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Research

Effects of feeding tuna oil on the lipid composition of pig spermatozoa and *in vitro* characteristics of semen

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The aim of the present study was to characterize the effects of feeding tuna oil on the lipid and fatty acid composition of boar spermatozoa and to relate changes in composition to boar semen characteristics. Ten boars were paired by age and allocated to one of two diets (five boars per diet). The diets, which were offered for 6 weeks, consisted of a basal diet that was either unsupplemented or supplemented with 30 g tuna oil kg^{-1} diet. Adding tuna oil to the diet increased the ether extract concentration of the diets fed from 65 to 92 g kg^{-1} dry matter and supplied 10.5 g long chain polyunsaturated (n-3) fatty acids per 100 g total fatty acids. There were no changes in semen fatty acid composition after 3 weeks of feeding tuna oil. However, after 5 and 6 weeks, the proportions (g per 100 g total fatty acids) of 22:6(n-3) in sperm phospholipid fatty acids were increased from 34.5 to 42.9 g by feeding tuna oil and 22:5(n-6) decreased from 29.8 to 17.9 g. No changes were observed in other sperm lipids or seminal plasma phospholipids as a result of the diets fed. Feeding tuna oil increased the proportion of spermatozoa with progressive motility and with a normal acrosome score and reduced the proportion of spermatozoa with abnormal morphologies.

Introduction

The phospholipids of mammalian spermatozoa have a characteristic fatty acid composition, the most distinctive feature of which is a very high proportion of long chain (C_{22}) highly polyunsaturated fatty acids. In most mammals, docosahexaenoic acid (22:6(n-3)) is the dominant polyunsaturated fatty acid although, in several species, docosapentaenoic acid (22:5(n-6)) is also a major component (Neill and Masters, 1972; Poulos and White, 1973; Poulos *et al.*, 1973; Darin-Bennett *et al.*, 1974; Jain and Anand, 1976; Salem *et al.*, 1986; Lin *et al.*, 1993; Kelso *et al.*, 1997a). Pig spermatozoa share the above characteristics, although the reported proportions of polyunsaturated fatty acids in phospholipids and the relative amounts of the (n-6) and (n-3) fatty acids vary (Ahluwalia and Holman, 1969; Johnson *et al.*, 1972; Evans and Setchell, 1979; Paulenz *et al.*, 1995).

In human spermatozoa, 22:6(n-3) performs an essential function in promoting optimal fertility, since reductions in the amount of this fatty acid in sperm lipids have been correlated with reductions in sperm concentration and in spermatozoa with progressive motility and normal morphology (Nissen and Kreysel, 1983; Zalata *et al.*, 1998; Conquer *et al.*, 1999). During ageing of chickens (Kelso *et al.*, 1996, 1997b; Cerolini *et al.*, 1997) and bulls (Kelso *et al.*, 1997a), reductions in the output, quality and fertilizing ability of spermatozoa are associated with decreased proportions of $C_{20–22}$ polyunsaturated fatty acids in sperm phospholipids.

Diets fed to pigs commonly contain large amounts of cereals, with most supplementary protein being added in the form of oilseed meals such as soya-bean and rapeseed. Thus, dietary fatty acids typically have a (n-6):(n-3) ratio of greater than 6:1 and contain no long chain polyunsaturated (n-3) fatty acids. If 22:6(n-3) is essential for optimal fertility in pig spermatozoa, as it is in human spermatozoa (Nissen and Kreysel, 1983; Zalata *et al.*, 1998; Conquer *et al.*, 1999), then it is possible that pig diets provide a suboptimal supply of 22:6(n-3) for spermatogenesis. This suboptimal supply may arise either from a deficit of (n-3) fatty acids *per se* or a reduced synthesis of 22:6(n-3) from 18:3(n-3) owing to competition between (n-6) and (n-3) fatty acids for desaturase enzymes (Sprecher, 1989). Furthermore, when pregnant sows were offered diets containing either 18:3(n-3) or 22:6(n-3), feeding 18:3(n-3) did not result in increases in 22:6(n-3) in sow adipose tissue or piglet tissues at birth, indicating a limited biosynthetic capacity of 22:6(n-3) to 18:3(n-3) (Rooke *et al.*, 2000). To date, studies in which fish oil has been fed to boars (Paulenz *et al.*, 1995, 1999) have not improved fertility, although these studies were carried out against a background of good fertility. The aim of the current study was to characterize the effects of feeding tuna oil on the lipid and fatty acid composition of boar spermatozoa and to

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relate changes in composition to boar semen characteristics using boars of different ages with potentially different sperm quality.

Materials and Methods

Boars

Between June and August 1997, initial samples of semen were obtained from 12 Large White boars (*Sus scrofula*), which were in use for the production of semen for commercial artificial insemination at A. Simmers Ltd, Mains of Cairnbrogie, Old Meldrum, Aberdeenshire. The boars were maintained in individual pens (3.0 m × 3.2 m) in a ventilated building in which temperature was maintained below 25°C. Of the 12 boars, ten were paired by age and one of each pair randomly assigned to one of two diets (see below). At the start of the experiment, the age of the boars ranged from 395 to 761 days.

Diets

The diets were formed from a basal diet consisting of 660 g barley, 243 g wheatfeed, 34 g extracted rapeseed meal, 38 g whole rapeseed and 25 g mineral–vitamin supplement per kg fresh mass, which the boars had been fed before the experiment. With the exception of additional α-tocopherol acetate (as an antioxidant), the basal diet was fed alone as an unsupplemented control diet. The tuna oil (30 g kg^{-1}, Tuna Orbital Oil, Scotia Pharmaceuticals Ltd, Carlisle) was added to the basal diet as a supplement. The diets were fed as follows. The unsupplemented boars were fed 2.5 kg daily, consisting of 2.0 kg basal diet and 500 g of a premix obtained by mixing basal diet with 1.5 g α-tocopherol acetate kg^{-1}. Similarly, for the supplemented diet, boars were fed 2.575 kg daily, consisting of 2.0 kg basal diet and 575 g of a premix obtained by mixing basal diet with 150 g tuna oil and 1.5 g α-tocopherol acetate kg^{-1}. Therefore, the final concentrations of added α-tocopherol acetate were 300 and 291 mg α-tocopherol acetate kg^{-1} for the unsupplemented and supplemented diets, respectively. The premixes were prepared at the start of the experiment and stored at –20°C until required. The compositions of the diets as fed are given (Table 1) and they were fed to the boars for 6 weeks.

Semen collection

Semen was collected from all boars ($n = 12$) in duplicate at the start of the experiment and after 3, 5 and 6 weeks of feeding the experimental diets (single sample per week; $n = 10$). Semen was collected using the gloved hand technique (Hancock and Hovell, 1959); the boars were accustomed to the procedure, which was carried out once or twice per week by the same person. The total volume of the sperm-rich fraction was recorded and the sperm-rich fraction was filtered through gauze. The semen was maintained at 39°C until evaluation on the farm and during transport to the laboratory for centrifugation.

Semen evaluation

Raw semen density was estimated using a colorimeter and diluted for use with a commercial extender (Androhep, Minitub, Tiefenbach). The number of spermatozoa in diluted semen was determined in duplicate using a Makler counting chamber and phase-contrast microscopy. Sperm motility was also determined in duplicate using the Makler chamber: 100 spermatozoa were classified as having progressive motility, being motile but not progressive or being immotile.

Morphological analysis

Stained semen smears were prepared by mixing diluted semen with nigrosin–eosin stains to evaluate sperm

Table 1. Composition of diets fed to the boars

	Unsupplemented	Tuna oil
Dry matter (g kg^{-1})	916	918
Crude protein (g kg^{-1} dry matter)	150	143
Ether extract (g kg^{-1} dry matter)	65	92
Fatty acids (g per 100 g total fatty acids)		
16:0	15.8	16.2
16:1(n-7)	2.9	3.6
18:1(n-9)	33.9	28.8
18:2(n-6)	36.0	26.5
18:3(n-3)	7.9	6.3
20:4(n-6)	ND	0.7
20:5(n-3)	ND	2.0
22:6(n-3)	ND	8.5
Total (n-6)	36.0	28.1
Total (n-3)	8.0	17.3
Ratio (n-6):(n-3)	4.5	1.6

ND: none detected.

morphology and viability or with Giemsa stain to evaluate acrosome structures. The mixed semen and stain were incubated for 2–5 min at 37°C before preparing smears on microscope slides and then leaving them to dry. Stains were obtained from Sigma Chemical Co. (Poole). The nigrosin–eosin-stained slides were evaluated by examining 100 spermatozoa per slide in duplicate slides. Viable spermatozoa were defined as those that did not take up stain. Spermatozoa were examined for the following abnormal morphologies: detached head, abaxial head, malformed head, damage to acrosome cap, bent tail, coiled tail and presence of cytoplasmic droplets. Giemsa-stained slides were examined similarly to determine the occurrence of abnormal acrosome structures (Pursel *et al.,* 1972).

Feed analysis

Dry matter was determined by drying at 80°C for 24 h. Crude protein content was determined by the Kjeldahl method and ether extract according to the method of the Ministry of Agriculture, Fisheries and Food (1992).

Lipid extraction and analysis

Approximately 20 ml of the sperm-rich fraction of the ejaculate was transported to the laboratory at 39°C. The spermatozoa were separated from the seminal plasma by centrifugation at 700 ***g*** for 20 min at 4°C. The spermatozoa were re-suspended in the same volume of 9 g NaCl l^{-1} 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) (Christie, 1982). The lipids were fractionated into their major classes (phospholipid, free cholesterol, triacylglycerol, free fatty acids and cholesterol ester) by thin layer chromatography on silica gel G using a solvent system of hexane:diethyl ether:formic acid (80:20:1, v/v). After visualization under UV light after spraying with 0.1% (w/v) 2,7-dichlorfluorescein in methanol, the separated bands were scraped from the plates. Phospholipid was eluted from the plates by washing three times with 2 ml methanol, and the other lipid classes were eluted with diethyl ether. The esterified lipid fractions were subjected to transmethylation by refluxing with methanol:toluene:sulphuric acid (20:10:1, v/v) in the presence of a pentadecaenoic acid standard (Christie *et al.*, 1970). The resultant fatty acid methyl esters were analysed by a 1 µl injection via a CP9010 autosampler (Chrompack, London) onto a 30 m × 0.25 mm diameter, 0.25 µm film thickness Carbowax capillary column (Econo-Cap, Alltech UK Ltd, Carnforth). The following temperature program was used to separate the fatty acids: 185°C for 2 min; increase to 230°C at 5°C min^{-1} and 24 min at 230°C. Peak values were detected by flame ionization. Integration of the peaks using an EZ-Chrom Data Handling System (Speck Analytical, Alloa) enabled the determination of the fatty acid composition (percentage w/w of total fatty acids). The amount of each lipid class was calculated by comparison of the total fatty acid peak areas with that of the pentadecaenoic fatty acid internal standard (Christie *et al.*, 1970). Free cholesterol was determined by a colorimetric assay (Boehringer, Lewes).

Statistical analysis

Data for each individual abnormal morphology were summed to give a single value for cells with abnormal morphology in an ejaculate. The effects of feeding tuna oil were measured by a repeated measures ANOVA using Genstat (Lawes Agricultural Trust, 1987); sperm composition and characteristics at week 0 were used as covariates, and linear and quadratic orthogonal polynomials were used to assess the effects of time of feeding and of time × diet interactions. One boar was removed from the experiment for reasons unconnected with the diets fed and, therefore, data for 3, 5 and 6 weeks of feeding for the tuna oil supplemented group include an estimated missing value.

Results

Sperm characteristics *in vitro* are given (Table 2). Semen volume increased from week 3 to week 6 of the experiment (linear effect, $P < 0.05$), whereas the proportion of spermatozoa with abnormal morphology decreased (linear effect, $P < 0.01$). Feeding tuna oil significantly increased sperm viability ($P < 0.05$) and the proportion of spermatozoa with progressive motility ($P < 0.01$) and with a normal acrosome score ($P < 0.001$). For sperm viability and spermatozoa with a normal acrosome score, these changes became more marked as the experiment progressed (linear diet × time interaction; $P < 0.05$ for normal acrosome score).

The lipid and phospholipid fatty acid compositions of seminal plasma and spermatozoa at the start of the experiment are given (Table 3). The fatty acid compositions of cholesterol esters and triglycerides of spermatozoa are not shown. The fatty acid composition of cholesterol esters from spermatozoa were not influenced by the diets fed, and saturated (16:0 and 18:0, 54%) and monounsaturated (18:1, 38%) acids were quantitatively the most important fatty acids. Seminal plasma lipids and lipid fatty acid composition were more variable than those of spermatozoa (Table 3) and were not influenced by feeding tuna oil (data not shown). The predominant lipids in seminal plasma were phospholipids and free cholesterol. Phospholipid fatty acids of seminal plasma contained smaller amounts of long chain polyunsaturated fatty acids than did spermatozoa phospholipids.

As the experiment progressed (Table 4), the amounts of cholesterol ester (quadratic effect, $P < 0.01$) and free cholesterol (quadratic effect, $P < 0.05$) in the spermatozoa increased and the amounts of phospholipid decreased

Table 2. Effect of tuna oil supplementation on pig semen characteristics

Diet	Unsupplemented			Tuna oil					Significance of		
Week	0*	3	5	6	3	5	6	SED†	SED‡	Diet	Week	Diet × week
Semen volume (ml)	291	273	281	346	238	284	293	31.9	27.3	NS	L*	NS
Spermatozoa												
Concentration (10^8 cells ml^{-1})	2.81	2.13	2.78	1.97	4.02	3.78	4.12	0.690	0.581	NS	NS	NS
Viable (%)	70.9	75.3	71.0	71.3	76.4	77.7	80.4	1.97	2.35	**	NS	NS
Progressive motility (%)	62.9	65.4	70.1	72.0	77.8	78.0	76.6	1.80	1.68	**	NS	NS
Normal acrosome (%)	44.9	44.2	44.2	44.6	45.9	50.0	53.7	0.77	1.38	***	L*	L*
Abnormal morphology (%)	12.8	17.5	11.1	7.7	7.3	5.6	3.3	2.08	1.80	*	L**	NS

*Pre-experimental values used as covariate.
SED between † diets (15 observations) and § weeks (10 observations).
L, linear effect of time or time × diet interaction.

Table 3. Lipid composition of spermatozoa and seminal plasma from pig semen at beginning of experiment

	Spermatozoa		Seminal plasma	
	Mean	Standard deviation	Mean	Standard deviation
Total lipid*	149	42.7	16	2.9
Lipids (g per 100 g total lipid)				
Cholesterol	32.8	5.91	45.7	9.18
Cholesterol ester	1.2	0.61	9.4	5.86
Phospholipids	61.9	5.10	37.6	8.75
Phospholipid fatty acids				
Saturated	28.1	13.32	28.5	1.51
Monounsaturated	11.7	2.46	2.9	0.40
18:2(n-6)	9.5	3.36	2.1	0.21
20:4(n-6)	7.6	2.74	2.8	0.24
22:4(n-6)	6.2	2.13	1.9	0.12
22:5(n-6)	13.1	5.59	24.8	5.92
22:6(n-3)	13.9	9.95	32.9	4.80
Total (n-6)	40.7	9.38	33.5	4.89
Total (n-3)	18.0	11.35	33.6	5.19

Values are means for ten boars.
*μg per 10^8 spermatozoa; μg per ml of seminal plasma.

(linear effect, $P < 0.01$). When tuna oil was added to the diet, changes in fatty acid composition occurred only in phospholipid fatty acids. It should be noted that the method used to transmethylate phospholipid fatty acids measured only ester-linked fatty acids and not ether-linked fatty acids as occur in plasmalogens. Changes in phospholipid ester-linked fatty acids were noted after weeks 5 and 6 of the experiment and not after week 3. Therefore, there were marked diet × time interactions. Feeding tuna oil decreased the (n-6) fatty acid proportions, principally of 22:4(n-6) and 22:5(n-6), in total phospholipid fatty acids and increased the 22:6(n-3) proportions. Therefore, the ratio of total (n-6) to (n-3) fatty acids and of 22:5(n-6) to 22:6(n-3) significantly decreased after 5 weeks of feeding tuna oil.

Discussion

The objective of the present study was to evaluate whether feeding tuna oil changes sperm lipid composition and sperm characteristics *in vitro*.

The present evaluation of the composition of spermatozoa lipids and their fatty acids agreed broadly with previous studies on boar spermatozoa, with phospholipids being the most abundant lipid class (Komarek *et al.*, 1965; Johnson *et al.*, 1972). Phospholipids were also the dominant lipid in seminal plasma (Komarek *et al.*, 1965; Johnson *et al.*, 1969; Evans and Setchell, 1979). The most abundant fatty acids in sperm phospholipids were the long chain polyunsaturated fatty acids 22:5(n-6) and 22:6(n-3). There is some variance among studies in the amounts of 22:5(n-6) and 22:6(n-3) reported in sperm phospholipids. For example, Johnson *et al.* (1972) reported 34% 22:5(n-6) and 23% 22:6(n-3); Evans and Setchell (1979) reported 24% 22:5(n-6) and 43% 22:6(n-3); and Paulenz *et al.* (1995) reported 11% 22:5(n-6) and 29% 22:6(n-3) (none of these studies reported either the age or diet of the boars from which semen was obtained; both of these may be important factors influencing phospholipid fatty acid composition). The analysis at the start of the present study produced values of 25% 22:5(n-6) and 33% 22:6(n-3).

Another factor that might explain differences in sperm fatty acid composition among studies is the presence of ether-linked fatty acids in pig spermatozoa (Evans *et al.*, 1980; Brouwers *et al.*, 1998). In the present study, ether-linked fatty acids were not quantified by the derivitization procedure used. Brouwers *et al.* (1998) quantified the different molecular species of phosphatidyl choline from pig spermatozoa and found that approximately 60% of the phosphatidyl choline contained ether-linked fatty acids at the *sn*-1 position. However, the ether-linked fatty acids were saturated and predominantly 16:0, and the long chain polyunsaturated fatty acids were confined to the *sn*-2 position of the glycerol moiety. Therefore, changes in phospholipid fatty acid composition in response to feeding tuna oil, which primarily involved the replacement of 22:5(n-6) with 22:6(n-3), probably involved changes in the fatty acid esterified at the *sn*-2 position of both ether- and ester-linked fatty acids. The close relationship between 22:5(n-6) and 22:6(n-3) is confirmed by the highly signifi-

Table 4. Effect of tuna oil supplementation on pig sperm lipid and sperm phospholipid fatty acid (g per 100 g total fatty acids) composition

Diet	Unsupplemented			Tuna oil					Significance of		
Week	3	5	6	3	5	6	SED†	SED‡	Diet	Week	Diet × week
Lipids (g per 100 g total lipid)											
Free cholesterol	31.2	27.0	35.8	32.4	33.1	48.5	3.61	4.38	NS	Q*	NS
Cholesterol ester	2.3	10.6	6.6	1.8	11.4	7.1	1.25	1.63	NS	L*** Q**	NS
Phospholipid	66.4	62.3	57.5	65.9	55.4	44.4	3.44	3.76	NS	L**	NS
Phospholipid fatty acids											
Total saturated	27.3	26.0	22.8	25.1	27.3	30.4	3.01	1.62	NS	NS	L**
Total mono-unsaturated	4.2	3.2	2.7	3.8	3.0	3.6	0.21	0.25	NS	L ** Q*	L*
18:2(n-6)	2.6	1.1	1.1	2.1	0.8	1.1	0.12	0.11	NS	L***Q***	L*
20:4(n-6)	3.1	3.0	3.1	3.0	2.9	3.0	0.07	0.10	NS	NS	NS
22:4(n-6)	1.9	1.4	0.2	2.0	0.6	0.1	0.31	0.38	NS	L***	NS
22:5(n-6)	25.9	29.5	30.2	27.4	19.3	16.1	1.54	1.01	**	L**	L*** Q*
22:6(n-3)	33.6	32.5	36.4	33.7	40.1	41.2	1.65	1.51	*	L**	NS
Total (n-6)	35.6	37.0	36.6	36.1	25.7	21.9	1.21	1.02	***	L***	L*** Q*
Total (n-3)	33.8	34.9	38.8	33.9	42.8	43.0	1.75	1.46	NS	L***	Q*
Ratio (n-6):(n-3)	1.07	1.07	0.97	1.09	0.58	0.51	0.040	0.041	***	L***Q*	L*** Q**
Ratio 22:5(n-6):22:6(n-3)	0.79	0.91	0.85	0.83	0.47	0.38	0.041	0.039	***	L***	L***Q*

SED between † diets (15 observations) or ‡ weeks (10 observations).
L, linear and Q, quadratic effects of time or time × diet interaction; NS, not significant.

cant inverse relationship between the proportions of these two fatty acids ($r = –0.98$; $n = 12$; $P < 0.001$) in sperm phospholipids in duplicate samples obtained from the boars before the start of the experiment.

Age-related changes in sperm lipid composition and sperm quality have been noted in cockerels (Kelso *et al.,* 1997b) and bulls (Kelso *et al.,* 1997a). In cockerels, the amounts of phosphatidyl ethanolamine and the dominant phospholipid fatty acid, 22:4(n-6) decreased with age, as did the fertility of the spermatozoa. In bulls, sperm concentration and motility decreased with age and there were associated changes in lipid composition. Total sperm phospholipids and phosphatidyl ethanolamine and the proportion of 22:6(n-3) in phosphatidyl ethanolamine and phosphatidyl choline all decreased with age. Kelso *et al.* (1997a) suggested that changes in the activities of desaturase enzymes required for the synthesis of the long chain polyunsaturated fatty acids may be responsible for the decline in 22:6(n-3) concentrations. Indeed Brenner (1989) has observed that, in rat testicles, Δ6-desaturase activity, the rate-limiting step in polyunsaturated fatty acid synthesis, decreased with age. Therefore, it appears that sperm lipid composition changes with age, with undesirable consequences for sperm quality. In the present study, the inverse relationship between 22:5(n-6) and 22:6(n-3) proportions in sperm phospholipids at the start of the experiment was related to the age of the boar, and the ratio of 22:5(n-6) to 22:6(n-3) was demonstrated to be correlated positively with the age of the boar ($r = 0.82$; $n = 12$; $P < 0.001$). Although the effects of age *per se* or long-term exposure to an inadequate diet could not be separated in the present study, these data imply a relationship between sperm phospholipid fatty acid composition and age in boars, as in other species (Kelso *et al.,* 1997a,b), which may have functional consequences.

After 5 weeks of feeding tuna oil, there were changes in sperm phospholipid fatty acid proportions that were not apparent after 3 weeks feeding. Paulenz *et al.* (1995, 1999) have also fed fish oil (cod liver oil) to boars and reported changes similar to those observed in the present study in total sperm lipid fatty acids, that is, increases in 22:6(n-3) and decreases in 22:5(n-6) proportions in total sperm fatty acids. As in the present study, these changes became apparent only after 5 weeks of feeding the marine oil (Paulenz *et al.,* 1995). Spermatogenesis and epididymal transport are reported to take 34 and 10 days, respectively, in boars (Swierstra, 1968). Therefore, it seems that there is a point during spermatogenesis after which exogenous 22:6(n-3) cannot be incorporated into sperm phospholipids. The present study (between days 11 and 23) and that of Paulenz *et al.* (1995; between days 15 and 21) both indicate that a similar stage of spermatogenesis is important.

Changes in semen characteristics were observed both in relation to the diets fed and to the duration of the experiment. The increase in progressive sperm motility and the decrease in the proportion of spermatozoa with abnormal morphology occurred in both control and tuna-oil-containing diets and, thus, may be related either to seasonal effects or to the inclusion of 300 mg α-tocopherol kg^{-1} as an antioxidant in both the control and tuna oil-containing diets. The α-tocopherol was added to the diets to prevent oxidation of polyunsaturated fatty acids before feeding and represented the only change made to the diet the boars had been maintained on before the experiment. Marin-Guzman *et al.* (1997) have also found that inclusion of 220 mg kg^{-1} α-tocopherol in the diet of boars improved sperm morphology but differences were not apparent until 8 weeks after feeding commenced. Therefore, it is difficult to establish reasons for the time-related improvements in sperm quality.

Supplementing the diet of the boars with tuna oil changed sperm characteristics in the present study favourably, increasing viability, and the proportions of spermatozoa with progressive motility and normal morphology. This finding is in contrast to that of Paulenz *et al.* (1999) who found no improvements in sperm motility or acrosome integrity after fish oil supplementation. However, initial values for motility and acrosome integrity were markedly higher in the study of Paulenz *et al.* (1999) than they were in the current experiment (motility: 78 versus 71%; acrosome integrity: 98 versus 45%), indicating that responses to the inclusion of fish oil in the diet may depend on initial sperm quality.

In conclusion, feeding tuna oil to working boars specifically changed sperm phospholipid fatty acid proportions and improved sperm quality *in vitro*. Since, in humans, Zalata *et al.* (1998) have reported significant positive correlations between phospholipid 22:6(n-3) status and the concentrations of motile spermatozoa and spermatozoa with normal morphology, nutritional supplements of fish oil may also improve the quality of human spermatozoa.

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