Journal of Livestock Science and Technologies, **2019, 7 (1): 39-44** ***http://lst.uk.ac.ir***

DOI: 10.22103/jlst.2019.13049.1251

Sperm quality parameters and fatty acid composition in Farahani rams fed pistachio by-products

M. Khodaei-Motlagh[1*], M. Kazemi-Bonchenari[1], M. Zhandi[2], M. H. Moradi[1] and A. Mohammadi-Sangcheshmeh[3]

[1]Department of Animal science, Faculty of Agriculture and Natural Science, Arak University, Arak 38156-8-8349, Iran.

[2]Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

[3]Department of Animal and Poultry Science, College of Aburaihan, University of Tehran, Pakdasht, Tehran, Iran.

*Corresponding author, E-mail address: m-motlagh@araku.ac.ir

Abstract Fifteen fertile rams (live weight 55.9 ± 1.21 kg, and 2.5-3 years old) were randomly allotted to three diets consisting of 0 (Control), 12.5 (PBP-12.5), and 25 % (PBP-25.5) pistachio by-products (PBP) in dry matter. Sperm motility parameters were detrimentally affected in animals feeding on PBP-25% diet (P<0.05). Sperm concentration of C17-1, C22-6-n-3, C18-2-Cis and C22-6 n-3 fatty acids decreased but that of C18-0 and C18-1-Cis fatty acids increased at the highest level of dietary PBP. The results indicated that 12.5% PBP in dry matter, as a cheap by-product, may be included in the ram diet for extended periods without any discernible detrimental effects on sperm characteristics. However, fertility trials need to be performed to substantiate this conclusion.

Keywords: Farahani ram, pistachio by-product, sperm parameters

Received: 15 Nov. 2018, *accepted:* 11 Feb. 2019, *published online:* 14 Oct. 2019

Introduction

Every year, approximately 500,000 tons of fresh pistachio by-products (PBP) are produced in Iran (Shakeri, 2016). These are high in crude fat (69.5 g/kg DM) and crude protein (158.2 g/kg DM).

Pistachio by-products can be used as a part of the forage constituent of the diet for sheep, especially when forage supply is low (Shakeri, 2016). There are several reports on the effects of PBP on milk production (Mokhtarpour et al., 2012), lamb performance (Shakeri, 2016) and growth (Shakeri et al., 2014) in ruminants; however, there is no published report on the effects of PBP on male reproduction. The use of highly fertile rams in breeding programs is important, especially under extensive conditions which can be affected by agricultural by-products that are included in the diet at the time of feed scarcity. Pistachio by-products that are copiously produced in Iran can be used in ruminant nutrition; however, they contain high concentrations of phenolic compounds and total tannins that may limit animal productivity (Sedighi-Vesagh et al., 2015). Therefore, the aim of this study was to investigate the effect of feeding PBP on sperm quality parameters in Farahani rams.

Materials and methods

Chemicals

Unless otherwise mentioned, all chemicals, reagents and media constituents were purchased from Sigma-Aldrich Chemicals, USA.

Animals and diets

Fifteen fertile rams of Farahani breed with a mean live BW of 58.9 ± 1.21 kg (2.5-3 years old) were fed with one of three iso-nitrogenous and iso-energetic diets (5 rams allotted randomly to each diet), containing 0 (Control), 12.5% (PBP-12.5), and 25% (PBP-25) PBP in dry matter. Composition of the diets (Table 1) was determined based on the Association of Official Analytical Chemists (Horwitz, 2000).

Semen collection

Semen was collected twice a week from each ram during the breeding season (October to January) using artificial vagina. Immediately after arrival at the laboratory, evaluation of diluted samples were performed.

Table 1. Ingredients (% in DM) and chemical composition of the control and diets containing 12.5 (PBP-12.5) and 25 (PBP-25) percent (DM basis) pistachio by-products

Item	Control	PBP-12.5	PBP-25
Ingredients			
Alfalfa hay	32	24	16
Wheat straw	15	15	15
Barley grain, ground	32	32	32
Pistachio by-product	0	12.5	25
Vitamin/mineral premix[1]	0.4	0.4	0.4
Salt	0.15	0.15	0.15
Soybean meal	3.2	3.2	3.2
Dicalcium phosphate	0.16	0.16	0.16
Wheat bran	16	16	16
Composition			
Crude protein	13.9	13.7	13.5
Metabolizable energy	2.30	2.25	2.20
Ca	0.6	0.6	0.6
P	0.4	0.4	0.4

[1]Contained per kilogram of supplement: 500,000 IU vitamin A, 50,000 IU vitamin D, 1,500 IU vitamin E, 120 g Ca, 30 g P, 20 g Mg, 25 g Na, 2000 mg Mn, 5000 mg Zn, 1500 mg Fe, 14 mg Co, 125 mg Cu, 56 mg I, and 10 mg Se.

Sperm motility

Sperm motility was determined using a phase-contrast microscope (400× magnification) equipped with a warm stage. A small drop of diluted semen was placed on a microscope slide and covered with a cover-slip. Sperm motility estimations were performed in five different microscopic fields for each semen sample. The mean of five successive estimations was recorded as the final motility score (Bucak et al., 2009).

Sperm viability

Equal volumes of semen and eosin-nigrosin stain were incubated at room temperature. One drop of the mixture was then put on a slide, smeared and air dried. A total of 200 sperm were evaluated under light microscope (1000 × magnification, oil immersion). Partially- and completely- stained sperm were considered as dead and sperm showing strict exclusion of the stain were considered to be alive (Evans and Maxwell, 1989).

Sperm abnormal morphology

For assessment of sperm abnormality (head, mid piece droplet, free tail, coiled tail and bent tail), at least 30 µL of the diluted sample was transferred into a microtube containing 300 mL of Hancock's solution (Schafer and Holzmann, 2000). A drop of the mixture was placed on a slide and at least 200 sperm were evaluated for sperm abnormality.

Determination of sperm fatty acid profile

Semen samples were diluted with an equal volume of 0.85 % NaCl (wt/vol) followed by centrifugation at 700 × g for 20 min at 4 °C. The supernatant (seminal plasma) was transferred to a clear test tube and the sperm pellet was washed with 1 mL of 0.85 % NaCl and re-centrifuged. The sperm pellet was re-suspended in 2 mL NaCl, and the resulting sperm pellet was washed twice with saline. Total lipid was extracted from the spermatozoa after homogenization in chloroform–methanol (2:1, v/v) (Folch et al., 1957). The fatty acid concentration was determined by GC (0.25×0.32, ID of 0.3 m WCOT Fused Silica Capillary, Agilent 6890, USA) with a 120 m silica-fused column (BPX-70). Nitrogen was the carrier gas. Initial and final temperatures were set at 140 and 240˚C, respectively, with detector and injector temperatures set at 280 and 260˚C. The fatty acid standard was obtained from Sigma-Aldrich.

Statistical analysis

The experiment was conducted as a completely randomized design. Semen was considered as the subject. The data were analyzed using the Proc Mixed (Version 9.1; SAS Institute, 2002, Cary, NC, USA).). The percentage data did not follow normal distribution and were arcsine –transformed before analysis. The results were expressed as mean ± SEM. The Tukey's test was used to compare the least squares means. Differences were considered statistically significant at $P<0.05$.

Results

Sperm viability (Figure 1) and motility (Total and progressive motility; Table 2) were not different between

Table 2. The Effect of diets containing 12.5 (PBP-12.5) and 25 (PBP-25) percent (DM basis) pistachio by-products on sperm characteristics in Farahani rams

Sperm characteristics	Control	PBP-12.5	PBP-25	SEM	P-value
Total motility	77.30[a]	77.20[a]	67.45[b]	1.30	0.0006
Progressive motility	8.30[a]	8.40[a]	7.37[b]	0.17	0.004
Normal sperm	77.6	76.3	75.2	0.34	0.32

[a,b] within rows, mean values with common superscript(s) are not different (P>0.05).

the control and PBP-12.5 diets. However, rams fed with 25% PBP showed decreased viability and motility (P<0.05) as compared to the control and PBP-12.5 groups. Sperm morphology was not affected by feeding PBP (Table 2). Sperm plasma membrane fatty acid profile was not different between the control and PBP-12.5 rams (Table 3). However, concentration of C17-1 and DHA, decreased and that of stearic and oleic acids increased by inclusion of 25% PBP in the diet.

Discussion

A dietary level of 25% PBP exerted a detrimental effect on sperm viability and motility. Feeding of PBP to ruminants should be limited due to high tannin content (Safarinejad, 2011). Tannins, as antioxidants, could enhance fatty acid peroxidation when used at high levels (Labavitch et al., 1982) which might explain the decrease in sperm motility in rams fed 25% PBP in their diet. It is likely that several reactions might occur between sperm and tannins based on the common tannin-protein reaction. Tannic acid inhibited acrosomal enzymes and reduced sperm motility in rams (Taitzoglou et al., 2001). Seminal proteins influence sperm motility (Yoshida et al., 2008). These proteins could either decrease (La Falci et al., 2002) or increase sperm motility (Qu et al., 2007). Sperm in most mammalian species contain a high level of C22:6n-3 docosahexaenoic acid (DHA) (Lin et al., 1993). In this study, sperm DHA content decreased at 25% PBP inclusion. Conquer et al. (2000) concluded that using docosahexaenoic acid in the food increased DHA level in sperm and dietary fatty acids seem to be transferred to sperm. Rooke et al. (2001) showed that sperm fertilizing ability, yield, quality, and total motility were lower in ageing bulls which was accompanied by decreased DHA proportion in sperm phospholipids. Decreased DHA proportion in might result in decreased docosahexaenoic acid percentage in the seminal fluid (Ghaffari et al., 2014a). Unsaturated fatty acids impact on the sperm membrane fluidity (Aksoy et al., 2006) that affect sperm motility (Surai et al., 2000).

Results of this study indicated that DHA, arachidonic acid, oleic acid, palmitic acid, linoleic acid, stea-

Table 3. The effect of diets containing 12.5 (PBP-12.5) and 25 (PBP-25) percent (DM basis) pistachio by-products on sperm fatty acid composition in Farahani rams

Numeric Name	Common Name	Control	PBP-12.5	PBP-25	SEM	P-value
C12	Lauric acid	0.63	3.80	0.69	1.29	0.19
C14-0	Myristic acid	3.85	2.41	2.19	0.86	0.38
C16-0	Palmitic acid	13.05	10.94	13.92	1.49	0.39
C16-1	None	3.80	4.23	5.50	0.53	0.10
C17-0	Margaric acid	0.64	0.58	.64	0.27	0.60
C17-1	None	4.28[a]	4.13[a]	2.19[b]	0.71	0.04
C18-0	Stearic acid	5.44[a]	4.12[a]	7.68[b]	0.76	0.05
C18-1	Oleic acid	6.16[a]	7.86[a]	10.75[b]	0.64	0. 002
C18-2 n-6	Linoleic acid	5.01	5.09	6.48	0.43	0.09
C18-3-n-3	γ-Linolenic acid	2.29	1	1.48	0.16	0.15
C20-0	Arachidic acid	4.17	6.37	8.17	0.72	0.16
C20-1	Gadoleic acid	5.71	7.66	5	0.91	0.61
C22_0	None	3.23	3.94	3.46	1. 30	0.61
C20-4 -n-6	None	10.13	6.33	8.52	1.46	0.23
C22-6 n-3	DHA[1]	9.73[a]	7.76[a]	3.33[b]	1.02	0.05
Saturated	-	31.01[a]	32.16[a]	36.75[b]	1.32	0.05
Unsaturated	-	68.85[a]	63.07[ab]	57.76[b]	2.82	0.05
Un-/Saturated ratio	-	2.22[a]	1.96[a]	1.57[b]	0.31	0.05

[a,b] within rows, mean values with common superscript(s) are not different (P>0.05).

[1]Docosahexaenoic acid.

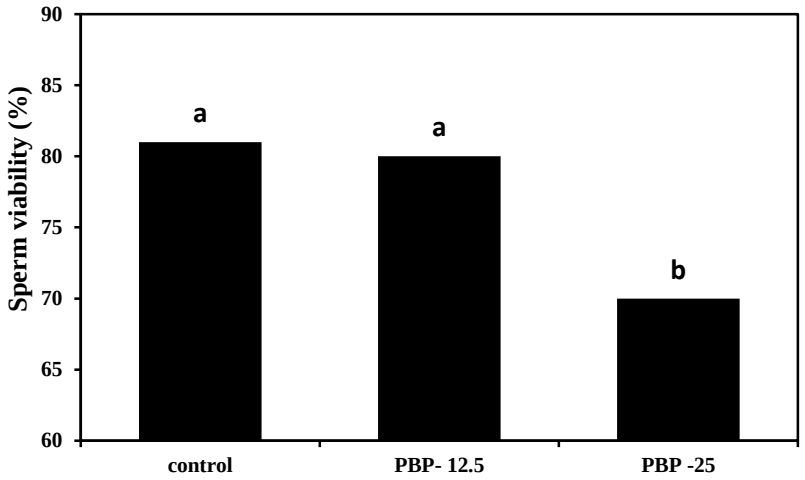

Figure 1. The effect of diets containing 12.5 (PBP-12.5) and 25 (PBP-25) percent (DM basis) pistachio by-products on the percentage of viable sperm in Farahani rams.
[a,b] Common letter(s) on bars indicate non-significance (P>0.05).

ric acid and linolenic acid are present in the ram sperm. In PBP-25 rams, stearic acid (C18-0) was significantly higher than in other groups. It has been shown that polyunsaturated fatty acids such as DHA are involved in the process of bending and flexing of sperm flagellum, and thereby in sperm motility (Qu et al., 2007). Am-In et al. (2011) found a negative correlation between sperm total lipids and saturated fatty acid content in abnormal boars. Blesbois et al. (1997) reported that the transfer of essential FAs from the diet to semen is effective and this transfer may have biochemical and biological effects on semen quality and fertilizing ability. Our results confirmed that the lipid composition of diets affected the fatty acids profile of ram sperm as also found in the trout sperm (Pustowka, 1998). Regarding the effect of PBP feeding on sperm characteristics, the present study showed that 12.5% PBP could be used as the dietary forage in the diet of rams without detrimental effects on sperm parameters. Field fertility trials are needed to confirm this conclusion.

Acknowledgements

The researchers gratefully acknowledge the financial and practical support provided by the Arak University. The authors thank the members of their own laboratories for their helpful discussions.

References

Aksoy, Y., Aksoy, H., Altınkaynak, K., Aydın, H.R., Özkan, A., 2006. Sperm fatty acid composition in subfertile men. *Prostaglandins Leukotrienes and Essential Fatty Acids* 75, 75-79.

Am-In, N., Kirkwood, R.N., Techakumphu, M., Tantasuparuk, W., 2011. Lipid profiles of sperm and seminal plasma from boars having normal or low sperm motility. *Theriogenology* 75, 897-903.

Ammerman, C., Henry, P., 1991. Citrus and vegetable products for ruminant animals. *Proceedings of the Alternative Feeds for Dairy and Beef Cattle*, St Louis, USA.

Blesbois, E., Lessire, M., Grasseau, I., Hallouis, J., Hermier, D., 1997. Effect of dietary fat on the fatty acid composition and fertilizing ability of fowl semen. *Biology of Reproduction* 56, 1216-1220.

Bucak, M.N., Sariozkan, S., Tuncer, P.B., Ulutas, P.A., Akcadag, H.I., 2009. Effect of antioxidants on microscopic semen parameters, lipid peroxidation, and antioxidant activities in Angora goat semen following cryopreservation. *Small Ruminant Research* 81, 90–95.

Conquer, J.A., Martin, J.B., Tummon, I., Watson, L., Tekpetey, F., 2000. Effect of DHA supplementation on DHA status and sperm motility in asthenozoospermic males. *Lipids* 35, 149-154.

Evans, G., Maxwell W.M.S., 1989. Salmon's Artificial Insemination of Sheep and Goats. University Press, Sydney, NSW, Australia.

Folch, J., Lees, M., Stanley, G.A., 1957. Simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry* 226, 497–509.

Ghaffari, M., Tahmasbi, A.M., Khorvash, M., Naserian, A.A., Ghaffari, A., Valizadeh, H., 2014a. Effects of pistachio by-products in replacement of alfalfa hay on populations of rumen bacteria involved in biohydrogenation and fermentative parameters in the rumen of sheep. *Journal of Animal Physiology and Animal Nutrition* 98, 578-586.

Ghaffari, M.H., Tahmasbi, A.M., Khorvash, M., Naserian, A.A., Vakili, A.R., 2014b. Effects of pistachio by-products in replacement of alfalfa hay on ruminal fermentation, blood metabolites, and milk fatty acid composition in Saanen dairy goats fed a diet containing fish oil. *Journal Applied Animal Research* 42, 186-193.

Horwitz, W., 2000. Official Methods of AOAC International. 17th Ed., Association of Official Analytical Chemists, Gaithersburg.

Labavitch, J.M., Heintz, C.M., Rae, H.L., Kader, A.A., 1982. Physiological and compositional changes associated with maturation of 'Kerman' pistachio nuts. *Journal of the American Society for Horticultural Science* 107, 688-692.

La Falci, V., Tortorella, H., Rodrigues, J., Brandelli, A., 2002. Seasonal variation of goat seminal plasma proteins. *Theriogenology* 57, 1035-1048.

Lin, D., Connor, W., Wolf, D., Neuringer, M., Hachey, D., 1993. Unique lipids of primate spermatozoa: desmosterol and docosahexaenoic acid. *Journal of Lipid Reserch* 34, 491-499.

Mokhtarpour, A., Naserian, A.A., Tahmasbi, A.M., Valizadeh, R., 2012. Effect of feeding pistachio by-products silage supplemented with polyethylene glycol and urea on Holstein dairy cows performance in early lactation. *Livestock Science* 148, 208-213.

Pustowka, C.V., 1999. Effects of source of Ddietary lipid on the fatty acid composition and cholesterol content of tissues, semen cryopreservation and embryo survival in Rainbow Trout (Oncorhynchus mykiss), National Library of Canada Bibliothèque nationale du Canada.

Qu, F., Ying, X., Guo, W., Guo, Q., Chen, G., Liu, Y., Ding, Z., 2007. The role of Zn-α2 glycoprotein in sperm motility is mediated by changes in cyclic AMP. *Reproduction* 134, 569-576.

Rooke, J., Shao, C., Speake, B., 2001. Effects of feeding tuna oil on the lipid composition of pig spermatozoa and in vitro characteristics of semen. *Reproduction* 121, 315-322.

Safarinejad, MR., 2011. Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic anti-oxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: a double-blind, placebo-controlled, randomised study. *Andrologia* 43, 38-47.

Shakeri, P., 2016. Pistachio by-product as an alternative forage source for male lambs: Effects on performance, blood metabolites, and urine characteristics. *Animal Feed Science and Technology* 211, 92-99.

Shakeri, P., Riasi, A., Alikhani, M., 2014. Effects of long period feeding pistachio by-product silage on chewing activity, nutrient digestibility and ruminal fermentation parameters of Holstein male calves. *Animal* 8, 1826-1831.

Schafer, S., Holzmann A., 2000. The use of transmigration and spermac stain to evaluate epididymal cat spermatozoa. *Animal Reproduction Science* 59, 201-211.

Sedighi-Vesagh, R., Naserian, A.A., Ghaffari, M.H., Petit, H.V., 2015. Effects of pistachio by-products on digestibility, milk production milk fatty acid profile and blood metabolites in Saanen dairy goats. *Journal of Animal Physiology and Animal Nutrition* 99, 777-787.

Surai, P.F., Noble, R.C., Sparks, N.H., Speake, B.K., 2000. Effect of long-term supplementation with arachidonic or docosahexaenoic acids on sperm production in the broiler chicken. *Journal of Reproduction and Fertility* 120, 257–64.

Taitzoglou, I., Tsantarliotou, M., Zervos, I., Kouretas, D., Kokolis, N., 2001. Inhibition of human and ovine acrosomal enzymes by tannic acid in vitro. *Reproduction* 121, 131-137.

Yoshida, M., Kawano, N., Yoshida, K., 2008. Control of sperm motility and fertility: diverse factors and common mechanisms. *Cellular and Molecular Life Sciences* 65, 3446-3457.

Communicating editor: *Mohammad Javad Zamiri*

فراسنجه‌های کیفی اسپرم و ترکیبات اسید چرب در قوچ‌های فراهانی تغذیه شده با پسماند پسته

م. خدائی مطلق[1*]، م. کاظمی بنچناری[1]، م. ژندی[2]، م. ح. مرادی[1] و ع. محمدی سنگ چشمه[3]

[1]گروه علوم دامی دانشکده کشاورزی و منابع طبیعی دانشگاه اراک، کدپستی ۳۸۱۵۶-۸۳۴۹، اراک، ایران.

[2]گروه علوم دامی پردیس کشاورزی و منابع طبیعی دانشگاه تهران، کرج، ایران.

[3]گروه علوم دامی پردیس ابوریحان، دانشگاه تهران، پاکدشت، ایران.

[*]نویسنده مسئول، پست الکترونیک: m-motlagh@araku.ac.ir

چکیده پانزده قوچ بارور (وزن زنده ۱/۲۱ ± ۵۵/۱ کیلوگرم و ۲/۵–۳ ساله) به صورت تصادفی به سه جیره غذایی شامل: ۰ (کنترل)، ۱۲/۵ (PBP-12.5) و ۲۵٪ (PBP-25.5) پسماند پسته (PBP) در ماده خشک تقسیم شدند. فراسنجه‌های حرکتی اسپرم، در حیوانات تغذیه شده با جیره غذایی PBP-25٪ تحت تأثیر قرار گرفتند ($P < 0.05$). غلظت اسیدهای چرب C17-1، C22-6-n-3، C18-2-Cis و C22-6 n-3 اسپرم کاهش یافت، اما اسیدهای چرب C18-0 و C18-1-Cis در جیره دارای بالاترین سطح پسماند پسته، افزایش یافت. نتایج نشان داد که ۱۲/۵٪ PBP در ماده خشک، به عنوان یک محصول جانبی ارزان، ممکن است در جیره قوچ برای دوره‌های طولانی بدون هیچ گونه تاثیر منفی شایانی بر ویژگی های اسپرم استفاده شود. با این وجود، آزمایش های باروری برای اثبات این نتیجه مورد نیاز است.