

ZINC AND SELENIUM IN TISSUES OF
YOUNG ATLANTIC SALMON *SALMO SALAR*
FED DIETS CONTAINING DIFFERENT LIPID SOURCES
AT TWO LEVELS OF VITAMIN E

By

AMUND MAAGE and RUNE WAAGBØ
Institute of Nutrition, Directorate of Fisheries
P.O. Box 1900, Nordnes, N-5024 BERGEN, NORWAY

ABSTRACT

Six duplicate groups of young Atlantic salmon weighing about 30 g, were fed extruded dry feeds with three lipid sources (soyabean oil, capelin oil and sardine oil) with or without addition of 300 mg α -tocopheryl acetate per kg diet.

The three lipid sources were chosen to provide three levels of dietary (n-3) polyunsaturated fatty acids. After about five months feeding in tanks supplied with brackish water the fish were transferred into net pens in the sea and the feeding regime was continued for another seven months before sampling.

During the experiment there were no significant differences in growth between the groups.

The zinc status in the fish was measured as zinc concentrations in serum, liver, muscle, spleen and vertebrae in addition to serum alkaline phosphatase activity. There were no significant differences in zinc status between the six dietary treatments.

Selenium status was measured as hepatic, serum, muscle, spleen and vertebrae selenium concentrations. There was a marked difference in selenium status depending on the dietary lipid source, soyabean oil giving the highest and capelin oil the lowest selenium status. Selenium status was not affected by the supplementation of dietary α -tocopheryl acetate.

INTRODUCTION

The trace elements zinc (Zn) and selenium (Se) are necessary for good growth and health in fish (Lall, 1989), even though exact dietary requirements are difficult to quantify (NRC, 1981). The intestinal absorption of the elements are susceptible to dietary interactions with other micronutrients as well as with other feed components (Hilton, 1989; Maage *et al.*, 1990a). Such interactions may give poor growth and negatively affect fish health (Ketola, 1979).

There is evidence of substantial interactions between zinc and fatty acids (Hambidge *et al.*, 1986) and it has been suggested that increased levels of essential or unsaturated fatty acids enhance zinc absorption in rats (Hamilton *et al.*, 1981; Cunnane, 1982; Knudsen *et al.*, 1989). Interactions between trace elements and dietary lipids in fish nutrition have, to our knowledge, not been reported.

The dietary requirement of vitamin E (as α -tocopherol) in fish is affected by the dietary lipids (Cowey *et al.*, 1981, 1983; Watanabe *et al.*, 1977, 1981). Further, there is an interaction between dietary vitamin E and selenium in the body's defence against lipid oxidation in fish (Poston *et al.*, 1976). A high content of dietary polyunsaturated fatty acids is a potential risk of *in vivo* oxidation which enhance the dietary vitamin E requirement.

Elevated levels of (n-3) fatty acids and vitamin E have been suggested to improve fish health (Hardie *et al.*, 1990; Salte *et al.*, 1988).

The present study reports on the effect of three dietary lipid sources with different levels of (n-3) unsaturated fatty acids and vitamin E on the tissue levels of zinc and selenium in Atlantic salmon (*Salmo salar*).

A preliminary account was given orally at the Third International Symposium on Feeding and Nutrition in Fish, Toba, Japan, August 28th-Sept 1st, 1989.

MATERIALS AND METHODS

Fish

Six duplicate groups of Atlantic salmon (*Salmo salar*) were used. Initially 750 fish per tank (1.5m x 1.5m x 1.5m), weighing approximately 30 g were set up in brackish water. After 5 months the fish (n = 450 per group) were transferred to net sea pens (3m x 6 m x 6m) and fed the experimental diets for an additional seven months.

The fish were fed continuously by automatic feeders the first seven months and when transferred to net sea pens, they were fed to satiety twice a day.

At the end of the feeding period five fish were randomly sampled from each net pen (medio March). The fish were killed by a blow on the head, weighed, blood samples were collected from the caudal vein and organs dissected, weighed, freeze dried and homogenized for mineral analyses. Serum samples were stored at -80 °C until analyses.

At the time of sampling (March) the water temperature was about 8 °C.

Diets

Extruded dry pellets containing per kg: 330 g fish meal, 290 g soyabean protein concentrate, 160 g extruded wheat and 60 g vitamin/mineral mix

Table 1. Fatty acid composition of the different experimental diets.

Analyses	Lipid sources		
	Soyabean oil	Capelin oil	Sardine oil
Sum saturated (%)	16.6	21.3	24.9
Sum Monoenes (%)	24.8	51.1	27.1
Sum Polyenes (%)	58.4	25.0	42.1
(n-3) PUFA (%)	11.7	19.8	36.4

were made by Skretting A/S, Norway. The pellets were coated with 16% (w/w) of either soyabean oil (A/S DeNoFa Lilleborg Fabrikker, Norway), capelin oil (Norsildmel, Norway) or sardine oil (J.C. Martens & Co., Norway) each with (300 E) or without (0 E) addition of 300 mg vitamin E as α -tocopheryl acetate (Rovimix E 50, Roche) per kg feed. The sardine oil was stabilized with 0.2 g ethoxyquin/L oil.

Proximate analyses showed the content of protein, fat and ash to be 480, 180, 60 g/kg, respectively. The element composition of the diets were as follows: Ca: 10.1 g/kg, P: 9.1 g/kg, Mg: 1.7 g/kg, Zn: 352 mg/kg, Fe: 193 mg/kg, Cu: 17.5 mg/kg and Se: 1.3 mg/kg.

The fatty acid composition (summed classes of lipids) of the feeds are given in Table 1. The analysed content of vitamin E as -tocopherol in the feeds were about 50 and about 270 mg/kg feed in the 0 E and 300 E group, respectively. For further details on experimental procedures see Waagbø *et al.* (1990).

Analyses

Freeze dried samples of muscle, liver and vertebrae as well as serum and feed samples were digested in nitric/perchloric acid (9:1) according to Julshamn and Andersen (1982).

Calcium, phosphorous, magnesium, zinc, iron and copper were analysed by flame atomic absorption spectroscopy (Perkin Elmer 3030). Selenium analyses were performed by graphite furnace atomic absorption spectroscopy (Perkin Elmer 5000 AAS or 5000 Zeeman equipped with a Perkin Elmer 500 HGA) as described by Maage *et al.* (1990b). All element analyses were controlled by concomitant analyses of Oyster Tissue and Bovine Liver standards from the National Institute of Standards and Technology, USA.

Determination of blood hemoglobin, serum alkaline phosphatase and total protein were carried out according to Sandnes *et al.* (1988). Analyses of total fatty acids and α -tocopherol in the feeds were performed as described by Waagbø *et al.* (1990).

Statistical analyses

Statistical analyses were performed by use of Student's t-test.

RESULTS

Growth

There were no differences in weight gain between fish given the six dietary treatments. Fish from all groups grew from about 30 g to about 400 g (mean range 373-418 g; S.D. range 47-100) during the experimental period of 12 months.

Zinc status

There were no significant differences in zinc concentration in serum or any organ between the groups (Table 2). The serum alkaline phosphatase (AP) activity was in the normal range (504-722 U/L) for all groups.

Selenium status

As seen from Table 3 significant differences were found for the hepatic selenium concentrations between the dietary groups. However, no effect was observed for the addition of vitamin E.

The hepatic selenium concentrations varied from about 4.8 mg Se/kg wet weight in the fish fed the soyabean oil diet to about 3.1 mg Se/kg in the capelin oil groups, with sardine oil groups in between. The same pattern was also seen in vertebrae and muscle tissue while an opposite relation was found in the spleen.

Serum selenium concentrations were less affected by the dietary treatments, but the fish fed sardine oil added vitamin E had significantly higher serum selenium concentration than the fish fed capelin oil added vitamin E.

Other analyses

The mean blood hemoglobin (8.2- 9.1 g/100 mL) and serum protein concentrations (31.6-37.3 g/L) were not significantly different between the groups and were in the normal ranges of Atlantic salmon as described by Sandnes *et al.* (1988).

Table 2. Zinc status indicators. Mean concentrations (\pm SD, n=10) of zinc in serum (mg/L) and organs (mg/Kg wet weight) in Atlantic salmon given different dietary lipids at two vitamin E levels.

Lipid sources Vit E addition (mg/Kg)	Soyabean oil		Capelin oil		Sardine oil	
	0	300	0	300	0	300
Serum	16.5 \pm 5.0 ^{a1)}	16.6 \pm 5.1 ^a	17.0 \pm 3.7 ^a	16.5 \pm 3.9 ^a	17.7 \pm 2.9 ^a	17.0 \pm 4.9 ^a
Liver	24.3 \pm 1.4 ^a	28.1 \pm 5.9 ^a	24.1 \pm 2.2 ^a	24.4 \pm 1.6 ^a	24.9 \pm 3.4 ^a	26.0 \pm 2.4 ^a
Vertebrae	73 \pm 15 ^{ab}	75 \pm 29 ^{ab}	74 \pm 30 ^{ab}	82 \pm 24 ^a	70 \pm 16 ^{ab}	59 \pm 16 ^b
Muscle	4.5 \pm 0.6 ^a	5.5 \pm 1.1 ^a	5.4 \pm 1.4 ^a	5.0 \pm 0.7 ^a	4.8 \pm 0.6 ^a	4.9 \pm 0.6 ^a
Spleen ²⁾	74	77	83	74	78	73

1) Means in one line sharing a common superscript are not significantly different ($p>0.05$).

2) Mean of two pooled samples of five fish each.

Table 3. Selenium status indicators. Mean concentrations (\pm , n=10) of selenium in serum (mg/L) and organs (mg/Kg wet weight) in Atlantic salmon given different dietary lipids at two vitamin E levels.

Lipid sources Vit E addition (mg/Kg)	Soyabean oil		Capelin oil		Sardine oil	
	0	300	0	300	0	300
Serum	0.14 \pm 0.02 ^{ab1)}	0.14 \pm 0.03 ^{ab}	0.14 \pm 0.02 ^{ab}	0.13 \pm 0.02 ^a	0.14 \pm 0.02 ^{ab}	0.15 \pm 0.02 ^b
Liver	4.8 \pm 1.0 ^c	4.8 \pm 1.3 ^{bc}	3.2 \pm 0.5 ^a	3.1 \pm 0.8 ^a	4.3 \pm 0.8 ^{bc}	3.8 \pm 0.7 ^b
Vertebrae	0.21 \pm 0.05 ^b	0.19 \pm 0.06 ^{ab}	0.17 \pm 0.05 ^{ab}	0.16 \pm 0.03 ^a	0.19 \pm 0.05 ^{ab}	0.19 \pm 0.04 ^b
Muscle	0.19 \pm 0.03 ^d	0.16 \pm 0.03 ^{bc}	0.14 \pm 0.02 ^{ab}	0.13 \pm 0.01 ^a	0.17 \pm 0.05 ^{bcd}	0.18 \pm 0.03 ^{cd}
Spleen ²⁾	4.2	3.9	5.2	5.0	4.7	4.5

1) Means in one line sharing a common superscript are not significantly different ($p>0.05$).

2) Mean of two pooled samples of five fish each.

DISCUSSION

Zinc status

Serum zinc concentration is the most widely used clinical indicator of zinc status in experimental animals (Anonymus, 1987). The activity of some zinc containing enzymes, especially serum alkaline phosphatases, are also good status indicators especially when zinc deficiency is involved (Everett and Apgar, 1987).

Neither the serum zinc concentration nor the zinc concentrations in the organs analysed differed between the diets used in this experiment. This suggests that at the high dietary zinc levels (more than 300 mg Zn/kg) used in commercial salmon feeds, negative effects on zinc metabolism may not be expected on zinc status in salmon by changes in feed composition such as reported in this work. Nevertheless it should be emphasized that this might not be the case at lower dietary levels of zinc.

Selenium status

Surprisingly, the selenium status in the fish varied significantly with the dietary lipid source. Capelin oil gave the lowest selenium status, soyabean oil the highest status and sardine oil in between. This was especially pronounced in the liver.

Oxidized lipids may increase the requirement of functional GSH-Px and thus the requirement of Se. The selenium levels found were not related to the intake of (n-3) PUFA. One possible explanation for these results may be the high concentration of monoene fatty acids, especially the long chain monoenes, 20:1 (n-9) and 22:1 (n-9), in capelin oil. In mammals, it has been shown that these fatty acids in an adaptable way are partly oxidized outside the mitochondria, in small organelles called peroxisomes. The main resulting metabolic products are acetic acid and hydrogen peroxide (Anonymus, 1984; Bremer, 1989). The enzyme catalase in the peroxisomes normally takes care of the hydrogen peroxide formed, but it is possible that this reaction also leads to a higher demand for GSH-Px outside the peroxisomes thereby lowering the selenium status compared to the other dietary groups.

The hepatic selenium concentration in the fish with the lowest values (3.1 mg Se/kg wet liver or 12.3 mg Se/kg dry liver) were in good agreement with the levels found by Hilton *et al.* (1980) who gave about the same dietary selenium concentration to rainbow trout. This concentration is well above the levels where any deficiencies would be expected. This is supported by the fact that selenium from fishmeal have been shown to be readily available for fish (Bell and Cowey, 1989).

There were no obvious effects of adding vitamin E on the selenium status in the fish regardless of the lipid source in this experiment. It has been shown that selenium deficiency is less likely to occur when sufficient vitamin E is present (Poston *et al.*, 1976). The diets with lowest vitamin E concentration (40-50 mg vit E /kg) contained all more than the proposed dietary requirement of 30 mg/Kg (Halver, 1989) for salmonids. Further studies are required to find out whether interactions between dietary vitamin E and selenium status occur in vitamin E deficient fish.

The concentration of zinc (350 mg/kg) and selenium (1.4 mg/kg) found in the feeds were in accordance with levels earlier reported in commercial salmon feeds (Maage *et al.*, 1989). It should be noted that the dietary levels in this experiment were much higher than the levels suggested as dietary requirements for salmonids (Lall, 1989).

In conclusion, no effects of the lipid sources and vitamin E levels were found on the zinc status, but some clear effects were found on the selenium status of the lipid sources. However, the dietary changes in this experiment with similar levels of zinc and selenium as generally found in commercial salmon feeds, did not have negative effects or lead to any deficiencies of the trace elements investigated.

ACKNOWLEDGEMENTS

The study was supported by a grant from the Norwegian Fisheries Research Council. We also wish to thank A/S DeNoFa Lilleborg Fabrikker, Norsildmel, and J.C.Martens & Co. for generous gifts of oils and Trouw Research Center A/S for supporting us the feed basis.

REFERENCES

- ANONYMUS, 1984. Dietary fat, Acyl CoA levels and peroxisomal β -oxidation. *Nutr. Rev.*, *42*: 385-387.
- ANONYMUS, 1987. Mobilization of tissue zinc for growth and reproduction. *Nutr. Rev.*, *45*: 346-348.
- BELL, J.G. and COWEY, C.B., 1989. Digestibility and bioavailability of dietary selenium from fishmeal, selenite, selenomethionin and selenocystine, in Atlantic salmon (*Salmo salar*). *Aquaculture*, *81*: 61-68.
- BREMER, J. 1989. Metabolic effects of longchain unsaturated fatty acids in the diet. *In* Fish, fats and your health. Proceedings of the International Conference on Fish Lipids and their Influence on Human Health. Svanøy Foundation, Norway, p. 27-34.
- COWEY, C.B., ADRON, J.W, WALTON M.J., MURRAY, J., YOUNGSON, A. and KNOX, D. 1981. Tissue distribution, uptake, and requirement for α -tocopherol of rainbow trout (*Salmo gairdneri*) fed diets with a minimal content of unsaturated fatty acids. *J. Nutr.*, *111*: 1556-1567.

- COWEY, C.B., ADRON, J.W. and YOUNGSON, A. 1983. The vitamin E requirement of rainbow trout (*Salmo gairdneri*) given diets containing polyunsaturated fatty acids derived from fish oil. *Aquaculture*, 30: 85-93.
- CUNNANE, S.C., 1982. Maternal essential fatty acid supplementation increases zinc absorption in neonatal rats: relevance to the defect in zinc absorption in acrodermatitis enteropathica. *Pediatr. Res.*, 16: 599-603.
- EVERETT, G. and APGAR, J., 1987. Enzymes as indicators of zinc status. In *Trace element-Analytical Chemistry in Medicine and Biology (Vol.4)*. Edited by P. Braetter and P. Schramel. Walter de Gruyter & Co., Berlin, p. 283-288.
- HALVER, J.E. 1989. The Vitamins. In *Fish nutrition*. Edited by J.E. Halver. Academic press. San Diego, p. 31-109.
- HAMBIDGE, K.M., CASEY, C.E. and KREBS, N.F. 1986. Zinc. In *Trace elements in human and animal nutrition*. Vol 2. Edited by W. Merts. Academic press. San Diego, p. 1-137.
- HAMILTON, R.M., GILLESPIE, C.P. and COOK, H.V. 1981. Relationship between levels of essential fatty acids and zinc in plasma of cystic fibrosis patients. *Lipids*, 16: 374-376.
- HARDIE, L.J., FLETCHER, T.C. and SECOMBES, C.J. 1990. The effect of vitamin E on the immune response of the Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 87: 1-13.
- HILTON, J.W., HODSON, P.V. & SLINGER, S.J. (1980). The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *J. Nutr.*, 110: 2527-2535.
- HILTON, J.W. 1989. The interaction of vitamins, minerals and diet composition in the diet of fish. *Aquaculture*, 79: 223-244.
- JULSHAMN, K. and ANDERSEN, K.J. 1982. Subcellular distribution of minor elements in unexposed molluscs in Western Norway. I. The distribution of zinc and copper in the liver and the digestion system of the oyster (*Ostrea edulis*). *Comp. Biochem. Physiol.*, 75A: 9-12.
- KETOLA, H.G. 1979. Influence of dietary zinc on cataracts in rainbow trout (*Salmo gairdneri*). *J. Nutr.*, 109: 965-969.
- KNUDSEN, E.R., MAAGE, A. and JULSHAMN, K., 1989. Absorption and organ retention of zinc from fish in the rat. *Fisk. Dir. Skr., Ser. Ernæring*, 2: 245-253.
- LALL, S.P. 1989. The minerals. In *Fish nutrition*. Edited by J.E. Halver. Academic press. San Diego, p. 219-257.
- MAAGE, A., SVEIER, H. & JULSHAMN, K. 1989. A comparison of growth rate and trace element accumulation in Atlantic salmon (*Salmo salar*) fry fed four different commercial diets. *Aquaculture*, 79: 267-273.
- MAAGE, A., WAAGBØ, R., OLSSON, P.-E., JULSHAMN, K. and SANDNES, K. 1990a. Ascorbate-2-sulfate as a dietary vitamin C source for Atlantic salmon (*Salmo salar*): 2. Effects of dietary levels and immunization on the metabolism of trace elements. *Fish Physiol. Biochem.*, 8: 429-436.
- MAAGE, A., JULSHAMN, K. and ANDERSEN, K.-J. 1990b. Graphite furnace atomic absorption spectrometric determination of selenium in acid digested solutions of marine samples with continuum-source background correction and nickel as matrix modifier. *J. Anal. At. Spectrom.*, (Submitted).
- NATIONAL RESEARCH COUNCIL. 1981. Nutrient requirements of coldwater fishes. National Academy Press, Washington DC. 63 pp.
- POSTON, H.A., COMBS, JR., G.F. & LEIBOVITZ, L. (1976). Vitamin E and selenium interrelations in the diet of Cap. Atlantic salmon (*Salmo salar*): Gross, histological and biochemical deficiency signs. *J. Nutr.*, 106: 892-904.
- SALTE, R., THOMASSEN, M.S. and WOLD, K. 1988. Do high levels of dietary polyunsaturated fatty acids (epa/dha) prevent diseases associated with membrane degeneration in farmed Atlantic salmon at lower water temperatures? *Bull. Eur. Ass. Fish Pathol.*, 8: 63-66.

- SANDNES, K., LIE, Ø. and WAAGBØ, R. 1988. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. J. Fish. Biol., 32: 129-136.
- WAAGBØ, R., SANDNES, K., SANDVIN, A. and LIE, Ø. 1990. Increasing levels of omega-3 polyunsaturated fatty acids (n-3 PUFA) and vitamin E in feed to Atlantic salmon (*Salmo salar*): 1. Growth and chemical composition. To be published.
- WATANABE, T., TAKEUCHI, T., MATSUI, M., OGINO, C. and KAWABATA, T. 1977. Effect of α -tocopherol deficiency on carp. VII. The relationship between dietary levels of linoleate and α -tocopherol requirement. Bull. Jpn. Soc. Sci. Fish., 43: 935-946.
- WATANABE, T., TAKEUCHI, T., WADA, M. and UEHARA, R. 1981. The relationship between dietary lipids and α -tocopherol requirement of rainbow trout. Bull. Jpn. Soc. Sci. Fish., 47: 1463-1471.