Effect of Fish Oil On-Top Feed Supplemented on Boar Spermatozoa Lipid Composition and Semen Quality: A Preliminary Study

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Introduction
Lipids are the main source of energy in metabolism of spermatozoa. The fatty acids are both structural elements and bioactive compounds (1). In most mammals, docosahexaenoic acid (DHA) is the dominant polyunsaturated fatty acid, although, in several species docosapentaenoic acid (DPA) is also a major component of the sperm cell membrane (2). The high quantity of long chain polyunsaturated fatty acids (LCPUFAs) in the membrane phospholipids of spermatozoa are known to play an important role in membrane fluidity and flexibility. The importance of polyunsaturated fatty acids in relation to male fertility has been illustrated by studies in humans demonstrating that the amount of docosahexaenoic acid in spermatozoa is positively correlated with sperm motility (3). In addition, it is believed that LCPUFAs contribute actively in the regulation of cellular movement, lipid metabolism and fusion capacity. LCPUFAs which are concentrated in the head and tail membrane regions of spermatozoa have been shown to play an important role in both sperm capacitation (4) and the interaction between spermatozoa and uterine surface environment (5). There were the previous studies showed that feed supplementation affected the fatty acid composition of boar spermatozoa by increasing the n-3 LCPUFAs at the expense of n-6 LCPUFAs in the sperm plasma membranes (6,7). The objective of the present study was to investigate the effects of fish oil supplemented in the boar feed on the lipids (fatty acid) composition of ejaculated spermatozoa, semen quality and on storability at 18°C.

Materials and Methods
Three sexually mature boars were assigned into “before and after” experimental design. They were kept in 2x3 m individual pen and received 2.5 kg standard diet per day. Fish oil (14 g), vitamin E (480 iu) and vitamin C (2400 mg) were given once a day by on-top feeding for 10-week period. Semen collection was performed for each boar once a week starting from 4 week prior to the supplementation and continues for a total of 15 collections per boar. All semen samples were evaluated for semen quality e.g. volume, concentration, pH, motility, sperm morphology and acrosomal defects. Sperm viability and membrane integrity of stored semen at 0 and 12 h were also evaluated. Approximately 30 ml of the sperm-rich fraction of week -4, 0, 5 and 10 was transported to the laboratory at 37°C. The spermatozoa were separated from the seminal plasma by centrifugation at 700 g for 20 min at 48°C. The spermatozoa were re-suspended in the same volume of normal saline and re-centrifuged. This process was repeated twice. The washed spermatozoa and the seminal plasma were stored at -20°C to await analysis. Total lipid was extracted from the spermatozoa after homogenization in a suitable excess of chloroform-methanol (2:1, v/v) (8). Both spermatozoa and seminal plasma were analyzed for lipid composition by gas chromatography.

Results and Discussion
The average of motility, % normal sperm, % intact acrosome were no any changes; however, the average of total number of sperm per ejaculate increase after week 4 through week 10 comparing with week 3 (27.4 x10^9 vs 25.3x10^9 spz) (Table 1.). There is the study which also found no improvements in sperm motility or acrosome integrity after fish oil supplementation (6). This finding is in contrast to Rooke’s study which found improvements in sperm motility or acrosome integrity after LCPUFAs supplementation. However, initial values for motility and acrosome integrity were markedly lower in the study of Rooke than this study, indicating that responses to the inclusion of fish oil in the diet may depend on initial sperm quality (9). The sperm viability and intact plasma membrane of sperm stored for 12 h trended to higher after week 5 through week 10 comparing with week 4 (69.83% vs 65.67% (Fig. 1); 66.5% vs 62.33% (Fig. 2) respectively). No changes in the ratio of lipid content in spermatozoa comparing with protein and carbohydrate. The average ratio of omega-3 fatty acid in spermatozoa increased at week 5 and week 10 comparing week-4 and week 0 (38.97, 38.49% vs 18.09, 18.17%) (Fig. 3); however, no any changes were found for the ratio of omega-3 fatty acid in seminal plasma.

Acknowledgement
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References

Table 1 Parameter of fresh semen quality (n=3)

<table>
<thead>
<tr>
<th>Parameters (n=3)</th>
<th>week</th>
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<tbody>
<tr>
<td>% motility</td>
<td>70</td>
</tr>
<tr>
<td>Absolute total number (×10⁶ ml⁻¹)</td>
<td>20.6 20.1 20.9 20.3 20.4 20.5 20.7 20.8 20.9 21.0</td>
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<tr>
<td>% normal sperm</td>
<td>85</td>
</tr>
<tr>
<td>% intact sperm</td>
<td>71</td>
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Fig. 1 Results of % lived sperm at 0 and 12 hrs. of preservation period

Fig. 2 % intact plasma membrane of sperm at 0 and 12 hrs. of preservation period

Fig. 3 Ratio of n-3 PUFA in total lipids of sperm composition