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Original Article

Effect of Dietary Fish Oil on Semen Quality and Reproductive Performance of Iranian Zandi Rams

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Abstract

The current study was conducted to evaluate the effect of dietary fish oil on the semen quality and fertility potential of Zandi rams. For this purpose, a total of 15 Iranian Zandi rams were randomly assigned into three equal groups. The first group was a negative control and were fed without oil supplement. The second group was a positive control, and their diet contained palm oil, and the last group had a diet containing fish oil. All the diets were isocaloric and isonitrogenous. The rams were fed for 70 days, and the semen smaples were collected every 10 days. In experiment I, the evaluated parameters included semen volume, sperm concentration, motility, membrane integrity, and viability. In experiment II, 210 Iranian Zandi rams received CIDR for 12 days and 400 IU of equine chorionic gonadotropin at the time of CIDR removal. Then, they were assigned into three equal groups and artificially inseminated with semen samples. According to the obtained results, the supplementation of ram diet with fish oil as a source of omega-3 fatty acids improved the semen volume, sperm concentration, total motility, progressive motility, viability, membrane integrity, pregnancy rate, parturition rate, and lambing rate of the rams ($P \leq 0.05$). In conclusion, fish oil as a dietary supplement for rams could be an effective strategy to improve the semen quality of rams for artificial insemination and other goals.

Keywords: Artificial insemination, Fish oil, Sperm quality, Zandi rams

Effet de l'huile de Poisson Diététique sur la Qualité du Sperme et les Performances de Reproduction des Béliers Iraniens de Zandi

Résumé: Cette étude avait pour but d'évaluer l'effet de l'huile de poisson diététique sur la qualité du sperme et le potentiel de fertilité des béliers Zandi. Dans ce but, un total de 15 béliers iraniens Zandi ont été répartis au hasard en trois groupes égaux. Le premier groupe a été nourris sans supplément d'huile et représentait le témoin négatif. Le deuxième groupe (témoin positif) a reçu un régime alimentaire contenaant de l'huile de palme, alors que le régime du dernier groupe contenait de l'huile de poisson. Tous les régimes étaient isocaloriques et isonitrogènes. Les béliers ont été nourris pendant 8 semaines et les échantillons de sperme ont été prélevés une fois par semaine. Dans l'expérience I, les paramètres évalués comprenaient le volume de sperme, la concentration de sperme, la motilité, l'intégrité de la membrane et la viabilité. Dans l'expérience II, 210 béliers iraniens de Zandi ont reçu du CIDR pendant 12 jours et 400 UI de gonadotrophine chorionique équine au moment de l'élimination du CIDR. Ensuite, ils ont été répartis en trois groupes égaux et inséminés artificiellement avec des échantillons de sperme. Selon les résultats obtenus, la supplémentation du régime des béliers avec de l'huile de poisson comme source d'acides gras oméga-3 a amélioré le volume de sperme, la concentration de spermatozoïdes, la motilité totale, la motilité progressive, la viabilité, l'intégrité de la membrane, le taux de gestation ainsi queles taux de parturition et d'agnelage des béliers ($P \leq 0,05$). En

conclusion, l'utilisation de l'huile de poisson comme complément alimentaire pour les béliers, pourrait être une stratégie efficace pour améliorer la qualité du sperme utilisé pour l'insémination artificielle ainsi que pour d'autres objectifs.
Mots-clés: Insémination artificielle, huile de poisson, qualité du sperme, béliers Zandi

1. Introduction

Sperm membrane polyunsaturated fatty acids (PUFAs) play an important role in energy metabolism (1), fluidity of the plasma membrane (2), and some functions, which are related to fertilization (3). Docosahexaenoic acid (DHA) and eicosapentaenoic (EPA) are the main omega-3 sources in the sperm membrane (4). The body produces DHA and EPA via its precursors in the diet (5) or direct inclusion in the diet (4).

The useful effect of dietary fish oil as a source of omega-3 fatty acids has been recorded in the improvement of sperm quality among men (2, 6, 7), cows (8), pigs (9-12), goats (13), sheep (14), and roosters (15, 16). The addition of omega-3 fatty acids to animal diet protects the sperm plasma membrane against damages (14).

The sperm plasma membrane is sensitive to biochemical and anatomical damages in the storage process; accordingly, diet supplementation with DHA and EPA could be an effective method to improve sperm quality and fertility potential (8). Therefore, this study was performed to assess the effect of dietary fish oil on the sperm quality and fertility potential of rams. This experiment evaluated several sperm quality parameters, such as semen volume, sperm concentration, motility, membrane integrity, and viability. Finally, artificial insemination was conducted to investigate the reproductive performance of the rams fed with dietary fish oil.

2. Material and Methods

2.1. Animal Management and Sample Collection

In the current study, 15 Iranian Zandi rams (3-4 year-old) were randomly assigned into three equal groups fed with the three diets, including 1) negative control without oil supplement, 2) positive control group with the diet containing palm oil, and 3) treatment group with the diet containing fish oil (Table 1). The rams had the diets for 70 days, and the semen samples were collected every 10 days via an artificial vagina. The collected semen samples were maintained at 37°C and transferred to the laboratory for quality evaluation.

2.2. Semen Quality Assessment

Semen volume was measured using conical graduated tubes, and sperm concentration was examined using a hemocytometer after dilution with 3% (wt/vol) NaCl solution (1:200) (14). To evaluate motility, membrane integrity, viability, and fertility potential, the semen samples were diluted (1:10) in a Tris-based extender (17). Total motility and progressive motility were analyzed using a sperm class analyzer (Animal version 12.3 CEROS, Hamilton-Thorne Biosciences, Beverly, MA, USA).

To evaluate membrane integrity, the hypo-osmotic swelling test was used in this part, and 20 µl of the semen was added to 200 µl of hypo-osmotic solution (9.0 g of fructose and 4.9 g of trisodium citrate in 1,000 ml of H_2O with 100 mOsm/kg of water). The samples were assessed under a microscope at room temperature. Then, 200 sperm cells were recorded in four different microscopic fields, and the cells with swollen tails were recorded as intact membranes (18). The Eosin-Nigrosin staining assessed sperm viability by counting 200 spermatozoa under a light microscope, and the cells with unstained heads were recorded as live spermatozoa (19).

2.3. Artificial Insemination

For reproductive potential, 210 Zandi rams received CIDR (Easy-Breed™, CIDR®, New Zealand) for 12 days and 500 IU of equine chorionic gonadotropin

(Sanofi Animal Health, Libourne Cedex, France) at the time of CIDR removal. Then, they were assigned into three equal groups (70 rams per group) for artificial insemination with the last semen samples 54 h after CIDR removal. Pregnancy diagnosis was conducted using an ultrasound unit (Falco 100, Premedical, Romania) equipped with a 3.5-MHz sectorial transducer probe on the 50th day after insemination.

Pregnancy rate = number of pregnant rams/number of inseminated rams × 100

Lambing rate = number of born lambs/number of inseminated rams × 100

Parturition rate = number of lambed rams/number of inseminated rams × 100

Twinning rate = number of twins/number of parturitions × 100

2.4. Statistical Analysis

Changes in the sperm characteristics were analyzed for the effects of treatment, time, and treatment by time interaction using the MIXED procedure in SAS (SAS Institute, Cary, NC, USA) with a repeated-measures analysis. The pregnancy, parturition, lambing, and twinning rates were analyzed using the GENMOD procedure of SAS (9.1), and the significant difference between the groups was tested using the Chi-square test.

Table 1. Components of diets

	Ctrl	**PO**	**FO**
Alfalfa (% DM)	25.77	41.96	41.96
Corn silage (% DM)	28	28	28
Straw (% DM)	9.5	9.5	9.5
Barley (% DM)	22.33	6.04	6.04
Wheat bran (% DM)	12.54	10.18	10.18
Palm oil (% DM)	0	3	0
Fish oil (% DM)	0	0	3
CaCO3 (% DM)	1	0.5	0.5
NaCl (% DM)	0.36	0.32	0.32
Vitamin E (% DM)	0.5	0.5	0.5
Metabolizable energy (Mcal/kg DM)	2.24	2.24	2.24
CP (% in DM)	12	12	12
Ether extract (% in DM)	2.41	5.28	5.28
NDF (% in DM)	44.9	48.6	48.6
Calcium (% in DM)	0.75	0.79	0.79
Phosphorus (% in DM)	0.37	0.31	0.31

Ctrl: Control; PO: Palm oil; FO: Fish oil

3. Results

3.1. Quality Parameters

Table 2 tabulates the results of the effect of the treatment on semen quality parameters. The semen volume, sperm concentration, total motility, progressive motility, membrane integrity, and rate of live cells were higher (P≤0.01) in the rams fed with dietary fish oil, compared to those reported for other groups. The effects of time and time × treatment were also significant (P≤0.01).

Table 2. Effect of treatment on sperm quality parameters of rams

Trait	Treatment			SEM	P-value		
	Ctrl	PO	FO		Treatment	Time	Treatment × Time
SV (ml)	1.15^{b}	1.12^{b}	1.39^{a}	0.06	<0.0001	<0.0001	<0.0001
SC (×10⁹)	3.5^{b}	3.49^{b}	3.81^{a}	0.07	0.0003	<0.0001	<0.0001
TM (%)	47.81^{b}	48.5^{b}	64.4^{a}	2.9	<0.0001	<0.0001	<0.0001
PM (%)	16.21^{b}	16.77^{b}	39.97^{a}	3.6	<0.0001	<0.0001	<0.0001
MI (%)	60.22^{b}	60.51^{b}	73.4^{a}	2.9	<0.0001	<0.0001	<0.0001
LC (%)	60.41^{b}	59.94^{b}	73.31^{a}	2.9	<0.0001	<0.0001	<0.0001

Different letters within the same rows indicating significant differences among the groups (P≤0.05)
Ctrl: Control; PO: Palm oil; FO: Fish oil; SV: Semen volume; SC: Sperm concentration TM: Total motility; PM: Progressive motility; MI: Membrane integrity; LC: Live cells

3.2. Reproductive Performance

Table 3 shows the results of the effect of the treatment on ram's reproductive performance. The pregnancy, parturition, and lambing rates were higher (P≤0.1) in the rams fed with dietary fish oil, compared to those reported for other groups.

Table 3. Effect of treatment on reproductive performance of Iranian Zandi rams (P≤0.1)

Trait	Ctrl	PO	FO
Pregnancy rate (%)	52.85^{b} (37/70)	50^{b} (35/70)	70^{a} (49/70)
Parturition rate (%)	48.58^{b} (34/70)	42.85^{b} (30/70)	64.28^{b} (45/70)
Lambing rate (%)	48.58^{b} (34/70)	42.85^{b} (30/70)	64.28^{b} (46/70)
Twining rate (%)	0 (0/34)	0 (0/30)	2 (1/45)

Different letters within the same rows indicating significant differences among the groups (P≤0.1)
Ctrl: Control; PO: Palm oil; FO: Fish oil

3.3. Semen Volume

Figure 1 depicts the effect of dietary fish oil on the ram's semen volume during 70 days of feeding. Using fish oil improved the semen volume in comparison to palm oil and control groups from the 3rd sample collection up to the end of the experiment.

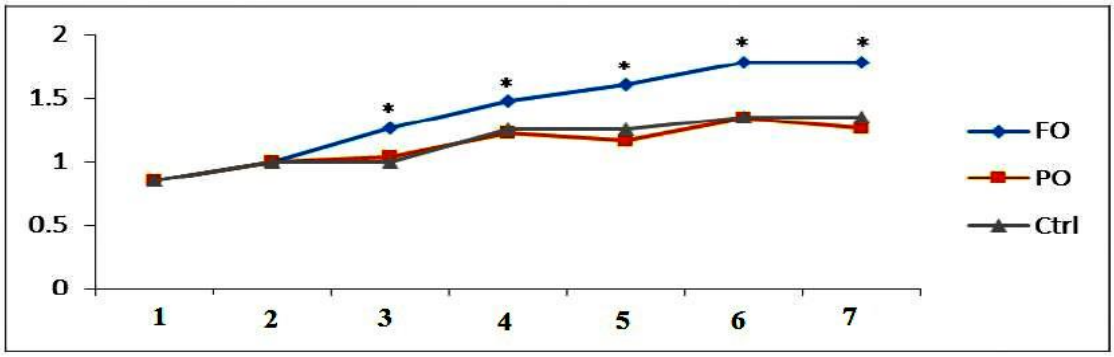

Figure 1. Effect of dietary fish oil on ram's semen volume during 70 days of feeding (P≤0.05)

3.4. Sperm Concentration

Figure 2 illustrates the effect of dietary fish oil on the ram's sperm concentration during 70 days of feeding. Using fish oil improved the semen volume in comparison to palm oil and control groups on the 4th, 6th, and 7th sample collections.

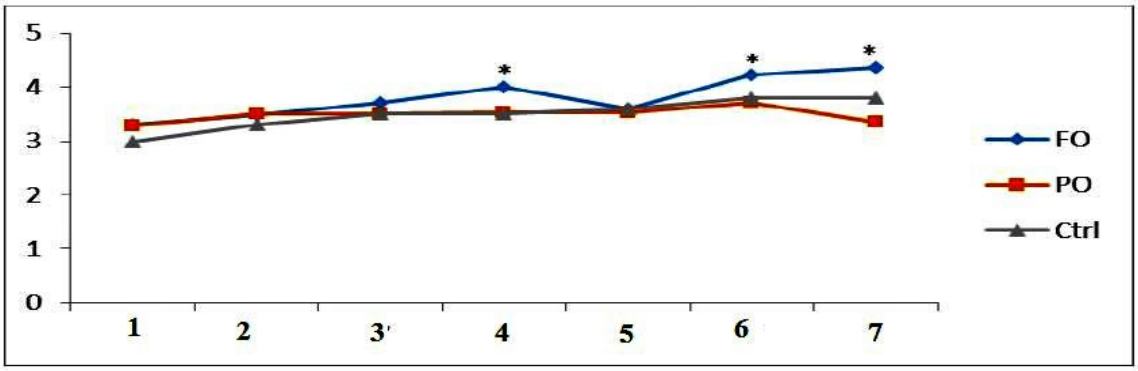

Figure 2. Effect of dietary fish oil on ram's sperm concentration during 70 days of feeding (P≤0.05)

3.5. Total Motility and Progressive Motility

Figures 3-5 illustrate the effect of dietary fish oil on the total motility, progressive motility, and membrane integrity of the ram's sperm during 70 days of feeding. Using fish oil improved the total motility, progressive motility, and membrane integrity of the ram's sperm in comparison to palm oil and control groups from the 3rd sample collection up to the end of the experiment.

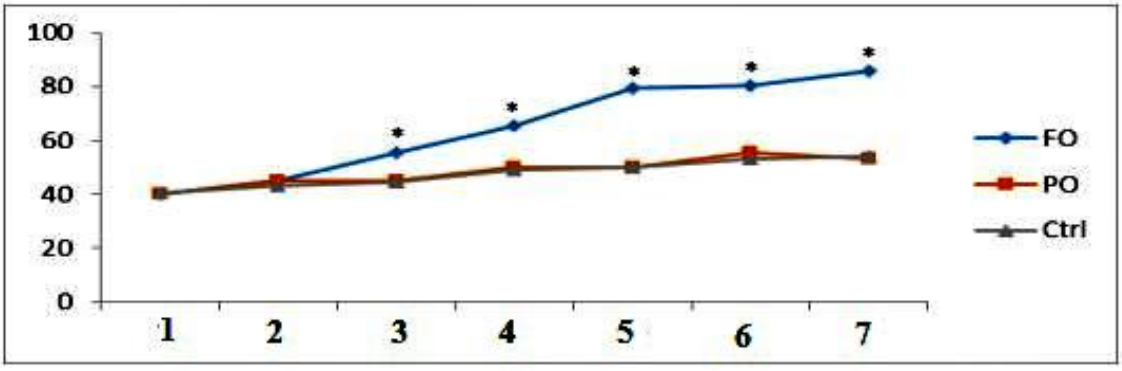

Figure 3. Effect of dietary fish oil on total motility of ram's sperm during 70 days of feeding (P≤0.05)

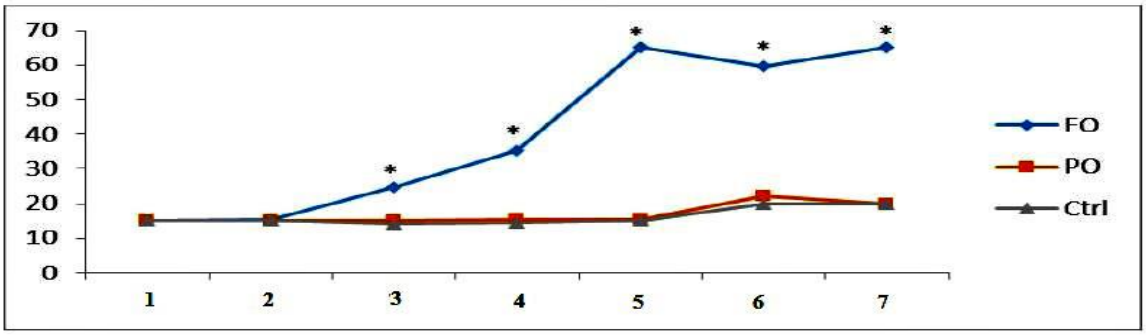

Figure 4. Effect of dietary fish oil on progressive motility of ram's sperm during 70 days of feeding (P≤0.05)

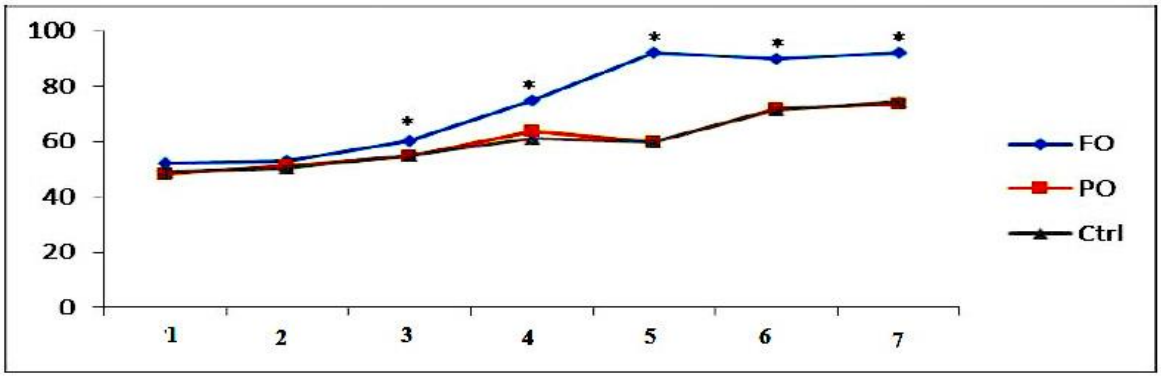

Figure 5. Effect of dietary fish oil on membrane integrity of ram's sperm during 70 days of feeding (P≤0.05)

3.6. Live Cells

Figure 6 depicts the effect of dietary fish oil on the viability of ram's sperm during 70 days of feeding. Using fish oil improved the semen volume in comparison to palm oil and control groups from the 2nd sample collection up to the end of the experiment.

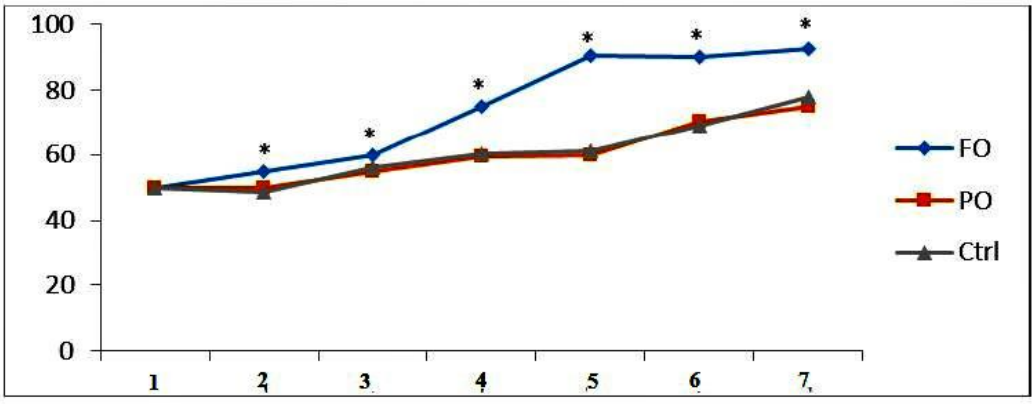

Figure 6. Effect of dietary fish oil on viability of ram's sperm during 70 days of feeding (P≤0.05)

4. Discussion

In the present study, using dietary fish oil significantly improved the sperm quality parameters, such as semen volume, sperm concentration, motility, membrane integrity, viability, and fertility potential, after 70 days of feeding. The reason for the improvement could be related to PUFA increment in the sperm membrane, especially in the head and tail, by feeding the rams with an n-3 supplemented diet. The presence of long-chain fatty acids is crucial for many sperm activities from spermatogenesis up to fertilization (20).

Dietary fish oil improved the motility, membrane fluidity, and flexibility of sperm tail (3); therefore, the improvement of viable spermatozoa in the current experiment could be justified due to the presence of an n-3 source (i.e., fish oil) in the diet of the rams. The results of the current study are in line with the findings of studies reporting helpful effects of fish oil for the fresh storage of goats' (13) and rams' (14) semen.

Omega-3 fatty acids in the phospholipid bilayers of sperm plasma membrane increase conversion between extended and loop conformation, which is the result of the improvement of sperm plasma membrane flexibility (21). In the current experiment, the rams which were considered control and fed with palm oil produced a higher significant number of dead spermatozoa. This result is in line with the findings of a study reporting that the increment of saturated fatty acids in the sperm membrane decrease membrane fluidity as well as sperm resistance against storage (22).

The reproductive evaluation was an important experiment in the present study for the verification of the in vitro results of sperm quality. The collected semen samples from the rams fed with fish oil as a treatment showed higher reproductive performance, such as pregnancy, parturition, and lambing rates, compared to those of other groups. These findings are in line with the results of a study (3) demonstrating that normal men have a higher concentration of omega-3 in seminal plasma in comparison to infertile men.

Although several studies were performed to assess the effects of fish oil on the sperm quality of different species (23, 24), artificial insemination was conducted in the current study for the evaluation of the spermatozoa of the rams fed with dietary fish oil. Finally, the results of the present study indicated that artificial insemination with the semen samples collected from the rams feeding with fish oil improved the pregnancy, parturition, and lambing rates. This improvement could be related to the higher parameters of the sperm quality in the group fed with fish oil.

5. Conclusion

Dietary supplementation with fish oil for 70 days improved semen quality and fertility potential of Iranian Zandi rams; therefore, it could be a practical method to improve the efficiency of sheep reproduction.

Authors' Contribution

Study concept and design: R. M.
Acquisition of data: N. D. D.
Analysis and interpretation of data: R. M.
Drafting of the manuscript: N. D. D.
Critical revision of the manuscript for important intellectual content: N. D. D.
Statistical analysis: R. M.
Administrative, technical, and material support: N. D. D.

Ethics

This project was evaluated in the Animal Ethics Committee, Animal Science Research Institute of Iran under the project number of 21313026980299

Conflict of Interest

The authors declare that they have no conflict of interest.

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