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Effects of vitamin E and phosphatidylcholine on qualitative and quantitative parameters of rainbow trout (*Oncorhynchus mykiss*) milt

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ABSTRACT

The aim of this work was to investigate the effects of vitamin E and phosphatidylcholine on rainbow trout (*Oncorhynchus mykiss*) milt. One hundred and twelve rainbow trout (RB) broodstock (2n, 1030± 20g body weight, male:female ratio = 50:50) were fed four isoproteic and isolipidic diets for 110 days. Diets were differing for the type of vitamin premix and phosphatidylcholine supplied: Control (vitamin premix without Vit. E, no phosphatidylcholine); Vit.E (a premix with Vit. E, no phosphatidylcholine); PhC, (vitamin premix without Vit. E, phosphatidylcholine 2.5%); Vit.E +PhC, (vitamin premix with Vit. E and phosphatidylcholine 2.5%).

Sperm total volume, in sexually mature males (3+; 966±114g body weight), ranged between 18.57ml (Control) and 34.31ml (Vit. E). Sperm density varied between $1.76x10^9$ Szoa/ml (Control) and $1.16x10^9$ Szoa/ml (Vit. E+PhC), while relative density (related to male body weight) tended to increase with Vit. E (>$50x10^9$ Szoa/ml) and to reduce with Vit. E + PhC (<$30x10^9$ Szoa/ml). Percentage motility was >85% in all treatments, while motility duration was around 2.37min for Vit. E and Control reached only 0.97min. After overnight storage (+4°C, for 18 hours) motility decreased, 75-80% in gamete motility and 3.39-56.7% in time motility.

PhC dietary supplements significantly increased arachidonic acid contents of sperm with respect to Control (>120 vs 73µg/ g), while Vit. E caused a huge increase in C20:3 n-3 (10.25 vs 2.27ppm). DHA/EPA ratio was significantly lower in Control (>2; $p<0.05$), while n-3/n-6 ratio was significantly the highest for Vit. E (9.46 vs <7.3).

Key words: Oncorhynchus mykiss, Vitamin E, Phosphatidylcholine, Milt, Fatty acid composition.

RIASSUNTO

EFFETTI DELLA VITAMINA E E DI FOSFATIDILCOLINA SUI PARAMETRI QUALITATIVI E QUANTITATIVI DI SPERMA DI TROTA IRIDEA (*ONCORHYNCHUS MYKISS*)

*Lo scopo di questa prova è stato di studiare gli effetti di vitamina E e fosfatidilcolina sullo sperma di trota iridea (*Oncorhynchus mykiss*). Centododici riproduttori di trota iridea (RB) (2n, 1030±120 g peso vivo, rapporto maschi: femmine = 50:50), sono stati alimentati, per 110 giorni, con quattro diete isoproteiche ed isolipidiche, tra loro differenti per il tipo di premix vitaminico apportato e la fosfatidilcolina aggiunta:*

Controllo (premix vitaminico senza Vit. E; no fosfatidilcolina); Vit. E (premix vitaminico con vitamina E; no fosfatidilcolina); PhC, (premix vitaminico senza Vit. E; fosfatidilcolina 2,5%); Vit. E +PhC, (premix vitaminico con vitamina E; fosfatidilcolina 2,5%).
Il volume totale di sperma nei maschi (3+ ; 966±114g peso vivo) sessualmente maturi è variato tra 18,5 ml (Controllo) e 34,31ml (Vit. E). La densità spermatica è variata tra 1,76x10^9 Szoa/ml (Controllo) e 1,16x10^9 Szoa/ml (Vit. E+PhC), mentre la densità relativa (al peso del maschio) tendenzialmente è cresciuta con Vit.E (>50x10^9 Szoa/kg p.v.) e si è ridotta con Vit.E+PhC (<30x10^9 Szoa/kg p.v.). La motilità % è risultata >85 per tutti i trattamenti, mentre la durata della motilità circa 2,37min per Vit. E e solo 0,97min per Controllo. Dopo refrigerazione notturna (a+4°C per 18 ore), la motilità spermatica si è ridotta del 75-80% come numero di spermi e del 3,39-56,64% come tempi di movimento.
L'aggiunta di PhC nella dieta ha aumentato significativamente il contenuto di acido arachidonico nello sperma rispetto al Controllo (>120 vs 73µg/g), mentre Vit.E ha causato un significativo aumento di C20:3 n-3 (10,25 vs 2,27 µg/g). Il rapporto DHA/EPA è risultato significativamente maggiore in Controllo (>2; p<0,05), mentre il rapporto n-3/n-6 è stato maggiore per Vit. E rispetto gli altri trattamenti (9,46 vs <7,3).

Parole chiave: Oncorhynchus mykiss*, Vitamina E, Fosfatidilcolina, Liquido seminale, Composizione acidi grassi.*

Introduction

The high concentration of polyunsaturated fatty acid in the spermatic membrane is essential for its fluidity and to assure the gamete fusion, even if it is more prone to the attacks by reactive compounds (Beckman and Ames, 1998). The harmful effect of the reactive oxygen substances (ROS) on spermatozoa consists on affecting motility, membrane fluidity, fertility and the ability to fuse with the oocyte (Sakai *et al.*, 1992). Besides, cytoplasmatic modifications, alterations in the mitochondrial functionality and malformation of the axoneme can be observed (Sakai *et al.*, 1992). Aitken (1999), in particular, underlined the tight relation between sperm quality and ROS concentration. In general, all fish tissues, typically characterised by high levels of polyunsaturated fatty acids, are particularly affected by this type of degeneration (Mourente *et al.*, 1999) and the role of vitamin E is very important in inhibiting the oxidative reactions and protecting the biomembrane from degenerative damages (Pickova *et al.*, 1999). Furthermore, in the reproductive sphere, this fat-soluble vitamin would stimulate the synthesis of the fertility hormones and would guarantee testicular functionality (Pickova *et al.*, 1999). In 1993, the Committee on Animal Nutrition of American NRC (1993) suggested a general vitamin E supplement around 50mg/Kg for fish in the diet, but Lygren *et al.* (2000) pointed out that the daily doses must be corrected, considering diet and environmental interactions. Recently it was demonstrated that a low concentration of vitamin E reduces the rate of fish broodstock fertility and affects gamete and fry quality (Roselund, 2003). During the last phase of gonad development, this vitamin is transferred from the muscular tissue (site of accumulation) to the reproductive apparatus (Roselund, 2003). This process happens during the physiological fasting before the final sexual maturation and for this reason, the amount of vitamin E accumulated in the previous months is very important (Roselund, 2003). Some experiments on Salmonids tested diets with different levels of polyunsaturated fatty acids, associated with 60 or 270mg of vitamin E. The results demonstrated that high levels of fatty acids (almost 60g/Kg) and the highest concentration of vitamin E had the best

reproductive performances in terms of fry survival rate. Furthermore the trials showed that the requirement of this vitamin is related also to the level of fatty acids in the diet (Roselund, 2003).

The importance of phosphatidylcholine, and, in general, of phospholipids in fish nutrition is related to several facts. First, it is an important component of biomembranes and of lipoproteins and also plays a special role in the metabolism of spermatozoa (Lahnsteiner *et al.*, 1993). Salmonids have an external fecundation so the spermatic cell, in the aquatic *medium*, employs exclusively its energetic deposits stored during the spermiation and the spermiogenesis (Stoss, 1983). Different experiments showed that the addition of phosphatidylcholine in the diet of fish enhances their productive and reproductive performances (Kanazawa, 1991; Orthoefer *et al.*, 1995; Koven *et al.*, 1998). Phosphatidylcholine also has important antioxidant properties, reducing the peroxidation and degradation of lipids (Koven *et al.*, 1998). Its limited use is mainly due to its cost, but also to the lack of data concerning the optimal levels of inclusion and the best type. In fact, the requirements vary with the species, the size, the physiological stage, the environmental conditions, the diet sources and composition (Wilson, 1991). Another role of phospholipids in fish physiology is related to the thermal adaptation, which causes a modification in lipid metabolism. A temperature change causes the modification of biomembrane lipid composition in order to preserve membrane fluidity. Phosphatidylcholine protects the cytoplasm from pressure and cold shocks (Pustowka *et al.*, 2000). A modification in fatty acid composition of phospholipids is possible and, consequently affects membrane characteristics, such as fluidity, protein transport ability, permeability and cryoconservation stress resistance (Bell *et al.*, 1996). Cytoplasmatic fluidity is fundamental for the biological functionality of sperm membrane because it affects both the fusion with the egg cytoplasm and the capacity to survive cryoconservation. This physical property varies with the unsaturation degree and the position of double bonds in fatty acids present in the membrane, phospholipid disposition, cholesterol concentration and the type of bonds between phospholipids and proteins (Drobnis *et al.*, 1993). For this reason, a dietary source of phospholipids can become an important tool in the enhancement of sperm quality.

The aim of the present work was to evaluate the effects of these two compounds: vitamin E and phosphatidylcholine, alone or combined, on qualitative and quantitative parameters of rainbow trout (*Oncorhynchus mykiss*) milt.

Material and methods

Fish and facilities

One hundred and twelve rainbow trout (RB) broodstock, born and farmed in the experimental farm of the Animal Husbandry Department of Turin University were used. The batch was 2n, 3+, 1030±120g body weight, with male:female ratio = 50:50 and was randomly distributed in 8 fibreglass tanks (3m^3 of capacity). A well (80m depth) supplied water with constant temperature (13°C) and dissolved oxygen (7.6mg L^{-1}). The water flow in each tank was 0.2-0.3lsec^{-1}. Fish were submitted to natural photoperiod during the year and manual stripping in mid-December. Male body weight was 966±114g.

Diets

Four isoproteic and isolipidic diets were formulated, differing for the type of vitamin

premix supplied and phosphatidylcholine addition.

C: premix without vitamin E and no phosphatidylcholine;

Vit. E: premix with vitamin E, no phosphatidylcholine;

PhC: premix without vitamin E, 2.5% phosphatidylcholine;

Vit. E +PhC: premix with vitamin E, 2.5% phosphatidylcholine;

In Table 1 the formulation of the diets and the proximate analysis, according to AOAC methods (1995) are reported.

The fatty acid composition was determined, after lipid extraction (Folch *et al.*, 1957), by gas liquid chromatographic separation on fatty acid methyl esters, using a 17 A Shimadzu gas-chromatograph (Owen: 1min at 180°C, from 180°C to 225°C at 5°C/min for 30min); Carrier: Nitrogen, 24ml/min, 17cm/sec; Detector: FID, 270°C; Injector: 0.3μl, 250°C; Column: DB-WAX

Table 1. Formulation and proximate composition of the experimental diets.

		Control	Vit.E	PhC	Vit.E+PhC
Ingredients (%):					
Fish meal		60	60	60	60
Corn meal		10	10	10	10
Cod liver oil		14	14	14	14
Wheat starch		14	14	11.5	11.5
Mineral premix (1)		1	1	1	1
Premix without Vit.E (2)		1	-	1	-
Premix with Vit.E (3)		-	1	-	1
Phosphatidylcholine		-	-	2.5	2.5
Chemical composition:					
Dry matter	%	91.50	94.10	94.10	93.80
Crude protein	% DM	49.60	48.20	48.80	49.00
Crude fats	"	21.10	21.20	22.06	22.20
Ash	"	9.50	8.90	9.10	9.40
Crude fibre	"	0.85	0.70	0.80	0.90
NF extract	"	18.95	21.00	19.24	18.50

[1] Mineral premix (g or mg/kg diet): bicalcium phosphate 500 g, calcium carbonate 215 g, sodium salt 40 g, potassium chloride 90 g, magnesium carbonate 124 g, iron sulphate 20 g, zinc sulphate 4 g, copper sulphate 3 g, potassium iodide 4 mg, cobalt sulphate 20 mg, manganese sulphate 3 g, sodium fluoride 0.98 g (Granda Zootecnica, Cuneo, Italy).

[2] Vitamin premix (U or mg/kg diet): sodium menadione bisulphate, 5 mg; retinyl acetate, 15,000 U; DL-cholecalciferol, 3000 U; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B_{12}, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium panthotenate, 50 mg; choline chloride, 2000 mg (Granda Zootecnica, Cuneo, Italy).

[3] Vitamin premix (U or mg/kg diet): the same ingredients as [2] Vitamin premix, DL-a tocopherol acetate, 60 U.

(J&L), 60m x 0.53mmID x 1μm film. In Table 2, the fatty acid composition of the diets is reported and it can be emphasized that the fatty acid composition of Control and Vit. E diets were similar, but not for linoleic acid, while diets with PhC supplements were quite different, in particular for oleic acid, total n-3, n-6 contents and n-3/n-6 ratio.

Fish were fed the experimental diets 6 days a week for 110 days. The feeding level during the experimental period was 1% of the average total biomass in the tanks.

Table 2. Fatty acid composition of the experimental diets (% total acids).

Diets	Control	Vit. E	PhC	PhC+Vit. E
C14:0	7.23	6.85	6.13	5.54
C14:1	0.30	0.25	0.28	0.29
C15:0	0.57	0.54	0.51	0.45
C15:1	0.14	0.17	0.14	0.15
C16:0	19.25	19.44	20.47	19.56
C16:1	7.81	7.44	6.46	7.11
C17:0	0.70	0.90	0.91	0.85
C17:1	0.50	0.69	0.56	0,.43
C18:0 ISO	0.45	0.59	0.49	0.43
C18:0	3.88	3.54	3.63	3.66
C18:1 n-7	0.94	0.83	0.86	2.12
C18:1 n-9	18.59	17.92	20.13	20.31
C18:2 n-6	2.37	6.23	8.00	8.94
C18:3 n-6	0.21	1.88	0.77	1.08
C18:3 n-3	1.52	1.39	0.98	0.73
C20:1 n-9	2.79	2.89	2.42	2.13
C20:5 n-3	8.17	5.72	6.58	6.46
C20:2 n-6	0.87	0.76	0.65	0.55
C20:4 n-6	0.83	0.87	0.94	0.79
C22:1 n-1	10.54	8.75	7.04	7.63
C22:6 n-3	12.33	12.31	12.05	10.81
∑ SATURATED	32.08	31.86	32.14	30.49
∑ MUFA	41.61	38.94	37.89	40.17
∑ PUFA	26.30	29.16	29.97	29.36
∑ UNSATURATED	67.91	68.10	67.86	69.53
∑ SAT./∑ UNSAT.	0.47	0.47	0.47	0.44
∑ n-3	22.02	19.42	19.61	18.00
∑ n-6	3.45	8.87	9.42	10.57
∑ n-9	21.38	20.81	22.55	22.44
∑ n-3/∑ n-6	6.38	2.19	2.08	1.70
DHA/EPA	1.51	2.15	1.83	1.67
DHA/AA	14.85	14.15	12.82	13.68

Reproductive parameters

Sexually mature males were sampled in order to determine the following variables:

1. Pre-stripping male body weight (g);
2. Total and relative sperm volume (ml and ml/kg bw);
3. Sperm density (Szoa*10^9/ml and Szoa*10^9/Kg bw);
4. Spermatozoan motility (% and min) of fresh or stored (for 18h at 4°C) samples;
5. Sperm fatty acid composition, with the same procedures previously described for diets.

After anaesthesia, 7-8 fish per treatment were rinsed with fresh water to wash out all the anaesthetic and, with a gentle abdominal pressure the total amount of sperm was collected in graduated glass tubes. During stripping, small samples of sperm were carefully collected in order to avoid any contamination with organic fluids. Glass tubes were kept on ice and immediately analysed. One µl for each male was mixed with a drop of distilled water and then observed on a microscope slide (400 x magnification) without the addition of specific extenders. The rate of spermatozoa showing forward motility (immediately after addition of salt water) and the duration of flagellar movements (of at least 5% of the observed spermatozoa in the sample) were evaluated at two different times after collection. The first evaluation was performed immediately after milt collection and the second after overnight (18h later) storage at +4°C, in order to determine the survival of sperm.

Density was measured by counting the number of spermatozoa of a diluted sample of sperm in distilled water (10,501 X) in a Bürker hemocytometer under 400 x magnification (Rainis *et al.*, 2003). To avoid bias, all tests on sperm quality was carried out by the same person.

Statistical analysis

Data were submitted to one-way ANOVA. The comparison between means was performed with LSD method (Snedecor and Cockran, 1982).

Results and discussion

In this trial the effects of vitamin E were not observed on milt volume and on relative density of spermatozoa. The total volume of sperm ranged between 18.57 ml (Control) and 34.31ml (Vit. E) (Table 3). The relative volume presented the same trend, with a tendency to decreased values for Control (19.79ml/Kg bw) and highest for Vit. E treatment (38.13ml/Kg bw) (Figure 1). The density of sperms varied between 1.76x10^9 Szoa/ml (Control) and 1.16x10^9 Szoa/ml (Vit. E + PhC). The rela-

Figure 1. Sperm relative volume of rainbow trout fed Vitamin E and phosphatydilcholine.

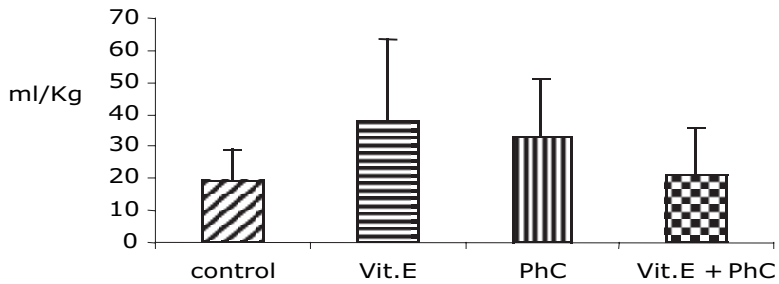
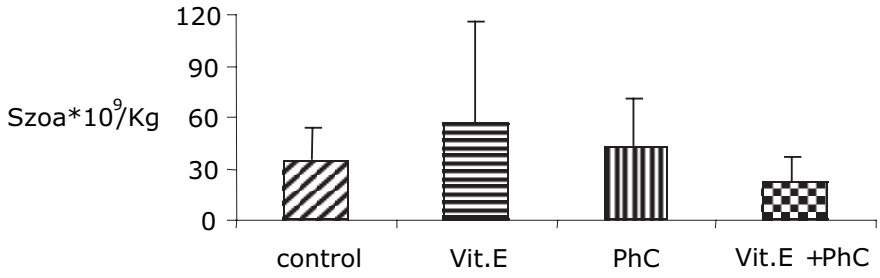


Figure 2. Sperm relative density (Szoa*10^9/Kg) of rainbow trout fed Vitamin E and phosphatidylcholine.



tive density, on the other hand, tended to increase with Vit. E and to reduce with Vit. E + PhC (Figure 2). The absolute densities of RB milt obtained in this trial agree with those reported by Campbell et al. (1994) (13-16x109 Szoa/ml), and Ciereszko et al. (1996) (11,8x10^9 Szoa/ml) for 2nd or 3rd spawning seasons. In fact, the first spawning season is usually modest for RT males, both for semen volume and length of the reproductive phase (Buyukhatipoglu and Holtz, 1984).

Motility of fresh sperm was high for all treatments; Control reached 100% of motile spermatozoa, while Vit. E and PhC were <90% (Table 3). In any case, the

Table 3. Total volume, density and motility of sperm (% and duration, min) of RB fed vitamin E and phosphatidylcholine.

	Control	Vit. E	PhC	Vit.E +PhC	SE
Volume of sperm ml	18.57	34.31	26.38	24.64	15.43 (26 df)
Density Szoa/ml x 10^9	1.76	1.18	1.41	1.16	0.58 (26 df)
Motility:					
%	100.00	89.90	87.50	95.70	22.71 (26 df)
min	0.99	2.37	1.55	2.11	1.02 (25 df)
Motility after 18h (overnight):					
%	25.00	11.86	21.63	16.57	19.71 (22 df)
min	0.92^{ab}	0.55^{a}	0.84^{ab}	1.20^{b}	0.34 (22 df)
Reduction of motility:					
%	75.00	75.28	77.56	80.37	27.23 (22 df)
min	3.39	56.64	41.60	34.80	34.48 (21 df)

a, b, : P <0.05; df: degree of freedom.

*[1] ((Fresh sample motility- storage sample motility)/fresh sample motility)*100.*

motility duration was around 2.37min for Vit.E, while Control reached only 0.97min. Nevertheless, no significant difference for both parameters was stressed out by statistical analysis.

The motility observed after overnight storage of the samples was characterised by a clear reduction compared to fresh sperm: more as percentage of motile gametes (75-80.37%) than time of motility (3.39-56.64%). In Control males the fresh and after storage values were quiet similar (only a reduction around 3.39%). Statistically, Vit. E showed a duration of motility significantly lower than Vit.E + PhC (0.55 *vs* 1.20min). Duration times for the other two treatments were in the middle: 0.92 and 0.84 for Control and PhC diets, respectively ($p<0,05$). Seventy-five % motility is considered the limit for a good quality sperm, by Lahnsteiner *et al.* (1998) and in this trial motility was always higher than 85% for all treatments. There was a positive trend to increase the duration of spermatozoa motility with Vitamin E, alone or associated with phosphatidylcholine. According to the positive effects of vitamin E on biomembranes, reported by Roselund (2003), this vitamin should positively affect also spermatic cell resistance to fresh storage, but this hypothesis was not confirmed in our trial. The physiological reduction of the percentage and duration of motility after overnight refrigeration were quite similar for all treatments, although statistically significant for the duration of motility (0.55min) in Vit. E diet. Probably sperm of fish that fed on the diet enriched with vitamin E (alone or with phosphatidylcholine) was stimulated to move longer immediately after spawning, but the effect of Vitamin E does not last (at least when sperm is stored at +4°C), probably because spermatozoa quickly consume their energetic reserves. This would explain why the analysis after 18 hours for the duration of motility in Vit. E treatment was the lowest. The supplement of phosphatidylcholine associated with Vit. E, tends to reduce this effect and the duration of the motility after storage was the highest (1.2min). It has to be understood if it was Vit. E to protect phosphatidylcholine from catabolism or if phosphatidylcholine plays, in some way, an energetic role that lets sperm move for a longer period, also hours after spawning.

In this trial, the specific vitamin or lipid supplements affected the fatty acid composition of RB trout sperm. In general, it is possible to observe a trend toward lower single fatty acid contents of Control with respect to the other dietary treatments (Tables 4 and 5). In particular, dietary PhC supplements significantly increased arachidonic acid (AA) contents of sperm with respect to Control (>120 *vs* 73μg/g), whilst Vit. E caused a huge increase in C20:3 n-3 (10.2 *vs* 2.27μg/g). DHA/EPA ratio was significantly higher than 2 in Control ($p<0.05$), while n-3/n-6 ratio was significantly higher for vit.E with respect to the other treatments (9.46 *vs* <7.3). Also worth noting in this case is the general trend to low contents of the main fatty acid families (SFA, MUFA, PUFA, n-3, n-6, n-9) for Control with respect to the other treatments (Table 5). Also Pustowka *et al.* (2000) observed that the dietary acidic profile directly affects the composition of the cytoplasmatic membrane of sperm, which in turn affects spermatic cell functionality (fusion ability with egg) and its resistance to freezing/thawing stress. The same authors concluded that dietetic lipids are very important in determining membrane characteristics and that the differences in the phospholipid composition reflects

Table 4. Sperm fatty acid composition of RB fed vitamin E and phosphatidylcholine (μg/g of tissue).

	Control	VIT. E	PhC	Vit. E +PhC.	SE (17df)
C10:0	1.11	0.96	0.99	1.97	1.58
C12:0	0.38	0.07	1.04	0.94	0.70
C14:0	23.56	49.83	71.97	55.42	34.00
C15:0	4.61	12.37	12.96	10.51	5.82
C16:0	347.96	711.90	853.98	692.84	351.00
C16:1n-7	21.74	53.77	65.84	41.91	33.35
C16:2n-4	2.35	5.62	3.52	2.01	5.03
C17:0	4.75	11.14	9.21	8.08	3.65
C16:3n-4	2.16	5.54	6.73	4.00	3.47
C16:4n-1	3.64	3.32	5.39	3.85	2.92
C18:0	85.98	170.36	159.95	133.60	63.15
C18:1n-9	154.19	329.68	336.86	261.93	145.89
C18:1n-7	90.05	180.63	182.21	156.75	0.70
C18:2n-6	31.13	83.58	110.77	76.22	51.50
C18:2n-4	0.24	3.77	2.32	1.17	4.89
C18:3n-6	3.71	9.85	7.21	3.33	6.80
C18:3n4	1.99	4.42	5.22	5.08	3.98
C18:3n-3	5.34	12.60	12.83	9.60	6.58
C18:4n-3	6.13	8.56	10.58	4.43	6.12
C18:4n-1	4.39	3.57	8.05	3.84	6.50
C20:0	2.14	0.97	2.10	3.07	2.19
C20:1n-9	17.40	29.84	38.04	33.07	19.80
C20:3n-9	2.93	14.99	5.37	6.56	8.68
C20:2n-6	6.96	16.21	16.96	20.08	9.63
C20:3n-6	3.95	4.68	5.93	8.07	6.46
C20:4n-6	73.57^{B}	40.92^{C}	127.09^{A}	125.04^{A}	55.00
C20:3n-3	2.27^{B}	10.25^{A}	2.99^{B}	1.91^{B}	32.31
C20:4n-3	10.43	24.95	19.66	15.93	7.38
C20:5n-3	305.57	670.17	606.39	553.68	239.00
C22:0	3.32	3.04	3.31	1.41	2.29
C22:1	7.81	15.44	15.44	7.03	10.60
C21:5n-3	6.05	9.02	7.16	3.86	5.19
C22:4n-6	21.19	50.16	22.23	38.75	21.42
C22:5n3	49.96	101.43	77.04	96.20	36.93
C24:0	3.49	19.48	4.00	8.60	14.70
C22:6n-3	627.60	1075.94	979.30	807.58	352.06
C24:1	3.09	187.89	5.31	1.85	188.30

A, B, C: P<0.01.

Table 5. Main sperm fatty acid classes of RB fed vitamin E and phosphatidylcholine (μg/g of tissue).

	Control	VIT. E	PhC	VIT. E +PhC.	SE (17df)
Σ SFA	477.32	980.12	1119.51	916,49	549.12
Σ MUFA	294.29	797.25	643.70	502.57	3221.77
Σ PUFA	1171.65	2169.02	2187.72	1791.28	926.88
Σ USFA	1465.95	2726.39	2871.25	2293.85	1239.98
Σ SFA/USFA	0.33	0.32	0.41	0.41	0.05
Σ n-3	1013.40	1945.01	1715.99	1529.04	616.60
Σ n-6	140.53	205.4	290.12	278.78	121.80
Σ n-9	174.52	374.51	380.27	307.86	166.69
Σ n-3/S n-6	7.25^{B}	9.46^{A}	5.91^{B}	5.49^{B}	3.06
DHA/EPA	2.04^{a}	1.64^{b}	1.63^{b}	1.49^{b}	0.20
DHA/AA	8.54	58.93	7.67	6.41	28.68

a, b: P<0.05; A, B: P<0.01.

those among diets. Sargent *et al.*, (2002) affirmed that fatty acid composition of phosphatidylcholine is more affected by diet than phosphatidylinositol and phospahtidyletanolamine. Gasco *et al.* (1999) demonstrated that the concentration of each fatty acid in rainbow trout sperm was influenced by the modification in lipid source. For example, broodstock males feeding on peanut oil produced a sperm characterised by an increased level in C18:2n-6, while diets with soybean determined the production of a milt with higher concentrations of C18:3n-6. In this trial, a significant increase in C20:3n-3 was observed in fish fed Vit. E diet and, in general, a tendency to increase n-3 single fatty acids. Consequently the n-3/n-6 ratio significantly increased for Vit. E.

No direct comparison of diet and sperm fatty acid classes can be made due to the endogenous biosynthesis and bioconversions, regarding also the different lipids composing the cell membranes (Sargent *et al.*, 2002). Vitamin E action could explicate its protective function on different lipid classes, for example on phosphatidylcholine. Unfortunately, the lack of class analysis of the sperm lipid in this work does not allow us to highlight a sharp positive effect of this vitamin on spermatozoa motility.

Conclusions

Diet is an important tool for modifying gametes characteristics, not only in female but also in male fish broodstock (density, motility, membrane fatty acid composition). Vitamin E (60 IU/kg diet) seems beneficial on sperm only when it is fresh. No positive effect of Vitamin E was observed on stored samples. The combination of Vitamin E and phosphatidylcholine seems to assure longer motility duration of stored sperm than Vitamin E alone. Further experiments are necessary to determine the most effective levels of Vitamin E and phosphatidylcholine to enhance the possible synergy among these two compounds and to define the more suitable moments of administration to the broodstock male.

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