Research Article

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Influence of Different Feeding Ratio of Pomegranate Peels Powder on Testicular Dimensions, Body Weight and Testosterone Concentration in Karadi Male Lambs

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Abstract

The current study was presented to conclude the influence of different ratio of pomegranate peel powder (PP) in feed of Karadi male lambs, they divided to 4 groups (0% as a control, 1% treatment 1, 2% treatment 2 and 4% as treatment 3), on scrotal circumference (SC), scrotum height (SH), body weight (BW) and blood serum testosterone concentration (T). In this study, sixteen (16) of Karadi male lambs used with 24.30 ± 0.40 kg weight and with 4-5 months old, all lambs have been randomly divided equally into four (4) groups and individually penned in a box for during 63 days. Results in our study showed that SC was significantly different and recorded higher 17.875±0.54 cm in group that fed on 1% PP, also SH recorded higher and significant different in group fed on 1% PP compared with other groups 10.000±0.32 cm. Average blood serum testosterone reduces in all groups compared to control group that fed on 0% of PP (11.418±0.15 n.mol/L). Final body weight in our study had significantly different ($P < 0.05$) between groups, 1% PP recorded higher weight (34.9 ± 0.58) kg. Each average daily gain, total body gain and initial body weight had not significant different in all groups. Correlation coefficient between SC, SH, blood serum testosterone concentration and final BW computed and ranged between (-0.397 to + 0.914).

Keywards: Pomegranate Peel, testicular dimension, testosterone, Karadi Lambs

تأثير استخدام مستويات مختلفة من مسحوق قشور الرمان على قياسات الخصية ووزن الجسم وتركيز هرمون التستستيرون في ذكور الحملان الكرادية

الخلاصة

هدفت هذه الدراسة تأثير استخدام مستويات مختلفة من قشور الرمان في تغذية ذكور الحملان الكرادية تم توزيعها الى 4 مجاميع (0% مجموعة السيطرة, 1% المعاملة الاولى, 2% المعاملة الثانية و 4% المعاملة الثالثة) على قياس محيط كيس الصفن, ارتفاع الخصية, وزن الجسم و تركيز هرمون التستستيرون. استخدمت 16 من ذكور الحملان الكرادية تراوحت اوزانها من (24.29±0.24) كغم واعمارها من (4-5) شهرا وتم توزيعها بصورة عشوائية الى اربعة مجاميع واستمرت الدراسة لمدة حوالي63 يوما. اظهرت النتائج وجود فرق معنوي لقياس محيط كيس الصفن وسجل (17.875±0.54) في المجموعة التي تتغذى على 1% من مسحوق قشور الرمان, وايضا ارتفاع الخصية لها فروقات المعنوية وسجل اعلى في المجموعة التي تتغذى على 1% من مسحوق قشور الرمان مقارنة بالمجاميع الاخرى (10.000±0.32). انخفاض معدل تركيز هرمون التستستيرون في كل المجاميع مقارنة بمجموعة السيطرة حيث بلغ (11.418±0.15). سجل وزن الجسم النهائي فرقا معنويا ($P < 0.05$)حيث بلغ (34.9±0.58) كغم. ولم يسجل كل من زيادة الوزن اليومي, معدل وزن الكلي و وزن الجسم البدائي اي فروقات معنوية في كل المجاميع. بلغ معامل الارتباط بين محيط كيس الصفن, ارتفاع الخصية, تركيز هرمون التستستيرون و وزن الجسم النهائي مابين (-0.397 الى +0.914).

Introduction

Punica granatum L. (Pomegranate) defined as a family of Punicaceae has been cultivated in tropical and subtropical regions, three portions of Pomegranate had separated into: 3% seeds, 30% juice and 67% total weight are the remaining of the peels and flash. Negi and Jayaprakasha (1) recorded that PP has been as antioxidant and antibacterial properties, and Viuda-Martos *et al* (2) including many types of minerals like as sodium, potassium, calcium, phosphorus, nitrogen and magnesium. PP are widely used in sexual organs to treat infections in human, also to treat mastitis, diarrhea, allergic dermatitis, dysentery, acne, scalds, pile, tympanitis, and folliculitis (3), also Abdel Al-Agha *et al* (4) showed that Pomegranate is rich in antioxidant of polyphenolic class which contains anthocynins, tannins and flavonoids, and it is usually demonstrated that Pomegranate exhibits antioxidant, antivirus, anticancer, antidiabetic, antidiarrheal and antiproliferative activities. Nigriz *et al* (5) demonstrated that in the middle east, many culture used the PP for the folk medicine. *Punica granatum* indicates fertility and health, and has been assumed to improve sexual effort. Scrotal circumference and testicular measurements like testicular length, width and height have been reported by Öztürk *et al* (6) as indirect criteria to get better fertility. Turk *et al* (7) reported that juice of pomegranate intake, increases level of testosterone and sperm quality in animals. Zeweil *et al* (8) recorded in male rabbits that, PP increased volume of semen, plasma fructose of semen, improved motility of sperm, total sperm production and decrease percentage of dead sperm significantly with feed that containing 1.5%, 3% and 4.5% of PP compared to control group. Al-Dujaili (9) showed that juice of pomegranate reducing blood pressure by reducing factors of stress and cardiovascular risk. There is another study by Shabtay *et al* (10) showed that dietary were contained PP as a supplementation stimulating the increase in feed intake, with favorable tendency that lead to rise in body weight gain in male calves.

Thus, the purpose of this study was to calculate the useful influence of PP on testicular dimensions, body weight and testosterone blood serum concentration and its relationship in Karadi male lambs.

Materials and Methods

This study was carried out at the farm of animal, animal science Dept., College of Agricultural engineering Science University of Sulaimani, Bakrajo/ Sulaimani, Kurdistan, Iraq. Pomegranate fruits were cleaned, washed and cut manually to separate the peel and seeds. Separated peels were cut into small parts using a sharp knife and dried in a drier at 60^0C for 8 h till its moisture reached into 12-14%. All dried pieces of peels were cooled and powdered by disc mill and packed in polyethylene bags and kept until use at 25±5ºC (11). In this study, the initial weight of 16 Karadi male lambs were used was 24.30±0.40 kg and 4- 5 months old. After treated all lambs against endo and ecto parasites were

individually penned and randomly were divided equally in to 4 treatments for 63 days after 14 days for an adaptation periods, four rations were used in this study, were contained (0, 1, 2, or 4%) of PP. All lambs were feeding on 3% of their body weight of fed concentration that contain 15.5% crude protein and 12.66 MJ/Kg dry matters. In the next morning, before offering the feed the refusal diet was collected and weighed twice. Barley straw was set freely and water stayed available constantly. Weekly intervals, all lambs were weighed. Table 1 showed chemical compositions of powder of PP namely: Crude protein, ash, crude fibers, ether extract, nitrogen free extract, organic matter and Metabolic energy.

Table (1) Chemical compositions of barley straw and pomegranate peels powder PP

Item %	Barley straw	Pomegranate peels powder (PP)
Crude protein	4.1	5.1
Ash	8.4	3.8
Crude fiber	41.0	11.22
Ether extract	1.2	4.9
Nitrogen free extract	45.3	80.5
Organic matter	91.6	96.2
Metabolic energy (MJ/Kg DM)*	6.6**	27.92

* Metabolic energy, calculated by (12).
** Metabolic energy, calculated by (13).

Blood sample collection & determination of testosterone concentration:

In the last week of the experiment (60 days of the experiment), after 6 hours post feeding, blood samples (10 ml) were withdrawn from jugular vein, by using anti-coagulant plastic tube the blood were deposited in and allowed to clot at room temperature then centrifuged at 6000 rpm for 10 min for serum separation. Serum were separated at once for concentration of testosterone was measured by radioimmunoassay (Model accent 200, Poland).

Measurement of testicular dimensions:

The testicular dimensions (scrotal circumference and scrotum height) were obtained by a procedure described by (14), after pushing the testes firmly into the scrotum, a flexible cloth tape used to measure the scrotal circumference and height in centimeters (cm) as the largest diameter of the scrotum and testes.

Statistical analysis:

In the present study all data were evaluated according to XLSTAT (15), to detect the differences among means also used Duncan's (16).

Formulation of data analysis: $Yij = \mu + ti + eij$

Where:

Yij = the dependent variable,

μ = overall mean,

ti = effect of the treatment (i = control, 1%PP, 2% PP or 4% PP),

eij = random residual error.

Results and Discussion

Testicular dimensions, testosterone and body weight gain of Karadi male lambs were

shown in table 2. Testicular dimension (Scrotum circumference, Scrotum height) significantly differed ($P \leq 0.05$), were recorded higher (17.875±0.54, 10.000±0.32) in lambs that fed 1% PP, respectively. Blood serum testosterone concentration of Karadi male lambs of this study recorded higher value in control group (11.418±0.15) compared into another groups that fed on 1%, 2% and 4% PP. Thus, there was not significant different ($P \leq 0.05$) in initial body weight, average daily gain and total body gain in control group and other groups were fed on different levels of PP, while there is significant different between groups in final body weight, group that fed on 4% PP recorded lower value compared into other groups.

The relationship between scrotum circumference, scrotum height, blood serum testosterone and final body weight were shown in table 3, Correlations coefficient among different traits were computed and ranged between (-0.397 to + 0.914). Scrotum circumference was positively and significantly correlated with scrotum height (r= 0.914) and final body weight (r= 0.746), respectively and similar observations were found between scrotum height with final body weight (r= 0.700). Testosterone had negative correlation with scrotum height (r= -0.562), while testosterone also was negative and not significant correlation with scrotum circumference and final body weight (r= -0.475, -0.397).

Discussion

In our present study, there is increase in final body weight by adding of PP in the diets by 1% to 2% PP supplementation in Karadi male lambs. Like other results were found by (7,17,18,19), reported that PP is an important basic of the antioxidant and scavenging of free radical activity of pomegranate phenolic compounds, gallic and ellagic acids, vitamin C, respectively. Amani *et al* (20), confirmed that PP is a good source of tannin, flavonoids and phenolic compounds, tannin is the main component of the peels by 25-28%. Also Aerts *et al* (21) reported that diet of sheep that contain 2% to 4% of tannins concentration increased feed intake, milk yield and ovulation rate, that lead to improve production efficiency in ruminants. Many evidences showed that, flavonoids interact with several biological systems Lairon and Amiot (22). However, in table 1 showed that the numerically increased the final body weight in lambs fed on 1% PP toward 2% PP, it appears that lambs had a better response to adding of 1%PP.

In our study, SC and SH had significant different in lambs that fed 1% PP (Table 2). During this study 1% PP increase final body weight significantly ($P \leq 0.05$), all testicular measurement also increased. In young rams, Omer (23) reported that testicular development more closely related with body weight than with its age. Also Mekasha *et al* (24) showed that testicular dimensions with scrotal circumference are excellent indicators of sperm role, also it has related to semen volume and body weight with other variables.

The effect of PP on blood serum testosterone

concentration significantly different ($P \leq 0.05$) in lambs fed 4% PP, (Table 2). This results agree with Mohamed *et al* (25), an increase in cortisol as a stress hormone, leading to a subsequent decrease in gonadotropin releasing hormone (GnRH), it has an important role of key hormones (LH and FSH) that able to affect the quality and quantity of sperm. Testosterone (T) is the male gonadal hormone that produced by the Leydig in the testes that should boost androgen-dependent parameters such as spermatogenesis mating performance, it also has an important role to increase muscle mass and strength and maintaining body shape. Chauhan and Dixit (26) reported that FSH influence on Sertoli cells that motivates spermatogenesis and LH effect on Leydig cells that encourages synthesis and release of testosterone. Sharma *et al* (27) showed that testosterone may be help male sexual activities. Increase in testosterone level in this study due to PP can be ability by pomegranate to reduce cortisol level as demonstrated by Hong *et al* (28).

In this study, our calculating showed that correlation coefficient between SC, SH, T. and BW were ranged between (-0.397 to + 0.914), Table 3. This result was similar with Allaoui *et al* (29). Boussena *et al* (30) recorded a significant correlations coefficient between testicular dimensions and body weight. There are many factors that effect on developing of testicular parameters (length, scrotal circumference and volume) in Awassi lambs from weaning age (2-3) months up to (17) months, recorded that the biggest increase in testicular parameters is recorded between 7 and 10 months of age Salhab *et al* (31). Agga *et al* (32) reported that testicular dimension and body weight have an important indicator for evaluating breeding healthy. Testis size in male reproductive success has an important reason, Gomendio *et al* (33) showed that larger testes produce highest quality and highest rates of sperm production. Other results recorded that the various testicular dimensions were more related with body weight than age (31). Mekasha *et al* (24) demonstrated that testicular dimensions with scrotal circumference have best indicators of sperm activity, while body weights with other variables have been found to be related to semen volume. There was positively and significantly correlated between SC and BW, this is agreement with (34) reported that SC and BW have positive relationship and this traits make improvement in both traits. Testosterone was negative and not significant correlation with scrotum circumference and final body weight table 3. Similar results described by Fourie *et al* (35) that showed a weak correlation coefficient between scrotal circumference and testosterone levels in rams. Preston *et al.* (36) showed that the variation between the sizes of testes is a great predictor of level of testosterone concentration during the breeding season. It is possible that large testes have more Leydig cells or higher concentration of testosterone level that stimulate the development and growth of spermatogenic tissues, which affect mass of the testes.

Table (2) effect of different level of PP on testicular dimension, blood serum testosterone and body weight in Karadi male lambs

Parameters	Control group	Treatment 1	Treatment 2	Treatment 3
Scrotum circumference (cm)	11.500±0.32 b	17.875±0.54 a	11.750±0.43 b	10.250±0.60 b
Scrotum height (cm)	6.750±0.43b	10.000±0.32 a	7.750±0.29b	6.750±0.32b
Testosterone (n.mol/L)	11.418±0.15 a	0.897±3.52b	1.193±0.07b	6.400±2.86a b
Initial body weight (kg)	23.6 ± 0.66a	23.4 ± 1.29a	23.5 ± 1.61a	23.3 ± 1.55a
Final body weight (kg)	33.3 ± 0.38ab	34.9 ± 0.58a	34.2 ± 0.62a	31.7 ± 0.48b
Average daily gain (g/day)	153.97 ± 11.80a	182.54 ± 24.80a	169.84 ± 24.90a	133.33 ± 28.60a
Total body gain (kg)	9.7 ± 0.75a	11.5 ± 1.56a	10.7 ± 1.57a	8.4 ± 1.80a

a,b Means with a different letter superscripts within a row are significant ($P \leq 0.05$)

Table (3) Correlation coefficients between scrotum circumference, scrotum height, testosterone and final body weight in Karadi male lambs

Parameters	Scrotum circumference (cm)	Scrotum height (cm)	Testosterone (n.mol/L)	Final body weight (kg)
Scrotum circumference (cm)	1			
Scrotum height (cm)	0.914*	1		
Testosterone (n.mol/L)	-0.475 $^{n.s}$	-0.562*	1	
Final body weight (kg)	0.746*	0.700*	-0.397 $^{n.s}$	1

*: $P \leq 0.05$, n.s: not significant

Conclusion

The result of this study revealed that 1 % PP effect on testicular dimensions and final body weight significantly, while 4% PP increase blood serum testosterone compare to other groups that fed on 1% and 2% PP, respectively. This study shows that there is a positive and significant correlation coefficient between SC with SH and final body weight, similar result recorded between SH with final body weight. Testosterone had not significant correlation with each SC and final body weight, also had negative correlation with SH.

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References

1. Negi PS., Jayaprakasha GK. Antioxidant and antibacterial activities of Punica granatum peel extracts. J. Food Sci. 2003; 68:1473–1477.

2. Viuda-Martos M., Fern´andez-L´oaez J., P´erez-´alvarez J. Pomegranate and its many functional components as related to human health: a review, Comprehensive Reviews in Food Science and Food Safety. 2010; (9) 6: 635–654.

3. Singh RP., Chidambara MK., Jayaprakasha GK. Studies on the antioxidant activity of

pomegranate (Punica granatum) peel and seed extracts using in vitro models. J. Agri. Food Chem. 2002; 50: 81–6.

4. Al-Agha AG., Al-Khafaji NJ. Antimicrobial activity of methanol and aqueous extracts of some medicinal plants against Klebsiella pneumonia. Al-Anbar Journal of Veterinary Sciences. 2017;10(1):86-95.

5. Nigriz F., Maria L., Sharon W., Francesco P., D'Armiento C., Carmela F., Louis J., Ignarro D., Claudio N. The influence of Pomegranate fruit extract in comparison to regular pomegranatr juice and seed oil on nitric oxide and arterial function in obese Zucker rats. Nitric Oxide. 2007; 17: 50-54.

6. Öztürk A., Dağ B. and Zülkadir U. Akkaraman ve İvesi koçların bazı testis özelliklerinin döl verimine etkisi. Türk Veterinerlik ve Hayvancılık Dergisi. 1996; 20: 127–130.

7. Türk G., Sönmez M., Aydin M., Yüce A., Gür S., Yüksel M., Aksu E. and Aksoy H. Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clin Nutr. 2008; 2:289-96.

8. Zeweil HS., ElNagar S., Zahran SM., Ahmed MH., El-Gindy Y. Pomegranate Peel as a Natural Antioxidant Boosts Bucks' Fertility under Egyptian Summer Conditions.World Rabbit Sci. 2003; 21: 33-39.

9. Al-Dujaili NS. Pomegranate juice consumption reduces salivary cortisol levels and improves mood and positive affects at work in healthy volunteers. 5th International Conference on Polyphenols and Health, Barcelona. 2011; 17-20: 145.

10. Shabtay A., Eitam H., Tadmor Y., Orlov A., Meir A., Weinberg P., Weinberg Z., Chen Y., Brosh A., Izhaki I., Kerem Z. Nutritive and Antioxidative Potential of Fresh and Stored Pomegranate Industrial Byproduct as Novel Beef Cattle Feed. Journal of Agricultural and Food Chemistry. 2008; 56: 10063-10070.

11. Devatkal SK., Naveena BM. Effect of Salt, Kinnow and Pomegranate Fruit By Product Powders on Color and Oxidative Stability of Raw Ground Goat Meat during Refrigerated Storage. *Meat Science*. 2010; 85: 306-311.

12. Mirzaei-Aghsaghali A., Maheri-Sis N., Mansouri H., Razeghi M., Mirza-Aghazadeh A., Cheraghi H., Aghajanzadeh-Golshani A. Evaluating Potential Nutritive Value of Pomegranate Processing By-Products for RuminantsUsing in Vitro Gas Production Technique. Journal of Agricultural and Biological Science. 2011; 6: 45-51.

13. Hassan SA., Sadq SM., Hassan KM. Evaluation of Fungal or Chemical Treatments for Barley Straw in Ruminants Feeding 1-Chemical Composition, *in Vitro*, *in Vivo* Digestibility and Voluntary Intake. *Jordan Journal of Agricultural Sciences*. 2012; 8: 232-241.

14. Sönmez R., Kaymakçı M. Koyunlarda Döl Verimi. Ege Üniv. Zir. Fak.Yay. 1987; No. 404.

15. XLSTAT. Statistical Software for Excel. 2007; https://www.xlstat.com

16. Duncan, DB. Multiple Ranges and Multiple "F" Test. *Biometrics*. 1955; 11: 1-42.

http://dx.doi.org/10.2307/3001478.

17. Sadq S., Dereen O., Hozan J., Karzan A. Growth Performance and Digestibility in Karadi Lambs Receiving Different Levels of Pomegranate Peels. Open Journal of Animal Sciences. 2016; 6: 16-23.

18. Rosenblat M., Hayek T., Aviram M. Anti-oxidative effects of pomegranate juice (PJ) consumption by diabetic patients on serum and on macrophages. Atherosclerosis. 2006; 187: 363-371.

19. Lansky E. and Newman R. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J. Ethnopharmacol. 2007; 109: 177-206.

20. Amani S., Giles E., Mohammed A., Ghanim A., Ahmed S., Mohammed S. Phenolic Controlstituents of Pomegranate Peels (Punica granatum L.) Cultivated in Oman. European Journal of Medicinal Plants. 2014; 4(3): 315-331.

21. Aerts RJ., Barry TN., McNab WC. Polyphenols and Agriculture: Beneficial Effects of Proanthocyanidins in Forages. Agriculture, Ecosystems & Environment. 1999; 75: 1-12.

22. Lairon D., Amiot M. Flavonoids in food and natural antioxidants in wine. Curr. Opin. Lipidol. 1999; 10: 23-28.

23. Omar CA. Study of some testicular dimensions and their relationship to body weight in karadi ram lambs. Assiut vet. Med. J. 2016; (62), 150: 31-38.

24. Mekasha Y., Tegegne A., Abera A., Rodriguez-Martinez H. Body size and testicular traits of tropically-adapted bucks raised under extensive husbandry in Ethiopia. Reprod. Domest. Anim. 2008; 43(2):196-206.

25. Mohamed A., Saleh Q., Ahmed E. Effect of Pomegranate (Punica granatum L.) Juice and Methanolic Peel Extract on Testis of Male Rats. Pakistan J. Zool. 2013; 45(5): 1343-1349.

26. Chauhan N.S., Dixit VK. Effects of Bryonia laciniosa seeds on sexual behaviour of male rats. Int. J. Impot. Res. 2010; 22: 190-195.

27. Sharma V., Boonen j., Chauhan N S., Thakur M., De Spiegeleer B., Dixit VK. Spilanthes acmella ethanolic flower extract: LC-MS alkylamide profiling and its effects on sexual behavior in male rats. Phytomedicine. 2011; 18:1161-1169.

28. Hong MY., Seeram NP., Heber D. Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor. J. Nutr. Biochem. 2008; 19: 848-855.

29. Allaoui A., Safsaf B., Laghrour W., Tlidjane M. Factors Affecting Scrotal Measurements and Weight of Ouled Djellal Rams in Eastern and South-Eastern Algeria. APCBEE Procedia. 2014; (8): 260 – 265

30. Boussena S., Bouaziz O., Zerrougui S., Derqaoui L., Tainturier D. Performances de croissance corporelle et testiculaire avant le sevrage chez les agneaux de race Ouled Djellal. Revue Méd Vét. 2013; 164 (4): 191-199.

31. Salhab SA., Zarkawi M., Wardeh MF., Al-Masri MR., Kassem R. Development of testicular dimensions and size, and their relationship to age,

body weight and parental size in growing Awassi ram lambs. Small Rumin. Res. 2001; 40: 187-191.

32. Agga G., Udala U., Regassa F., Wudie A. Body measurements of bucks of three goat breeds in Ethiopia and their correlation to breed, age and testicular measurements. Small Rumin. Res. 2011; 95(2-3):133-8.

33. Gomendio M., Harcourt A., Roldan E. Sperm competition in mammals. In: Birkhead, T. R. & Møller, A. P. (Eds.). Sperm Competition and Sexual Selection. San Diego, Academic Press. 1998; 667-751.

34. Pourlis A F. A review of morphological characteristics relating to the production and reproduction of fat-tailed sheep breeds. Trop. Anim. Health Prod. 2011; 43(7):1267-87.

35. Fourie JP., Schwalbach ML., Neser CW., Greyling, CP. Relationship between body measurements and serum testosterone levels of Dorper rams. Small Rum Res. 2005; 56: 75–80.

36. Preston TB., Stevenson RI., Lincoln AG., Monfort LS., Pilkington GJ., Wilson K. Testes size, testosterone production and reproductive behaviour in a natural mammalian mating system. J Anim Ecol. 2012; 81: 296–305.