites, and growth performance in fattening lambs. Animals, 9(9), 652.