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Reproductive Performance of Barki Rams Fed on Different Omega-6: Omega-3 Ratios

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Abstract

The current experiment intended to investigate the impact of various dietary omega- 6/ omega -3 fatty acids ratios (FAs) on the reproductive performance and serum lipid profile in male Barki sheep over two months' experimental period. Twelve males were randomly allotted into 4 equal groups receiving 4 different ratios of omega-6/ omega- 3 FAs including 5.40 (higher ratio, HR), 4.56 (medium ratio, MR), 3.13 (lower ratio, LR) and 1.76 (very low ratio, VLR):1. Feeding rams on diets with HR or VLR did not exhibit substantial impact on the sperm motility, sperm viability and testosterone hormone, however these parameters were non-significantly improved in the MR and LR- fed rams. The semen volume was significantly increased ($P < 0.05$) with the MR in comparison with the remaining groups. Serum biochemical parameters, including total lipids, HDL concentrations did not exhibit significant differences ($P > 0.05$) among the different ratios. VLR- fed rams showed the higher serum total cholesterol, triglyceride, LDL and VLDL ($P < 0.05$), while the MR fed rams showed the highest CHO/HDL ratio ($P < 0.05$) in comparison with the other ratios. In summary, Barki rams fed different omega-6/ omega- 3 PUFA ratios (5.4, 4.56, 3.13 and 1.76:1) containing diets exhibited no significant difference in their reproductive performance parameters, however the medium (4.56:1) and low (3.13:1) ratios showed potential improving effect.

KEYWORDS
Barki rams, Omega-6/Omega-3 ratios, Testosterone hormone, Reproductive performance

INTRODUCTION

The improvement of the conception rate and overall yearly birth rate for each herd are largely dependent on the semen quality in order to increase farm economic profit (Tran *et al*., 2016). Male reproductive capability is determined by sperm production, and success of artificial insemination depends upon number and quality of sperms in the ejaculate (Berndtson, 2008). The quality of the gametes produced (oocytes and spermatozoa), ovulation, and fertilization, as well as the levels of hormones and metabolites necessary for reproduction, can all be impacted by a variety of aspects, including genetics, management, environment, and nutrition, which indirectly affect the mentioned aspects of the reproductive process (Wathes *et al*., 2007).

Polyunsaturated fatty acids (PUFAs) play a crucial role in regulating the fluidity of cell membranes, intracellular signaling, and vulnerability to oxidative damaging (Wathes *et al*. 2007; Santos *et al*., 2008; Gulliver *et al*., 2012). Growing evidence specifies that dietary PUFAs regulate reproductive processes through modifying the fatty acid profile and maintaining the sperm membrane fluidity and integrity, particularly in conditions of cryopreservation or cold shock (Hammerstedt *et al*., 1990; Moallem *et al*., 2015; Khoshvaght *et al*., 2016). PUFAs are very important for the production of prostaglandin, and could also modify the expression of a number of vital enzymes included in steroid and prostaglandin metabolism (Tran *et al*., 2017). PUFAs including eicosapentaenoic acid (EPA, 20:5n-3), alpha-linolenic acid (ALA, 18:3n-3), linoleic acid (LA, 18:2n-6), and docosahexaenoic acid (DHA, 22:5n-3) were found to be involved in the reproductive function and fertility (Thatcher and Staples, 2007). The omega-6 and omega-3 PUFAs represent around 30-50 percent of the total quantity of fatty acids found within the mammalian sperm membrane (Poulos *et al*., 1973). Maintaining the right balance between omega-3 and omega-6 PUFAs within animal diet is essential as this ratio contributes significantly in many attributes of animal production, health, and reproductive function (Abayasekara and Wathes 1999).

Unsaturated fatty acids (UFAs) exhibit comparatively short half-lives within the ruminal contents due to rapid microbial hydrogenation, which converts them into more saturated end products (biohydrogenation) (Tran *et al*., 2017). Several techniques aiming the protection of UFAs have been established such as lipid-protein matrix treated with formaldehyde (Ashes *et al*., 1979), water-insoluble lipid layer on the microencapsulation (Putnam *et al*., 2003), FA amides formation (Fotouhi and Jenkins 1992), and Ca salts FA formation (Jenkins and Palmquist, 1984). So far, insufficient information for studies about the impacts of various omega-6/ omega-3 PUFAs ratios on the reproductive performance in rams is available. Therefore, the current investigation was conducted to investigate the impacts of different ratios of

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omega-6 and omega-3 PUFA on the reproductive performance in male Barki sheep.

MATERIALS AND METHODS

The Animal Care and Ethics Committee of the Faculty of Veterinary Medicine, Alexandria University, Egypt approved the management procedures followed during the experiment (AU 013-2020/12/13-3-4-68).

Animals care and experimental design

Twelve Barki rams (77.63 kg average body weight) were randomly allotted into 4 groups (3 rams /group). Each group was housed in a separate open yard (5 m^2), with a sandy ground, provided with a suitable umbrella of iron sheet, one basin for fresh water, and one stilt for feeding. Rams received the experimental diets containing 4 omega-6/omega-3 fatty acids ratios including 5.40:1 (higher ratio), 4.56 :1 (medium ratio), 3.13:1 (lower ratio) and 1.76 :1 (very lower ratio). These ratios were provided in the diet by the dietary inclusion of a combination of three different sources of by-pass fat including palm fat (dry fat) (Bergafat T-300), produced by Berg + Schmidt Company and each 1 Kg contain palm fat (99.25%), antioxidant (BHT 0.25%) and moisture (0.5%) , salmate (produced by The Ballard Group, Inc. (contain approximately 45% fish oil with total n-3 FA about 35.53% and total n-6 FA about 3.55% and ebilac-fort (locally produced contain dry fat 84.85% (palm oil 37%, soybean oil 37% and linseed oil 10.85%), calcium oxide 15%, antioxidant 0.15% and moisture 4%) commercial products. Experimental diets used were isoenergetic and isonitrogenous, which were designed to fulfill the nutrient requirements of sheep following NRC (1985). Table 1 illustrates the ingredient composition and chemical analysis of the experimental diets used in the current study. Fatty acid concentrations of the used basal diets throughout the experimental period are presented in Table 2.

Diets were offered to animals two times per day at 7:00 a.m. and 4:00 pm., while drinking water was provided ad libitum. Animals were fed at a feeding rate of 3 % of animal body weight on as fed basis. Rams were vaccinated against clostridial disease (Ultrabac® 8 CD by Zoetis U.S). Each animal was injected with 2.5 ml subcutaneous, followed by a second dose after 4-6 weeks. Rams were treated with an anthelmintic drug for internal and external parasites prior to the experiment.

Semen collection and sperm quality assessment

Semen samples collection (three rams/ group) was acquired by electro-ejaculator four times through the experiment (at the start, after 2 weeks, after 1 month and at the end of breeding season). One ejaculate per ram was obtained during each collection and the ejaculates volume was directly measured using a calibrated collection tube. Accordingly, semen quality was then determined.

The semen quality was assessed through evaluation of the following parameters: mass motility (the overall motility of the spermatozoa), individual motility, viability of sperms (live and dead spermatozoa percentages).

Mass motility was estimated through light microscope according to Barth and Waldner (2002). For each semen sample, sperm motility was calculated in 3–7 distinct microscopic areas. The intangible estimates were roughly rounded to the closest 5%. The ultimate motility score was determined by averaging the subsequent calculations (Evans and Maxwell, 1987). The procedure for measuring individual motility was as follows: a drop from the diluted semen was dispersed onto a glass slide over a hot stage microscope, covered by a cover slide, and inspected under a high microscope. The percentage of a progressive active motile spermatozoa was estimated by counting 100 to 200 spermatozoa. The sperm viability percentage (live and dead), was determined using a one-step eosin nigrosin staining according to Björndahl *et al.* (2003).

Table 1. Ingredient composition and chemical analysis of the used basal diets.

Ingredient %	Diet 1	Diet 2	Diet 3	Diet 4
Berseem hay	22	22	22	22
Wheat straw	28	28	28	29
Yellow corn	18.75	18.75	18.75	18.15
SBOM (46%)	8	8	8	6.6
wheat bran	16	16	16	17
Salmate [a]	0	0	0.2	1
Dry fat (palm oil) [b]	1.5	0.9	0	0
Ebilac- fort [c]	0	0.6	1.3	0.5
Limestone [d]	1	1	1	1
Molasse	3	3	3	3
Sod. bicarbonate	0.5	0.5	0.5	0.5
Ammonium chloride	0.3	0.3	0.3	0.3
Mg oxide	0.2	0.2	0.2	0.2
Premix [e]	0.3	0.3	0.3	0.3
Common salt	0.25	0.25	0.25	0.25
Anti-mycotoxin [f]	0.1	0.1	0.1	0.1
Yeast [g]	0.1	0.1	0.1	0.1
Chemical analysis (% DM basis)				
Crude Protein	13.05	12.43	12.47	12.66
Ether Extract	2.84	2.76	2.68	2.58
Organic matter	89.5	88.87	88.62	88.95
NDF[h]	53.59	50.33	54.79	51.09
ADF [i]	24.52	22.49	25.49	24.53
ADL[j]	9.11	8.88	8.74	10.01
HC[k]	29.08	27.84	29.29	26.56
Cellulose	17.21	15.32	18.91	16.32
Calcium	0.79	0.74	0.77	0.89
Phosphorus	0.35	0.31	0.29	0.35

[a] SALMATE® produced by The Ballard Group, Inc. (contain approximately 45% fish oil with total n-3 FA about 35.53% and total n-6 FA about 3.55%). [b] Palm fat (Bergafat T-300®), produced by Berg + Schmidt Company and each 1 Kg contain palm fat (99.25%), antioxidant (BHT 0.25%) and moisture (0.5%) .[c] Ebilac-fort® locally produced contain dry fat 84.85% (palm oil 37%, soybean oil 37% and linseed oil 10.85%), calcium oxide 15%, antioxidant (6443) 0.15% and moisture 4%. [d] Limestone contains 37% calcium & locally produced. [e] Each 3 kg contains: Vit A (10000000IU), Vit D (2000000IU), Vit E(10g), Iron (50g), Copper (8g), Zinc (30g), Manganese (40g), Iodine (0.5g), Selenium (0.1g), Cobalt (0.1g) and carrier calcium carbonate up to 3 kg. [f] Fer Mos®, each 1 kg contain Saccharomyces cerevisiae cell wall 500gm, Beta glucan112.5 gm, Mannan 112.5 gm and carrier silicate up to 1kg and produced by Micron Bio-Systems Company. [g] Beta Mos®, each 1 kg contain dried extract of Saccharomyces cerevisiae 1000 gm, Beta-glucan 250 gm, mannan oligosaccharide (MOS) 200 gm and produced by New Gene International Trading Company. [h] Neutral Detergent Fiber. [i] Acid Detergent Fiber. [j] Acid Detergent Lignin. k Hemo cellulose.

Estimation of some blood serum metabolites

Blood samples (three rams / group) were collected at the start of experiment, 4 weeks later, and at the end for blood biochemistry and serum testosterone. Blood samples were subjected to centrifuging (1006 g) for 10 minutes, then serum was preserved in the freezer (-20 °C) for analysis. Serum total lipids, total cholesterol, high density lipoprotein (HDL-cholesterol), low-density

lipoprotein (LDL-cholesterol) and triglyceride were estimated utilizing commercial kits obtained from Bio-diagnostic Co. (Diagnostic and Research reagents). VLDL-cholesterol = total cholesterol – (HDL-cholesterol + LDL-cholesterol).

Serum hormonal assay

Serum concentration of testosterone hormone was estimated utilizing enzyme linked immunosorbant assay commercial kits (Rat T (Testosterone) ELISA Kit, Fine Test®, Wuhan Fine Biotech Co., Ltd., China) The testosterone assay detection lower limit was 0.188ng/ml. The inter- and intra-assay coefficients were CV<10% and CV<8% respectively. All commercial assays were accomplished following the guidelines of the manufacturer.

Statistical analysis

Analysis of the obtained data was done using one-way ANOVA to test the impact of various n-6/n-3 ratios, followed by Tukey's multiple comparison test. At P < 0.05 significance level was considered to delineate the statistical differences.

RESULTS

Reproductive performance

The reproductive performance of Barki rams fed various omega-6/omega-3 ratios is shown in Tables 3 and 4. Motility % of Barki rams fed various omega-6/omega-3 ratios is illustrated in Table 3. Mass motility %, no significant (p>0.05) effect was observed between different ratios. Barki rams fed HR decreased the mass motility % after 2 weeks, 4 weeks and at end of breeding season (6 weeks), while MR decreased the mass motility % after 4 weeks and 6 weeks. Rams fed LR and VLR increased the mass motility % after 2 weeks, 4 weeks and at end of breeding season. Individual motility % of Barki rams fed various omega-6/omega-3 ratios is presented in Table 3. Individual motility %, no significant (p>0.05) effect was observed between different ratios. HR- fed rams increased the individual motility % 4 weeks and at end of breeding season. MR- fed rams had no effect on the individual motility % from start to at end of breeding season while, LR and VLR increased the individual motility % after 4 weeks of breeding season. Feeding of rams on different omega-6/omega-3 ratios containing diets did not exhibit considerable impact on the average sperm motility (either mass or individual) (P > 0.05), however these parameters were insignificantly enhanced in the MR and LR- fed rams. Semen volume (ml) of Barki rams fed various omega-6/omega-3 ratios is illustrated in Table 3. The semen volume responded differently as it revealed significant increase (P < 0.05) in the MR relative to the other groups. The higher significant (P≤0.05) sperm volume was observed in MR compared to VLR - fed rams at the start, after 2 weeks. Furthermore, higher significant sperm volume was found in Barki rams fed MR and VLR compared to HR and LR after 4 weeks of breeding season. For sperm volume at end of breeding season, no significant (P>0.05) effect was observed between ratios.

Sperm viability % of Barki rams fed various omega-6/omega-3 ratios is shown in Table 4. Live and dead sperm %, no significant (p>0.05) effects were observed between different ratios. Feeding of rams on different omega-6/omega-3 ratios containing diets did not exhibit considerable impact on the average sperm viability (dead or live sperms) (P > 0.05), however these parameters were insignificantly enhanced in the MR and LR- fed rams.

The testosterone hormone of Barki rams fed various ome-

Table 2. Fatty acids concentrations of the used basal diets during the experimental period.

Ingredient %	Dietary omega 6: omega 3 ratios			
	HR [a]	MR [b]	LR [c]	VLR [d]
Caprylic acid C8:0	0.36	4.3	1.36	1.17
Capric acid C10:0	1.28	1.07	3.72	3.76
Lauric acid C12:0	5.44	6.32	4.33	2.67
Tridecanoic acid C13:0	0.04	0.14	0.31	0.11
Myristic acid C14:0	7.73	11.76	8.45	8.7
Pentadecylic acid C15:0	0.48	0.51	0.63	0.8
Palmitic acid C16:0	30.45	25.57	26.18	26.15
Heptadecanoic acid C17:0	0.63	0.29	0.86	0.75
Stearic acid C18:0	9.34	5.34	12.17	6.28
Arachidic acid C20:0	0.46	0.27	0.26	0.14
Docosanoic acid C22:0	0.13	0.49	0.45	0.48
Myristoleic acid C14:1n9c	0.19	0.17	0.34	0.47
Palmitoleic acid C16:1n9c	0.23	0.78	1.33	1.69
Elaidic acid (C18:1 trans-n-9) C18:1n9t	0.55	0.53	0.44	0.39
Oleic acid (C18:1 cis-n-9) C18:1n9c	29.56	35.3	34.11	34.28
Eicosanoic acid C20:1	0.44	0.66	0.99	1.25
Linoleic acid C18:2n6c	5.22	7.1	7.6	7.3
Gamma- linolenic acid C18:3n6	4.18	3.2	3.6	2.2
α-Linolenic acid C18:3n3	1.73	2.3	3.6	5.7
Total PUFA	11.13	12.6	14.8	15.2
total n-6	9.4	10.3	11.2	9.5
total n-3	1.73	2.3	3.6	5.7
n-6/n-3 ratio	5.4:1	4.5:1	3.13:1	1.67:1

[a] High n- 6/ n- 3 ratio (5.4:1). [b] Medium n- 6/ n- 3 ratio (4.56:1). [c] Low n- 6/ n- 3 ratio (3.13:1). [d] Very low n- 6/ n- 3 ratio (1.67:1).

ga-6/omega-3 ratios is shown in Table 5. The testosterone hormone concentration was non-significantly increased (P > 0.05) with rams fed on HR and VLR at the commencement of experiment, after 2 weeks and at completion of experiment, while rams fed on LR non-significantly increased (P > 0.05) testosterone level after 4 weeks. The average testosterone hormone levels were non-significantly increased (P > 0.05) with lowering the omega-6/omega-3 ratios in diet.

Serum lipid profile

Serum lipid profile in rams in response to different omega-6/ omega-3 ratios is displayed in Table 6. Serum total lipid and HDL levels were non-significantly different among the different ratios, however revealed an insignificant increase (P> 0.05) with lowering the ratio in diet. Additionally, lowering n-6/n-3 ratio in diet led to a significant increase (P< 0.05) in serum total cholesterol and triglyceride with the highest concentrations in rams fed on VLR. Moreover, feeding on VLR triggered significant elevation (P< 0.05) in the highest concentration on rams fed on LDL and VLDL meanwhile MR fed rams showed the highest CHO/HDL ratio (P< 0.05) compared to the other ratios. Serum NEFA showed no difference among the different experimental ratios (P> 0.05).

DISCUSSION

As far as we know, very few studies have been carried out to study the impacts of the omega-6/ omega-3 PUFA ratio on male reproductive function as most of the studies were concerned with the different sources of omega-6 and omega-3 added in diet.

In the current study, the reproductive performance param-

Table 3. Motility % and semen volume (ml) of Barki rams fed various dietary n-6/n-3 ratios during breeding season.

Items	Dietary omega- 6/ omega- 3 ratios				*P- value*
	HR [a]	MR [b]	LR [c]	VLR [d]	
Mass motility %:					
At the start	90.00± 5.77	90.00± 8.66	70.00± 5.77	80.00± 7.51	0.22
2 weeks	85.00± 6.92	90.00±7.22	80.00± 7.79	80.00± 8.37	0.76
4 weeks	80.00± 6.64	85.00± 8.66	88.70± 8.42	95.67± 7.80	0.58
6 weeks	80.00± 4.62	85.00± 7.51	87.67± 11.61	90.00± 6.35	0.83
Average Mass motility	83.75± 2.39	87.50± 1.44	81.59± 4.32	86.42± 3.88	0.58
Individual motility %:					
At the start	70.00± 7.21	80.00± 9.24	80.00± 8.08	70.00± 5.77	0.65
2 weeks	80.00± 12.12	80.00±8.66	71.67± 6.01	80.00± 7.51	0.88
4 weeks	70.00± 6.92	83.33± 5.45	85.00± 8.66	90.00± 9.23	0.36
6 weeks	80.00± 9.81	80.00± 8.66	85.00± 6.93	70.00± 7.51	0.65
Average individual motility	75.00± 2.88	80.83± 0.83	80.42± 3.14	77.50± 4.78	0.56
Semen volume (ml)					
At the start	0.70± 0.23[ab]	1.50± 0.28[a]	0.70± 0.12[ab]	0.50± 0.11[b]	0.03
2 weeks	1.00± 0.23[ab]	1.50±0.23[a]	0.70± 0.17[ab]	0.50± 0.17[b]	0.04
4 weeks	1.00± 0.23[ab]	3.00± 0.34[a]	0.50± 0.12[b]	2.00± 0.29[a]	0.00
6 weeks	1.00± 0.17	0.70± 0.17	0.70± 0.23	1.00± 0.24	0.57
Average semen volume	0.93± 0.10 [b]	1.67± 0.27[a]	0.65± 0.07 [b]	1.00± 0.21 [ab]	0.00

Values are means ± SE. Means designated with various letters at the same row vary significantly at P< 0.05. [a] High n- 6/ n- 3 ratio (5.4:1). [b] Medium n- 6/ n- 3 ratio (4.56:1). [c] Low n- 6/ n- 3 ratio (3.13:1). [d] Very low n- 6/ n- 3 ratio (1.67:1).

Table 4. Sperm viability% of Barki rams fed various dietary n-6/n-3 ratios during breeding season.

Items	Dietary omega- 6/ omega- 3 ratios				*P- value*
	HR [a]	MR [b]	LR [c]	VLR [d]	
Live sperm %:					
At the start	83.00± 6.93	86.00± 7.51	85.00± 5.51	83.00± 4.04	0.98
2 weeks	80.00± 3.46	85.00±2.88	86.00± 3.46	75.00± 2.89	0.13
4 weeks	86.00± 2.30	86.17± 2.02	91.17± 2.31	94.00± 2.31	0.09
6 weeks	83.00± 2.89	87.33± 1.45	91.00± 1.73	78.00± 5.19	0.09
Average live sperm %	83.00± 1.93	86.12± 1.81	88.29± 1.74	82.50± 2.71	0.18
Dead sperm %:					
At the start	17.00± 6.93	14.00± 7.51	15.00± 5.51	17.00± 4.04	0.98
2 weeks	20.00± 3.46	15.00±2.88	14.00± 3.46	25.00± 2.89	0.13
4 weeks	14.00± 2.31	13.83± 2.03	8.83± 2.31	6.00± 2.30	0.09
6 weeks	17.00± 2.88	12.67± 1.45	9.00± 1.73	22.00± 5.19	0.09
Average dead sperm %	17.00± 1.94	13.88± 1.81	11.71± 1.74	17.50± 2.71	0.18

Values are means ± SE. Means designated with various letters at the same row vary significantly at P< 0.05. [a] High n- 6/ n- 3 ratio (5.4:1). [b] Medium n- 6/ n- 3 ratio (4.56:1). [c] Low n- 6/ n- 3 ratio (3.13:1). [d] Very low n- 6/ n- 3 ratio (1.67:1).

eters evaluated including sperm motility and viability showed non-significant difference with the different dietary omega-6/ omega-3 ratios. However, they were non-significantly increased with the MR and LR (diets rich in omega-3 than omega-6). The obtained results are consistent with Masoudi and Dadashpour Davachi (2021) who found that supplementation of fish oil (FO) as a source for n-3 enhanced the sperm total and progressive motility, viability, and membrane integrity of rams (P≤ 0.05). On the same context, Esmaeili *et al.* (2012) observed increased total sperm motility and live sperms in rams raised on FO containing diet than those fed on soybean oil or saturated fatty acids containing diets. Also, Alizadeh *et al.* (2014) reported that throughout the feeding period, FO had a considerable positive impact on the sperm quality and quantity (P< 0.05). Shah *et al.* (2016) found higher sperm motility in buffalo bulls received supplemental linseed oil supplementation (n-3 PUFA rich source) in their diet and assumed that flaxseed oil PUFAs are participating in sperm flagellar movement, which consequently raises the proportion of motility. The increased sperm individual motility on rams fed on MR and LR (increased n-3 more than n-6 FAs) could be related with the sperm membrane's fatty acid content, especially the tail as pointed out by Mourvaki *et al.* (2010) who reported that flaxseed seed supplementation increased PUFAs in rabbit sperm with the major portion of PUFA n-3 found in the tail followed by the acrosome. Alternatively, Fair *et al.* (2014) found that rams' plasma and sperm content was successfully enhanced by dietary supplementation omega-3 PUFA, however the quality of their liquid stored semen was only slightly affected. Further research addressing the PUFAs composition in sperms with various omega-6/omega-3 ratios is required.

Additionally, the various ratios affected the semen volume as the higher semen volume was obtained with the MR. These results in line with Esmaeili *et al.* (2012) and Masoudi and Dadashpour Davachi (2021) who found enhanced semen volume with n-3 FA supplementation provided by FO in rams. Likewise, Shah *et al.* (2016) in Nilli-Ravi buffalo bulls with linseed oil supplementation (n-3 PUFA rich source). The previously mentioned reports documented the increased semen volume with n-3 PUFA rich sources, although this was not clear in the current study with different omega-6/ omega-3 ratios with increasing the n-3 in diet. The highest volume was recognized in rams fed diet with the MR, then unclear trend of decrease and increase with lowered n-6/n-3 ratio was obtained.

Testosterone is a steroid hormone produced from cholesterol and secreted by Leydig cells in the testes that is crucial for the establishment and conservation of spermatogenesis and other male traits (Tran *et al.*, 2016). According to studies (Wang *et al.*, 2000; Hughes *et al.*, 2011), UFAs can improve steroidogenesis in vivo by upregulating steroidogenic acute regulatory (StAR) protein expression and changing the way that transcription factors work (Wathes *et al.*, 2007). In the current investigation, lowering the omega-6/omega-3 ratio was accompanied with numerical increase in testosterone level. These results agree with Esmaeili *et al.* (2012) who found higher blood testosterone level in FO fed rams relative to those fed palm oil and sunflower oil during 35 days experimental period. Also, Tran *et al.* (2016) found higher testosterone levels in bulls supplemented with calcium salts from linseed (CaLFA) than those fed calcium salt from soybean or prilled saturated fat. The obtained result could suggest that higher n-3 PUFA in diet probably impacted the testicular plasma membranes' phospholipid composition, changed the expression and affinities of gonadotropin receptors, and impacted the testosterone production rate (Tran *et al.*, 2016).

Since most of the studies were concerned with the omega-6 and omega-3 PUFAs and their inclusion sources in diet, limited information's are available regarding the ideal ratio for male ruminant reproduction. In the same regard, Samadian *et al.* (2010) and Jafaroghli *et al.* (2014) found that ram sperm quality improved when the omega-6/omega-3 PUFA ratio was 0.96, while was 2.01 (Moallem *et al.*, 2015) and 2.39 (Khoshvaght *et al.*, 2016) in bulls. In the present study, feeding rams on diets containing medium and low ratios, (4.56 and 3.13, respectively) would help in improving their reproductive performance compared with the high ratio (5.40) and the very low ratio (1.76). In the same context, Moallem *et al.* (2015) documented that the ratio of 2.01 had a stronger impact on quality of the sperm than the ratio of 3.96. Am-In *et al.* (2011) specified that omega-6/omega-3 PUFA ratios were inversely accompanied with swine sperm viability, motility, normal morphology, and normal plasma membranes. Investigating the effects of PUFAs on semen quality and quantity in different animals showed differences in results as some reports showed positive effects while the others failed to show signifi-

Table 5. Impact of different dietary omega- 6/ omega- 3 ratios on testosterone levels (ng/ ml) during breeding season of Barki rams.

	Dietary omega- 6/ omega- 3 ratios				*P- value*
	HR [a]	MR [b]	LR [c]	VLR [d]	
At the start	2.10± 0.46	1.90± 0.28	1.90± 0.25	2.19± 0.14	0.88
After 2 weeks	1.88± 0.28	1.51± 0.30	1.80± 0.29	1.90± 0.17	0.73
After 4 weeks	2.50± 0.23	2.13± 0.20	2.92± 0.34	2.41± 0.28	0.31
After 6 weeks	3.17± 0.29	2.90± 0.23	3.26± 0.34	3.62± 0.40	0.51
Average hormone level	2.41± 0.28	2.11± 0.29	2.47± 0.36	2.53± 0.37	0.81

[a] High n- 6/ n- 3 ratio (5.4:1). [b] Medium n- 6/ n- 3 ratio (4.56:1). [c] Low n- 6/ n- 3 ratio (3.13:1). [d] Very low n- 6/ n- 3 ratio (1.67:1).

Table 6. Impact of different dietary n-6/n-3 ratios on serum lipid profile of male Barki rams.

Items	Dietary omega 6: omega 3 ratios				*P- value*
	HR [a]	MR [b]	LR [c]	VLR [d]	
Total lipids (mg/dl)	968.9± 4.05	998.2± 2.47	991.2± 8.17	1020± 20.97	0.08
Total Cholesterol (mg/dl)	116.00± 9.57 [c]	181.40± 7.83 [b]	192.30± 10.28 [b]	253.1± 9.98 [a]	<0.001
Triglycerides (mg/dl)	156.70±10.98[b]	135.20±10.94[b]	179.00± 11.38[ab]	210.90±12.13[a]	0.01
HDL[e] (mg/dl)	36.47± 5.32	42.71± 4.67	47.39± 5.21	55.67± 5.41	0.14
LDL[f] (mg/dl)	48.16± 2.06[c]	111.70± 1.18[b]	109.10± 2.79[b]	155.30±2.15[a]	<0.001
CHO/HDL ratio	3.49±0.17[b]	7.25±0.77[a]	3.31±0.18[b]	5.23±0.33[b]	0.00
VLDL[g] (mg/dl)	31.34± 2.19[b]	27.04± 1.18[b]	35.80± 2.27[ab]	42.17± 2.43[a]	0.01
NEFA[h] (mg/dl)	2.47± 0.04	2.19± 0.17	2.41± 0.23	2.27± 0.09	0.58

Values are means ± SE. Means designated with various letters at the same row vary significantly at P< 0.05. [a] High n- 6/ n- 3 ratio (5.4:1). [b] Medium n- 6/ n- 3 ratio (4.56:1). [c] Low n- 6/ n- 3 ratio (3.13:1). [d] Very low n- 6/ n- 3 ratio (1.67:1). [e] High-density Lipoprotein. [f] Low Density Lipoprotein. [g] Cholesterol/ High Density Lipoprotein. [h] Very Low-density Lipoprotein.[9] Non-esterified fatty acids.

cant effects. The differences in species, breed, source, quantity, or duration of PUFA feeding, male fertility rate, animal population, age, sperm chemistry, environmental factors, supplement composition, and other dietary components may all affect the results of different trials.

Regarding the serum biochemical parameters, various omega-6/ omega-3 ratios in rams' diet did not reveal substantial impact on serum metabolites including total lipids, HDL and NEFA. Partially in line, Fair *et al.* (2014) documented that in terms of a control diet containing 2.5% rumen protected and saturated fat, feeding rams fish oil with a rumen protection level of 2.5% did not affect plasma NEFAs, triglycerides, or cholesterol content. Other lipid profile parameters such as total cholesterol, triglyceride, LDL and VLDL were differentially increased with lowering the omega-6/ omega-3 ratio as the highest concentrations were recorded in the VRL-fed rams. Unlike the obtained results, Adibmoradi *et al.* (2012) found that growing male kids that received soybean oil or FO did not have different plasma triglycerides, total cholesterol, HDL and LDL, and cholesterol levels from those of the control group.

Feeding of the rams on the VLR was accompanied with higher cholesterol and TG concentrations. These findings agree with Gonthier *et al.* (2005) who detected raised plasma cholesterol in dairy cows fed on flaxseed as source of omega-3. Petit *et al.* (2004) recognized that dairy cows fed flaxseed, sunflower seeds, or Megalac (a rumen-protected fat supplement rich in saturated FAs) had higher plasma cholesterol levels. Seminal lipids, in particular phospholipids and cholesterol, which are crucial for the metabolism, structure, sperm capacitation, and female gametes fertilization, are particular vital to the structure and function of the spermatozoa plasma membrane (Cross, 1998). Because of its interactions with fatty acid chains, cholesterol affects the mechanical and thermodynamic characteristics of the lipid bilayer, stabilizing membranes (Mocé *et al.*, 2010). In the present result, lowering the omega-6/ omega-3 PUFA ratio was accompanied with elevated total cholesterol which would explain the associated increase of testosterone level. These result are inconsistent with Esmaeili *et al.* (2012) who reported that dietary UFA linearly decreased blood cholesterol despite the increase in testosterone level.

CONCLUSION

From the results indicated above, it could be summarized that the tested dietary omega-6/ omega-3 ratios (5.4:1 (high); 4.65 (medium):1; 3.13 (low) and 1.67:1(very low)) altered the serum lipid profile and semen volume while, did not reveal significant impact on semen quality (sperm motility and viability), testosterone hormone. Moreover, the obtained data concluded that feeding Barki rams on medium or low n-6/n-3 ratios containing rations improve reproductive performance. Further research is required addressing the effects of this ram's semen on the ewes' reproductive performance.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

Abayasekara, D.R.E.,Wathes, D.C., 1999. Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. Prostaglandins Leukot Essent Fat Acids 61, 275-287.

Adibmoradi, M., Najafi, M., Zeinoaldini, S., Ganjkhanlou, M.,Yousefi, A., 2012. Effect of dietary soybean oil and fish oil supplementation on blood metabolites and testis development of male growing kids. Egypt J. Sheep Goats Sci. 7, 1-8.

Alizadeh, A., Esmaeili, V., Shahverdi, A.,Rashidi, L., 2014. Dietary fish oil can change sperm parameters and fatty acid profiles of ram sperm during oil consumption period and after removal of oil source. Cell J. 16, 289.

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

Ashes, J., Gulati, S., Cook, L., Scott, T., Donnelly, J., 1979. Assessing the biological effectiveness of protected lipid supplements for ruminants. Am. Oil Chem. Soc. 56, 522-527.

Barth, A.D.,Waldner, C.L., 2002. Factors affecting breeding soundness classification of beef bulls examined at the Western College of Veterinary Medicine. Can. Vet. 43, 274.

Berndtson, W.E., 2008. Comparative reliability and sensitivity of different methods for assessing treatment effects on sperm production. Anim. Reprod. Sci. 105, 5-22.

Björndahl, L., Söderlund, I.,Kvist, U., 2003. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum. Reprod. 18, 813-816.

Cross, N.L., 1998. Role of Cholesterol in Sperm Capacitation1. Biol. Reprod. 59, 7-11.

Esmaeili, V., Shahverdi, A., Alizadeh, A., Alipour, H.,Chehrazi, M., 2012. 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.

Evans, G.,Maxwell, W.C., 1987. Handling and examinations of semen. Salamon's Artificial Insemination of Sheep and Goats, 93-106.

Fair, S., Doyle, D., Diskin, M., Hennessy, A.,Kenny, D.A., 2014. The effect of dietary n-3 polyunsaturated fatty acids supplementation of rams on semen quality and subsequent quality of liquid stored semen. Theriogenology 81, 210-219.

Fotouhi, N., Jenkins, T., 1992. Resistance of fatty acyl amides to degradation and hydrogenation by ruminal microorganisms. Dairy Sci. 75, 1527-1532.

Gonthier, C., Mustafa, A.F., Ouellet, D.R., Chouinard, P.Y., Berthiaume, R.,Petit, H.V., 2005. Feeding Micronized and Extruded Flaxseed to Dairy Cows: Effects on Blood Parameters and Milk Fatty Acid Composition. Dairy Sci. 88, 748-756.

Gulliver, C., Friend, M., King, B.,Clayton, E., 2012. The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. Anim. Reprod. Sci. 131, 9-22.

Hammerstedt, R.H., Graham, J.K., Nolan, J.P., 1990. Cryopreservation of mammalian sperm: what we ask them to survive. Andrology 11, 73-88.

Hughes, J., Kwong, W.Y., Li, D., Salter, A.M., Lea, R.G., Sinclair, K.D., 2011. Effects of omega-3 and -6 polyunsaturated fatty acids on ovine follicular cell steroidogenesis, embryo development and molecular markers of fatty acid metabolism. Reproduction 141, 105-118.

Jafaroghli, M., Abdi-Benemar, H., Zamiri, M., Khalili, B., Farshad, A., Shadparvar, A., 2014. Effects of dietary n− 3 fatty acids and vitamin C on semen characteristics, lipid composition of sperm and blood metabolites in fat-tailed Moghani rams. Anim. Reprod. Sci. 147, 17-24.

Jenkins, T., Palmquist, D., 1984. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. Dairy Sci. 67, 978-986.

Khoshvaght, A., Towhidi, A., Zare-Shahneh, A., Noruozi, M., Zhandi, M., Davachi, N.D.,Karimi, R., 2016. Dietary n-3 PUFAs improve fresh and post-thaw semen quality in Holstein bulls via alteration of sperm fatty acid composition. Theriogenology 85, 807-812.

Masoudi, R.,Dadashpour Davachi, N., 2021. Effect of Dietary Fish Oil on Semen Quality and Reproductive Performance of Iranian Zandi Rams. Arch. Razi. Inst. 76, 621-629.

Moallem, U., Neta, N., Zeron, Y., Zachut, M.,Roth, Z., 2015. Dietary α-linolenic acid from flaxseed oil or eicosapentaenoic and docosahexaenoic acids from fish oil differentially alter fatty acid composition and characteristics of fresh and frozen-thawed bull semen. Theriogenology 83, 1110-1120.

Mocé, E., Purdy, P.H.,Graham, J.K., 2010. Treating ram sperm with cholesterol-loaded cyclodextrins improves cryosurvival. Anim. Reprod. Sci. 118, 236-247.

Mourvaki, E., Cardinali, R., Dal Bosco, A., Corazzi, L., Castellini, C., 2010. Effects of flaxseed dietary supplementation on sperm quality and on lipid composition of sperm subfractions and prostatic granules in rabbit. Theriogenology 73, 629-637.

NRC, 1985. Nutrient requirements of sheep, National Academies Press.

Petit, H.V., Germiquet, C., Lebel, D., 2004. Effect of Feeding Whole, Unprocessed Sunflower Seeds and Flaxseed on Milk Production, Milk

Composition, and Prostaglandin Secretion in Dairy Cows. Dairy Sci. 87, 3889-3898.

Poulos, A., Darin-Bennett, A.,White, I., 1973. The phospholipid-bound fatty acids and aldehydes of mammalian spermatozoa. Comp. biochem. physiol. B. 46, 541-549.

Putnam, D., Garrett, J., Kung, L., 2003. Evaluation key to use of rumen-stable encapsulates. Feedstuffs 75, 10-12.

Samadian, F., Towhidi, A., Rezayazdi, K.,Bahreini, M., 2010. Effects of dietary n-3 fatty acids on characteristics and lipid composition of ovine sperm. Anim. Sci.Technol. 4, 2017-2022.

Santos, J., Bilby, T., Thatcher, W., Staples, C., Silvestre, F., 2008. Long chain fatty acids of diet as factors influencing reproduction in cattle. Reprod. Domest. Anim. 43, 23-30.

Shah, S.M.H., Ali, S., Zubair, M., Jamil, H., Ahmad, N., 2016. Effect of supplementation of feed with Flaxseed (*Linum usitatisimum*) oil on libido and semen quality of Nilli-Ravi buffalo bulls. Anim. Sci. Technol. 58, 25.

Thatcher, W.W., Staples, R., 2007. Using fats and fatty acids to enhance reproductive performance. Proceedings of the 5th Mid-Atlantic Nutrition Conference. Timonium, MD: University of Maryland, Citeseer.

Tran, L.V., Malla, B.A., Kumar, S.,Tyagi, A.K.J., 2017. Polyunsaturated fatty acids in male ruminant reproduction-a review. Asian Australas J. Anim. Sci. 30, 622-637.

Tran, L.V., Malla, B.A., Sharma, A.N., Kumar, S., Tyagi, N.,Tyagi, A.K., 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.

Wang, X., Walsh, L.P., Reinhart, A.J.,Stocco, D.M., 2000. The role of arachidonic acid in steroidogenesis and steroidogenic acute regulatory (StAR) gene and protein expression. J. Biol. Chem. 275, 20204-20209.

Wathes, D.C., Abayasekara, D.R.E., Aitken, R.J., 2007. Polyunsaturated fatty acids in male and female reproduction. Biol. Reprod. 77, 190-201.