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Effect of omega-3 fatty acids enriched diet on semen characteristics in Marwari horses

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The ω-3 docosahexaenoic acid (DHA; C22: 6n-3) content is high in the sperm plasma membrane of many domestic animals (Zachut *et al*. 2011). In contrast, equine sperm contains high levels of ω–6 docosapentaenoic acid (DPA; C22: 5n-6) (Ball *et al*. 2000). Proportion of DHA and DPA, within sperm membrane are important determinant of semen quality and fertility (Rooke *et al*. 2001). Previous studies demonstrated that the fatty acid profile of sperm membrane can be modified by diet (Castellano *et al*. 2010) to improve the sperm quality of boar (*Rooke et al*. 2001), stallion (Brinsko *et al*. 2005), turkey (Zaniboni *et al*. 2006) and buffalo (Adeel *et al*. 2009). However, others did not observe quantitative improvement on various seminal parameters in stallion (Grady *et al.* 2009), boar (Yeste *et al.* 2011*)* and ram (Fair *et al*. 2014). In the present study, it was hypothesized that supplementation of n-3 polyunsaturated fatty acids (PUFA) enriched fish oil in the diet of Marwari stallions would enhance their semen quality.

Apparently healthy Marwari horses (6) of 4–8 years weighing 350–400 kg were daily fed a standard diet that included 3 kg of concentrate, mineral mixture, salt and 9 kg fodder (green: dry in 3: 1 ratio) from week –13 to 0 (pre-supplementation phase). This was followed by supplementation of refined fish oil having 15.28% eicosapentaenoic acid (EPA; C20: 5 n-3) and 10.41% DHA in their diets to provide combined EPA and DHA @64 mg/kg BW from week 1 to week 14 (fish oil treatment phase). Ejaculates were collected on weekly basis from week –5 to 0 in pre-supplementation and week 9 to 14 during fish oil treatment phase for 6 weeks period / phase (total=36 ejaculates per phase). Ejaculate characteristics such as color and consistency, volume (total, gel and gel free), seminal pH, total and progressive sperm motility, sperm concentration, live and abnormal sperm % were evaluated in fresh semen. In frozen semen samples, post thaw motility (PTM), live and abnormal sperm and hypo osmotic swelling (HOS) reacted sperm % were recorded. Semen collection, evaluation and processing for vapor freezing were done as per the standard techniques.

Data were tested for normality using Shapiro-wilk test and transformed to logarithm, arc-sine, and square-roots wherever the data were not normal. Seminal parameters were analyzed first for variability within group using one-way ANOVA. Since the variability within group failed to achieve significance in any of the parameter under study, prior to and after fish oil supplementation in diet, the data were pooled and analyzed by independent sample t-test using SPSS.

To the best of our knowledge, this is a first report to study effect of fish oil supplementation on the seminal attributes of Marwari horse. Milky white to creamy appearance and variably thin consistency of ejaculates were not altered following fish oil supplementation in diet. Motion characteristics, viability, morphology and membrane integrity of sperm in fresh and frozen-thawed semen are presented in Table 1. There was no significant difference in semen volume, viz. total, gel and gel free semen. In concurrence, studies in goat (Dolatpanah *et al.* 2008), buffalo bull (Adeel *et al.* 2009) and pig (Yeste *et al.* 2011) also failed to achieve significant difference in semen volume following unsaturated fatty acid supplementation. Similarly, fish oil fed for 9 weeks did not affect the total semen volume in the ram (Fair *et al.* 2014). However, Strzezek *et al*. (2004) had shown positive effect of unsaturated fatty acid on semen volume in boars supplemented for a 24 weeks period. In the present study, there was no difference in seminal pH of stallions either at pre-supplementation or fish oil treatment phase.

We observed no improvement in total and progressive sperm motility in the fresh semen. This is consistent with

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earlier reports fed DHA enriched commercial nutriceutical to boar for 24 weeks (Strzezek *et al.* 2004) and for 14 weeks in stallions (Brinsko *et al.* 2005). Similar results were observed in boar fed top dressed fish oil for 26 weeks (Yeste *et al.* 2011) and fish oil supplementation in ram for 9 weeks (Fair *et al.* 2014). However, a significant correlation was reported between dietary DHA supplementation and the number of motile spermatozoa in boar (Rooke *et al.* 2001) and goat (Dolatpanah *et al.* 2008). Gholami *et al.* (2010) also observed higher total and progressive sperm motility assessed by computer assisted semen analyzer (CASA) in Holstein bulls fed DHA enriched nutraceutical. No improvement in mean sperm concentration in this study is supported by earlier reports in turkey (Zaniboni *et al.* 2006), buffalo (Adeel *et al.* 2009) and pig (Yeste *et al.* 2011) following unsaturated fatty acid supplementation. In contrast, Strzezek *et al.* (2004) indicated positive effect of unsaturated fatty acid supplementation on the sperm count in the boar. Higher sperm concentration as well as sperm production was observed in boar (Rooke *et al.* 2001) and stallion (Harris *et al.* 2005) supplemented with n-3 fatty acid. Supplementation of fish oil in the diet increased the sperm concentration in the ram (Fair *et al.* 2014).

Our observation of no change in live sperm% of both fresh and frozen semen with fish oil supplemented diet was similar to earlier results in fresh semen of pig (Yeste *et al.* 2011) and frozen-thawed semen of stallion (Brinsko *et al.* 2005) when fed diet high in DHA. Grady *et al*. (2009) also failed to observe any significant effect of n-3 PUFA on the percentage of live spermatozoa in fresh, cooled and frozen-thawed semen in stallions. On the other hand, increased number of viable sperm cells in fresh semen was reported with DHA supplementation in boar (Rooke *et al.* 2001), turkey (Zaniboni *et al.* 2006) and bull (Gholami *et al.* 2010). Our results on sperm abnormalities in the fresh and frozen semen are at par with study of Grady *et al*. (2009) who also reported no change in morphologically normal spermatozoa (%) in fresh, cooled and frozen-thawed semen of horses supplemented with n-3 PUFA. Similarly, Strzezek *et al*. (2004) also indicated no effect on number of abnormal sperm cells in boar fed with unsaturated fatty acid. In contrast, DHA enriched diet in stallions reduced the incidence of acrosome and mid piece abnormalities (Elhordoy *et al*. 2008). Similarly, dietary fat supplementation increased the percent of sperm with normal morphologies in boar (Yeste *et al.* 2011). Rooke *et al*. (2001) had also shown an increase in proportion of morphologically normal spermatozoa in boars supplemented with fish oil and antioxidants.

Fish oil supplemented diet failed to improve sperm PTM in the present study. This is consistent with previous results in stallion (Grady *et al.* 2009) and boar (Castellano *et al.* 2010). In contrast, feeding a DHA-enriched nutriceutical improved the sperm motility of frozen-thawed semen (Harris *et al.* 2005) and cooled stored stallion semen (Elhordoy *et al.* 2008). In the buffalo bull, diets containing long-chain PUFA (sunflower oil) improved sperm PTM

Table 1. Semen characteristics (mean ±SEM) of Marwari stallions fed diet supplemented with fish oil

Group	Number of ejaculates	Seminal parameters										
		Total semen volume (ml)	Gel volume (ml)	Gel free semen vol. (ml)	pH	Total sperm motility (%)	Progressive sperm motility (%)	Sperm conc. (10^6/ml)	Live sperm count in fresh semen (%)	Abnormal sperm count in fresh semen (%)	PTM (%)	Live sperm in frozen semen (%)	Abnormal sperm count in frozen semen (%)	HOS reacted sperm (%)
Pre-supplementation phase	36	60.75±6.17	20.64±3.24	40.11±3.85	7.74±0.06	79.58±2.26	72.50±2.67	182.0±11.89	79.97±1.40	11.42±0.68	38.83±1.85	51.70±2.96	14.23±0.69	35.22±1.86
Fish oil treatment phase	36	58.92±5.38	18.05±2.70	40.86±3.74	7.64±0.06	76.39±2.63	70.14±2.85	188.24±12.94	78.97±1.96	10.39±0.86	39.63±2.08	51.70±2.06	12.63±0.96	34.44±1.78
P value		0.886	0.699	0.864	0.242	0.309	0.536	0.772	0.552	0.126	0.775	0.574	0.063	0.765

Values bearing no superscripts do not differ significantly (P>0.05) within column.

(Adeel *et al*. 2009). There was no increase in the number of HOS reacted sperm in frozen-thawed samples following dietary fish oil supplementation which is consistent with earlier report of Gholami *et al.* (2010) in Holstein bulls supplemented with DHA enriched nutraceutical; however, they observed an improvement in HOS positive proportion of sperm in fresh semen. Feeding PUFA to pigs had shown an increase in the proportion of membrane intact spermatozoa (Strzezek *et al.* 2004). Similarly, Adeel *et al*. (2009) observed a higher number of HOS positive spermatozoa in frozen-thawed semen of buffalo fed with sunflower-enriched diets but did not find such difference in the fresh semen. Kaeoket *et al.* (2010) reported that supplementation of the semen extender with DHA was effective for freezing boar semen as it resulted in higher sperm PTM and plasma membrane integrity. In another study, Selvaraju *et al.* (2012) fed rams either with maize or sunflower oil enriched diet (linoleic acid) and found that PUFA enrichment influenced sperm quality by stabilizing membrane integrity.

It was concluded that supplementation of n-3 PUFA @64mg/kg BW to horses for 14 weeks had no effect on semen quality. However, the beneficial effects of n-3 PUFA on semen quality may require longer period of supplementation.

SUMMARY

The effect of ω-3 fatty acid supplementation in the diet on various characteristics of fresh and frozen semen was investigated in Marwari horses. Stallions (6) were fed a standard diet daily from week –13 to week 0 (pre-supplementation phase) followed by supplementation of fish oil, a rich source of n-3 PUFA @64 mg/kg BW from week 1 to 14 (fish oil treatment phase) in diets. Ejaculates were collected from all the stallions on weekly basis from week –5 to 0 and week 9 to 14 during pre-supplementation and fish oil treatment phase, respectively. Effect of n-3 PUFA on ejaculate volume, color and consistency; total and progressive sperm motility, seminal pH, sperm concentration, live sperm and abnormal sperm in fresh semen was non-significant. Similarly, there was no change in the percentage of sperm PTM, HOS reacted sperm, live and abnormal sperm in frozen semen. It was concluded that dietary n-3 PUFA supplementation @64mg/kg BW to horses for 14 weeks did not affect the semen quality. However, the beneficial effects of n-3 PUFA on semen quality may require longer period of supplementation.

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