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 ram semen quality and fertility potential. Fifteen Iranian Zandi rams were randomly assigned into three equal groups. The first group was negative control and received the diet without oil supplement. The second group was positive control group and received the diet containing palm oil and the last group received the diet containing fish oil. All diets were isocaloric and isonitrogenous. The rams were fed during eight weeks and the semen samples were collected once a week. In experiment I, the following parameters were evaluated: Semen volume, sperm concentration, motility, membrane integrity and viability. In experiment II, 210 Iranian Zandi ewes received CIDR for 12 days and 400 IU eCG at the time of CIDR removal and assigned into three equal groups and artificially inseminated with semen samples. In result, supplementation of ram diet with fish oil as a source of omega-3 fatty acids improved ram semen volume, sperm concentration, total motility, progressive motility, viability, membrane integrity, pregnancy rate, parturition rate and lambing rate (P≤0.05). In conclusion, addition of fish oil the ram diet
could be an effective strategy to improve ram semen quality for artificial insemination and other goals.

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

**Introduction**

Sperm membrane polyunsaturated fatty acids (PUFAs) play an important role in energy metabolism (Zaniboni et al., 2006), fluidity of plasma membrane (Conquer et al., 2000) and some functions, which are related to fertilization (Connor et al., 1998). Docosahexaenoic acid (DHA) and eicosapentaenoic (EPA) are the main omega-3 sources in sperm membrane (Gulliver et al., 2012). The body produces DHA and EPA via their precursors in the diet (Lands, 1992) or direct inclusion to the diet (Gulliver et al., 2012). The useful effect of dietary fish oil as a source of omega-3 fatty acids has been recorded in improvement of sperm quality in men (Nissen et al. 1981), cow (Gholami et al., 2010), pig (Mitre et al., 2004), goat (Dolatpanah et al., 2008), sheep (Samadian et al., 2010) and rooster (Surai et al., 2000). Addition of omega-3 fatty acids to animal diet protects sperm plasma membrane against damages (Samadian et al., 2010). Sperm plasma membrane is sensitive toward biochemical and anatomical damages in storage process, so diet supplementation with DHA and EPA could be an effective method to improve sperm quality and fertility potential (Gholami et al., 2010). Therefore, this study was performed to assess the effect of dietary fish oil on ram sperm quality and fertility potential. In this experiment several sperm quality parameters were evaluated such as semen volume, sperm concentration, motility, membrane integrity and viability. Finally, artificial insemination was conducted to investigate the reproductive performance of rams fed dietary fish oil.

**Material and methods**

**Animal management and samples collection**
In the current study, 15 Iranian Zandi rams (3-4 year-old) were randomly assigned into three equal groups and fed the following diets: 1) negative control the diet without oil supplement, 2) positive control group the diet containing palm oil and 3) treatment group the diet containing fish oil (table 1). The rams received the diets for 70 days and semen samples were collected each 10-days via artificial vagina. Collected semen samples were maintained at 37°C and transferred to the laboratory for quality evaluation.

**Semen quality assessment**

Semen volume was measured using conical graduated tubes and sperm concentration was examined using a hemocytometer, after dilution with 3% (w/v) NaCl solution (1 : 200) (Samadian et al., 2010).

To evaluate motility, membrane integrity, viability and fertility potential, semen samples were diluted (1:10) in a Tris based extender (Sharafi et al., 2009). Total motility and progressive motility were analyzed using sperm class analyzer (Animal Version 12.3 CEROS, Hamilton-Thorne Biosciences, Beverly, MA, USA).

To evaluate membrane integrity, hypo osmotic swelling test (HOST) was used an in this part, 20 µl of semen were added to 200 µl of hypo osmotic solution (9.0 g fructose, 4.9 g trisodium citrate, in 100 ml of H2O with 100 m osm/kg water). The samples were assessed under microscope at room temperature. Then, 200 sperm cells were recorded in 4 different microscopic fields and the cells with swollen tails were recorded as intact membrane (Forouzanfar et al., 2010).

The eosin–nigrosine stain assessed sperm viability by counting 200 spermatozoa under light microscope and cells with unstained heads were recorded as live spermatozoa (Salmani et al., 2013).

**Artificial insemination**
For reproductive potential, 210 Zandi ewes were received CIDR (Easy-Breed™, CIDR®, New Zealand) for 12 days and 500 IU eCG (Sanofi Animal Health, Libourne Cedex, France) at the time of CIDR removal and then assigned into 3 equal groups (70 ewes per group) for artificial insemination with the last semen samples 54 h after CIDR removal. Pregnancy diagnosis was conducted via an ultrasound unit (falco 100, premedical) equipped with a 3.5 MHz sectorial transducer probe in day 50 post insemination.

Pregnancy rate = number of pregnant ewes/number of ewes inseminated×100. Lambing rate = number of lambs born/number of ewes inseminated×100. Parturition rate = number of ewes lambed/number of ewes inseminated×100. Twinning rate = number of twins/number of parturitions×100.

**Statistical analysis**

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

**Results**

**Quality parameters**

The results of the effect of treatments on semen quality parameters are presented in table 2. Semen volume, sperm concentration, total motility, progressive motility, membrane integrity, and the rate of live cells were higher (P≤0.01) in rams which fed dietary fish oil compared to other groups. The effects of time and time×treatment were also significant (P≤0.01).
Table 2: Effect of treatments on sperm quality parameters of rams.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Treatments</th>
<th>Ctrl</th>
<th>PO</th>
<th>FO</th>
<th>SEM</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Time</td>
<td>Treatment×Time</td>
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<tr>
<td>SV (ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
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<tr>
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<td>1.12b</td>
<td>1.39a</td>
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<td>SC (x10^6)</td>
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<td>3.81a</td>
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<tr>
<td>TM (%)</td>
<td></td>
<td></td>
<td></td>
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<td>&lt;.0001 &lt;.0001 &lt;.0001</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>PM (%)</td>
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<td></td>
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<td>3.6</td>
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<td>16.21b</td>
<td>16.77b</td>
<td>39.97a</td>
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<tr>
<td>MI (%)</td>
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<td>2.9</td>
<td>&lt;.0001 &lt;.0001 &lt;.0001</td>
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<tr>
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<td>60.51b</td>
<td>73.4a</td>
<td></td>
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</tr>
<tr>
<td>LC (%)</td>
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</table>

Different letters within the same rows show 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.

Reproductive performance

The results of the effect of treatments on ram’s reproductive performance are shown in table 3. Pregnancy rate, Parturition rate and lambing rate were higher (P ≤ 0.1) in rams which fed dietary fish oil compared to other groups.

Table 3: Effect of treatments on reproductive performance of Iranian Zandisheeps (P ≤ 0.1).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Ctrl</th>
<th>PO</th>
<th>FO</th>
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<tbody>
<tr>
<td></td>
<td>(37/70)</td>
<td>(35/70)</td>
<td>(49/70)</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>52.85b</td>
<td>50b</td>
<td>70a</td>
</tr>
<tr>
<td>Parturition rate (%)</td>
<td>48.58b</td>
<td>42.85b</td>
<td>64.28b</td>
</tr>
<tr>
<td>Lambing rate (%)</td>
<td>48.58b</td>
<td>42.85b</td>
<td>64.28b</td>
</tr>
<tr>
<td>Twining rate (%)</td>
<td>0 (0/34)</td>
<td>0 (0/30)</td>
<td>2 (1/45)</td>
</tr>
</tbody>
</table>

Different letters within the same rows show significant differences among the groups (P ≤ 0.1).

Semen volume

The effect of dietary fish oil on ram’s semen volume during 70 days feeding is presented in Fig 1. Using fish oil improved semen volume compared to palm oil and control groups from the 3rd sample collection until the end of experiment.
Fig 1. Effect of dietary fish oil on ram’s semen volume during 70 days feeding (P≤0.05).

Sperm concentration

The effect of dietary fish oil on ram’s sperm concentration during 70 days feeding is shown in Fig 2. Using fish oil improved semen volume compared to palm oil and control groups on 4th, 6th and 7th sample collections.

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

Total motility and progressive motility

The effect of dietary fish oil on ram’s sperm TM, PM and membrane integrity during 70 days feeding are shown in figures 3-5. Using fish oil improved ram’s sperm TM, PM and membrane integrity compared to palm oil and control groups from the 3rd sample collection until the end of experiment.
Fig 3. Effect of dietary fish oil on ram’s sperm total motility during 70 days feeding (P≤0.05).

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

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

Live cells
The effect of dietary fish oil on ram’s sperm viability during 70 days feeding is presented in Fig 6. Using fish oil improved semen volume compared to palm oil and control groups from the 2nd sample collection until the end of experiment.

![Graph showing the effect of dietary fish oil on ram’s sperm viability during 70 days feeding.](graph.png)

Fig 5. Effect of dietary fish oil on ram’s sperm viability during 70 days feeding (P≤0.05).

**Discussion**

In this study, using dietary fish oil significantly improved the sperm quality parameters such as semen volume, sperm concentration, motility, membrane integrity, viability and fertility potential ability after 70 days feeding. The reason of improvement could be related to PUFA increment in the sperm membrane especially in the head and tail by feeding of rams with n-3 supplemented diet. Presence of long chain fatty acids is crucial for many sperm activities from spermatogenesis up to fertilization (Lenzi et al., 2000). Dietary fish oil improved the motility, membrane fluidity and flexibility of sperm tail (Conner et al., 1998), so improvement of viable spermatozoa in the current experiment could be justified due to presence of n-3 source (fish oil) in the ram’s diet. The results are in agreement with studies that reported helpful effects of fish oil for fresh storage of goat (Dolatpanah et al., 2010) and ram (Samadian et al., 2010) semen.
Omega-3 fatty acids in sperm plasma membrane phospholipids bilayers increase conversion between extended and loop conformation, which is the result of the improvement of sperm plasma membrane flexibility (Speake et al., 2003). In the current experiment, rams which were fed control and palm oil treatments produced higher significant number of dead spermatozoa which is in compliance with the study that reported that increment of saturated fatty acid in the sperm membrane decrease membrane fluidity as well as sperm resistance against storage (Avital-Cohen et al., 2013).

Reproductive evaluation was an important experiment in this study to verify in vitro results of sperm quality. Collected semen samples from rams were fed with fish oil treatment presented higher reproductive performances like pregnancy rate, parturition rate and lambing rate compared to other groups. These findings are in agreement with the results of a study (Conner et al., 1998) that declared normal men have higher concentration of omega-3 in seminal plasma compared to infertile men. Although several studies were performed to assess the effects of fish oil on sperm quality of different species (Cerolini et al., 2006; Castellano et al., 2010), we conducted artificial insemination to evaluate rams spermatozoa which were fed dietary fish oil. Finally, this study presented artificial insemination with semen samples were collected from rams fed fish oil improved pregnancy rate, parturition rate and lambing rate. This improvement could be related to the higher sperm quality parameters of fish oil group.

Conclusion

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


comparison of soybean lecithin based-extender with commercially available extender for ram semen cryopreservation. Int J FertilSteril, 3(3), 149-152.

