

## EFFECTS OF DIETARY SELENIUM SUPPLEMENTATION ON GROWTH, BLOOD CHEMISTRY AND TRACE ELEMENT LEVELS IN SERUM AND LIVER OF ADULT ATLANTIC SALMON (*SALMO SALAR*)

By

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### ABSTRACT

Two groups of adult Atlantic salmon (*Salmo salar*) held in sea pens were fed a fish silage-based diet which contained either 0.66 mg Se/kg (w. w.) (Diet 1) or 2.6 mg Se/kg (Diet 2) from September to May. Dietary treatment had no significant effects on growth, feed conversion, protein efficiency ratio (PER), protein productive value (PPV), hepatosomatic index, serum protein content, serum glutathione peroxidase (GSH-Px) activity, blood haemoglobin and haematocrit. The selenium concentrations in the serum and liver of fish fed Diet 2 were two and four times higher than of fish fed Diet 1, respectively. Significant positive correlations were found between the concentrations of selenium and copper in the liver in both groups. High dietary selenium did not affect the levels of zinc and iron in serum and liver significantly.

### INTRODUCTION

Selenium has recently received considerable attention in human, animal and fish nutrition. Selenium is an essential nutrient for domestic animals (Schwarz et al., 1957) as well as for fish (Poston et al., 1976). As a component of glutathione peroxidase (GSH-Px), selenium is related to the role of vitamin E but functions also independently (Rotruck et al., 1973).

Wild marine fish in general has high selenium concentrations in the fillet (0.2 - 0.6 mg/kg wet weight.) and in the liver (> 5 mg/kg wet weight) (Ringdal and Julshamn, 1986). Fish is among the food items in human nutrition containing the highest amounts of this element.

Farmed salmon has been reported to contain significantly lower levels of selenium, vitamin E and copper than their wild counterparts (Poppe et al., 1986). Still there is no evidence for deficiency symptoms in salmon fed com-

mercial fish meal based diets (Bell et al., 1986; Bell et al., 1987; Poston et al., 1976). Low concentration of selenium in the liver of farmed salmon may be due to difference in the dietary selenium between farmed and wild salmon as seen for copper (Julshamn et al., 1988).

The objectives of the present experiment was to study:

- 1) The physiological effects and tissue levels of Se in adult Atlantic salmon fed diets without and with Se supplementation.
- 2) The effects of dietary Se on the levels of Fe, Cu and Zn in serum and the liver of Atlantic salmon.
- 3) The levels of Se in the livers of farmed fish given a commercial dry pellet feed, and in wild caught salmon.

## MATERIALS AND METHODS

### *Fish and diets*

Two groups, each of six hundred Atlantic salmon with an initial weight of  $1.4 \pm 0.4$  kg (Mean  $\pm$  SD,  $n = 100$ ), were fed diets differing only in their content of selenium. The groups were held in seapens from September to May. The fish were fed their prescribed diet *ad libitum* three times a day. Water temperatures ranged from 2.4 to 10.0 °C during the feeding period.

The composition of the nutritionally well balanced fish silage-based diet employed in this study was described previously by Lie et al. (1988) (Diet A). The diet was a mixture of fish silage (50 %), a dry mix (49 %, Ewos, Norway) consisting mainly of fish meal and extruded wheat, and a vitamin and pigment mix (1 %, Skretting A/S). In the present study this basal diet was fed without Se supplementaion (Diet 1) or supplemented with 2 mg of Se (as sodium selenite) per kilogram (Diet 2). Several batches of each diet were prepared during the experiment.

### *Sampling*

The feeds and the fish were sampled eight times in the course of the experiment as described by Lie et al. (1988). Ten fish were randomly taken from each group and weighed. Blood samples and livers were taken for analysis. Livers from wild Atlantic salmon caught in bag-nets off the western coast of Norway and from fish fed a commercial pelleted dry feed were also taken.

### *Analytical Methods*

Feed, liver and serum samples for mineral element analyses were digested in a mixture of concentrated nitric and perchloric acid (suprapure 9+1) as descri-

bed by Julshamn et al. (1982). The elements were analyzed by atomic absorption spectrophotometry (AAS). Iron, copper and zinc were measured by flame AAS, selenium was determined by graphite furnace AAS (GFAAS). Selenium was analyzed by the use of uncoated graphite tubes with a L'vov platform inserted and stabilized by nickel as matrix modifier (Maage et al., 1990). The accuracy and precision of the element analyses were tested in an intercalibration study arranged by ICES (Berman, 1984) as well as by analysing standard reference material from the National Institute of Standards and Technology. All the methods used were found satisfactory by both tests.

Serum protein, blood haemoglobin and haematocrit were determined as described by Sandnes et al. (1988). Glutathione peroxidase activity (GSH-Px) was determined in serum as described by Bell et al. (1985). The GSH peroxidase activity was measured with two substrates: cumene hydroperoxide (Se dependent GSH-Px) and hydrogen peroxide (Se-independent GSH-Px).

#### *Data calculation and statistical analyses*

Results for feed conversion, protein efficiency ratio (PER), protein productive value (PPV) and hepatosomatic index (HSI) are shown in Table 2. Differences between sample means were tested by Student's t-test and correlations between analytical parameters were also tested.

## RESULTS

#### *Feed analyses*

Selenium analyses of the diets showed fairly constant values throughout the experiment (Table 1). The dietary concentrations of iron, copper and zinc varied widely between the different batches of feed the mean values are given in Table 1. The variation was probably due to new batches of fish meal and fish silage being obtained during the experiment.

#### *Growth study*

No significant differences were found between the samples taken from the groups for final fish weight, feed conversion, hepatosomatic index, protein efficiency ratio (PER) and protein productive value (PPV) (Table 2). Mortality was low throughout the experiment (2.6 % in group 1 and 5.7 % in group 2).

#### *Blood and serum analyses*

Serum selenium concentrations in fish fed Diet 1 remained fairly constant throughout the experiment (Fig. 1). Relative to the control group the serum

Table 1. Mean concentrations (mg/kg wet weight) of Se, Cu, Zn and Fe in the experimental diets fed to Atlantic salmon for 250 days<sup>1</sup>.

| Diet    | Se        | Cu      | Zn     | Fe     |
|---------|-----------|---------|--------|--------|
| 1 ..... | 0.66±0.12 | 8.5±3.7 | 140±63 | 250±70 |
| 2 ..... | 2.6±0.6   | 7.1±3.6 | 134±73 | 226±69 |

<sup>1</sup> Mean values ± SD (N = 10) of eight analyses of the test diets sampled in the course of the experiment.

Table 2. Effect of dietary selenium concentration on final weight, feed conversion, protein efficiency ratio, protein productive value and hepatosomatic index in Atlantic salmon. The fish were fed a test diet without (Diet 1) or with selenium supplementation (Diet 2) for 250 days.

| Diet    | N  | Mean final weight (kg) <sup>1)</sup> | Feed conversion <sup>2)</sup> | PER <sup>3)</sup> | PPV <sup>4)</sup> | HSI <sup>5)</sup> (mean ± n=20) |
|---------|----|--------------------------------------|-------------------------------|-------------------|-------------------|---------------------------------|
| 1 ..... | 30 | 4.2±1.1                              | 1.6                           | 1.5               | 0.23              | 1.24 (0.18)                     |
| 2 ..... | 30 | 3.7±0.8                              | 1.6                           | 1.5               | 0.21              | 1.15 (0.18)                     |

<sup>1)</sup> Initial weight of the fish was 1.4±0.4 kg.

<sup>2)</sup> Feed = dry food in take (kg)/ live weight gain (kg).

<sup>3)</sup> Protein efficiency ratio (PER) = weight gain (kg)/protein fed (kg).

<sup>4)</sup> Protein productive value (PPV) = protein gain (kg)/ protein fed (kg).

<sup>5)</sup> Hepatosomatic index (HSI) = liver weight (g) × 100/ total body weight (g).

selenium levels in the high selenium group (Diet 2) showed a 2-fold increase at the end of the experiment.

There were no obvious differences in the concentrations of iron, copper and zinc in serum between the low selenium and high selenium groups (Table 3). No significant differences were detected between the group samples in serum protein, serum GSH-Px activity, blood haemoglobin and haematocrit (Table 4). The GSH-Px activity was independent of the substrates used, suggesting that no Se-independent GSH-Px activity was present.

#### Liver analyses

The selenium concentrations in the liver found at the end of the experiment reflected the dietary level of selenium (Fig. 2). The liver selenium concentrations in fish fed Diet 2 showed a 3-fold elevation after 5 weeks increasing to approximately 12 mg/kg wet weight at the end of the study. The liver selenium

Fig. 1 The mean concentration of selenium in the experimental diets (mg/kg) and the corresponding mean serum concentrations (mg/L) in Atlantic salmon throughout the feeding period.

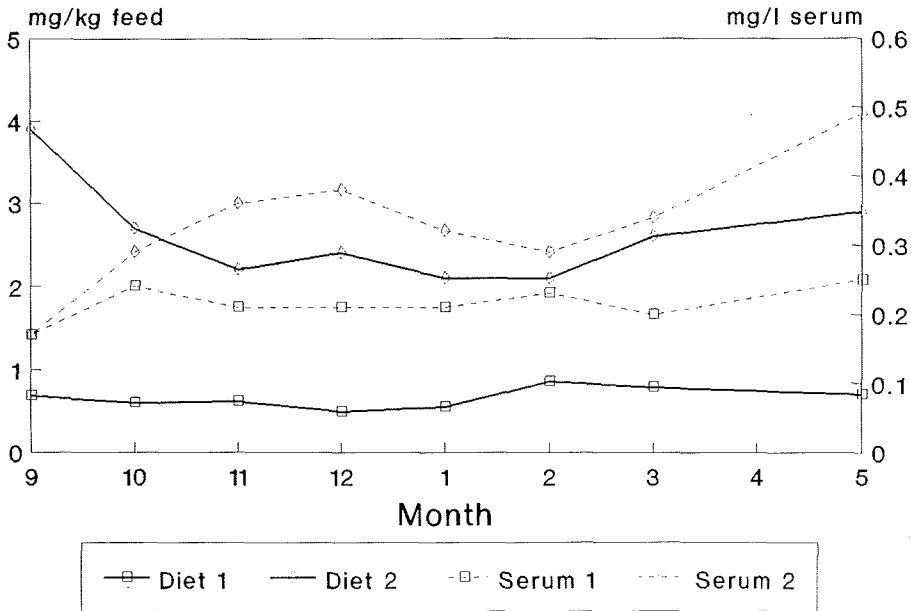


Fig. 2 The mean concentration of selenium in the experimental diets (mg/kg) and the corresponding mean liver concentrations (mg/kg) in Atlantic salmon throughout the feeding period.

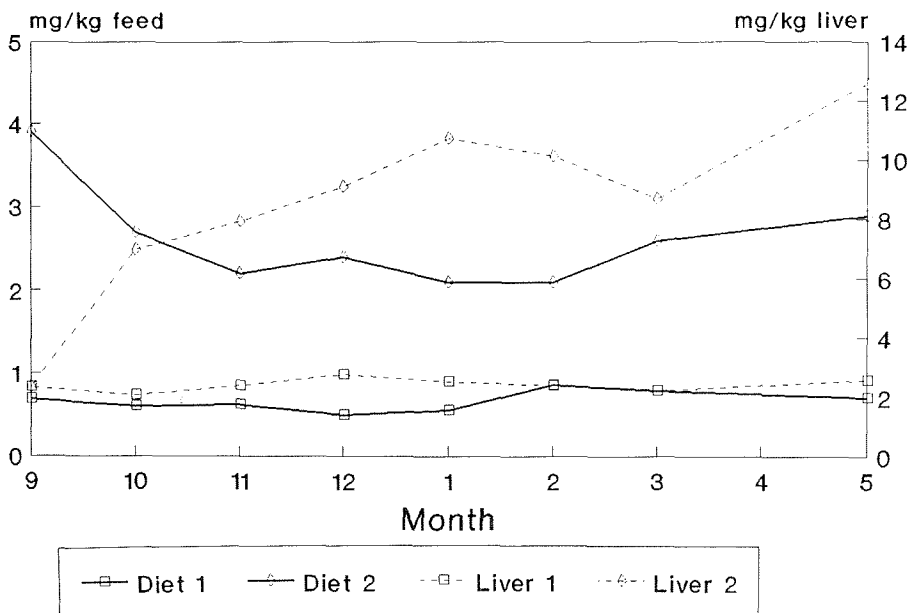


Table 3. Mean concentrations (N = 10; SD) of iron, copper and zinc in serum (mg/L) of Atlantic salmon fed diets containing two levels of selenium during an experimental period of 250 days.

| Analysis | Diet | Day:           |                | 20             | 11             | 12             | 15             | 19             | 26             | 20             | Mean |
|----------|------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|------|
|          |      | Month:         | 9              |                |                |                |                |                |                |                |      |
| Fe       | 1    |                |                | 2.06<br>(0.30) | 1.23<br>(0.32) | 1.44<br>(0.28) | 0.64<br>(0.25) | 1.37<br>(0.29) | 1.25<br>(0.27) | 1.35<br>(0.32) |      |
|          | 2    |                |                | 1.80<br>(0.45) | 1.46<br>(0.22) | 1.29<br>(0.30) | 1.19<br>(0.25) | 1.94<br>(0.39) | 1.26<br>(0.28) | 1.42<br>(0.35) |      |
| Cu       | 1    | 0.97<br>(0.26) | 1.34<br>(0.35) | 1.46<br>(0.17) | 1.18<br>(0.21) | 1.08<br>(0.31) | 1.16<br>(0.18) | 1.03<br>(0.12) | 0.76<br>(0.32) | 1.13<br>(0.29) |      |
|          | 2    |                | 1.22<br>(0.20) | 1.46<br>(0.18) | 1.21<br>(0.15) | 1.01<br>(0.11) | 1.11<br>(0.14) | 0.91<br>(0.16) | 0.86<br>(0.18) | 1.10<br>(0.23) |      |
| Zn       | 1    | 17.1<br>(5.2)  | 31.0<br>(3.7)  | 32.3<br>(3.5)  | 21.3<br>(5.9)  | 16.1<br>(2.8)  | 25.9<br>(5.2)  | 20.3<br>(2.9)  | 20.6<br>(3.3)  | 23.4<br>(7.2)  |      |
|          | 2    |                | 25.4           | 32.7<br>(4.3)  | 22.7<br>(3.7)  | 15.1<br>(2.9)  | 16.7<br>(3.3)  | 21.9<br>(3.8)  | 23.5<br>(4.4)  | 23.2<br>(5.9)  |      |

Table 4. Serum protein, haemoglobin, haematocrit and glutathione peroxidase activity (GSH-Px) in Atlantic salmon fed the test diets 1).

| Parameter                                          | Diet 1   | Diet 2   |
|----------------------------------------------------|----------|----------|
| Serum protein (g/L) .....                          | 46.6±5.4 | 48.3±6.3 |
| Haemoglobin (g/100 mL) .....                       | 9.0±0.9  | 9.9±0.7  |
| Haematocrit (%) .....                              | 47.0±4.7 | 47.0±3.4 |
| Glutathione peroxidase <sup>1</sup>                |          |          |
| H <sub>2</sub> O <sub>2</sub> .....                | 2.7±0.8  | 2.8±0.8  |
| Cumene H <sub>2</sub> O <sub>2</sub> -GSH-PX ..... | 2.5±0.6  | 2.6±0.5  |

<sup>1)</sup> Mean ± S.D.; N = 20.

<sup>2)</sup> Glutathione peroxidase activity expressed as nanomoles of NADPH oxidized per minute per milligram of protein (N = 10).

and copper concentrations showed significant positive correlations for fish fed Diet 1 at each sampling as well as for the material as a whole ( $r = 0.75$ ;  $p < 0.01$ ). This was also observed in fish fed Diet 2. No elevated liver copper concentrations were found in the high selenium group (Table 5). The dietary selenium levels had no significant effect upon the liver copper concentrations which initially declined and then remained fairly constant after the second sampling. The liver iron concentration in fish fed Diet 2 showed somewhat higher values than in fish fed Diet 1, but the mean values varied substantially in both groups throughout the experiment (Table 5).

Increased liver zinc concentrations were observed in both groups during the first part of the experiment (Table 5) which may be attributed to an elevated dietary zinc level during this period. Dietary selenium did not, however, influence the liver zinc concentrations significantly.

#### *Wild and commercial reared salmon*

Liver samples of wild Atlantic salmon were analysed for copper and selenium concentrations for comparison (Table 6). Selenium levels were on the average

Table 6. Copper and selenium levels in liver tissue (mg/kg wet weight) of wild and farmed Atlantic salmon <sup>1)</sup>.

| Type                | N  | Weight (Kg) | Cu mg/Kg | Se mg/Kg   |
|---------------------|----|-------------|----------|------------|
| Wild salmon .....   | 17 | 6.3 ± 1.3   | 225 ± 97 | 13.3 ± 3.9 |
| Farmed salmon ..... | 10 | 4.2 ± 1.1   | 77 ± 29  | 2.7 ± 0.8  |

<sup>1)</sup> Mean ± SD

Table 5. Mean concentrations (N = 10; SD) of iron, copper and zinc in liver (mg/kg wet weight) of Atlantic salmon fed diets containing two levels of selenium during an experimental period of 32 weeks.

| Analysis | Diet | Day<br>Month | 12<br>9       | 23<br>10      | 20<br>11      | 11<br>12      | 15<br>1       | 19<br>2       | 26<br>3       | 20<br>5       | Mean          |
|----------|------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Fe       | 1    |              | 231<br>(31)   | 191<br>(35)   | 184<br>(18)   | 187<br>(22)   | 172<br>(25)   | 163<br>(36)   | 194<br>(44)   | 214<br>(27)   | 191<br>(37)   |
|          | 2    |              |               | 234<br>(28)   | 184<br>(51)   | 173<br>(28)   | 184<br>(19)   | 209<br>(24)   | 202<br>(38)   | 266<br>(39)   | 205<br>(44)   |
| Cu       | 1    |              | 120<br>(66)   | 66<br>(32)    | 83<br>(22)    | 86<br>(37)    | 70<br>(22)    | 64<br>(14)    | 55<br>(18)    | 77<br>(29)    | 74<br>(34)    |
|          | 2    |              |               | 93<br>(25)    | 76<br>(21)    | 65<br>(29)    | 64<br>(21)    | 73<br>(20)    | 66<br>(27)    | 72<br>(18)    | 74<br>(25)    |
| Zn       | 1    |              | 30.2<br>(5.6) | 34.0<br>(3.8) | 40.5<br>(5.6) | 34.3<br>(3.2) | 30.3<br>(2.4) | 32.6<br>(4.4) | 28.2<br>(1.8) | 30.2<br>(2.4) | 32.5<br>(5.2) |
|          | 2    |              |               | 37.1<br>(5.6) | 40.0<br>(8.0) | 32.0<br>(3.2) | 31.5<br>(3.7) | 31.2<br>(4.2) | 30.9<br>(3.8) | 32.2<br>(4.4) | 34.1<br>(6.1) |



five times higher in liver of wild salmon than in farmed salmon. The copper concentrations were also significantly higher in wild salmon. Significant positive correlations were found between liver selenium and copper levels for both wild ( $r = 0.82$ ;  $p > 0.001$ ) and farmed salmon ( $r = 0.65$ ;  $p > 0.05$ ).

## DISCUSSION

Normal growth, feed utilization, haematological values and low mortality indicated that the selenium requirement for adult salmon was adequately met by Diet 1 used in this experiment. Further no differences were found in serum GSH-Px activity between adult Atlantic salmon fed a fish silage-based diet without selenium supplementation (Diet 1, 0.66 mg Se/kg w.w.) and the diet supplemented with selenium (Diet 2, 2.5 mg Se/kg). The selenium concentrations in the blood and liver of adult salmon fed Diet 1 were not correlated with body size. Similar findings were reported for small rainbow trout (Hilton et al., 1980). The mean selenium concentration factor was calculated to be 9.2 in the liver of fish fed Diet 1 (1.0 mg Se/kg dry weight). This value compares with the concentration factor of 9.8 found in trout (mean final weight 40 g) fed a diet containing 1.22 mg Se/kg dry weight for 16 weeks (Hilton et al., 1980). A higher concentration of selenium was found in the liver of the salmon fed Diet 2 and a plateau was reached after 16 weeks. A concentration factor of 11 was calculated for fish fed Diet 2 which corresponds well to the factor found for fish fed Diet 1. This shows that the amount of Se retained in the liver is practically proportional to the feed level within a wide range. The plateau found for liver selenium is in contrast to results with small rainbow trout fed diet with increasing levels of copper. In this case a steady accumulation of copper occurred in the liver with age (Julshamn et al., 1988). A relationship between dietary and liver selenium levels as found in salmon and trout may explain the high liver selenium levels found in adult wild salmon. Food items for wild salmonids include crustacea which contain high levels of selenium ( $> 3$  mg/kg dry weight). These levels are substantially higher than those found in whole fish used for fish meal production (Eisler, 1981). The optimal dietary selenium level for small salmonids is reported to be in the range of 0.15-0.3 mg/kg dry feed (Poston et al., 1976; Hilton et al., 1980). The selenium requirement of salmonids declines as the fish grows (Hilton et al., 1980). The basal diet used in this study contained 50 % fish silage and 49 % dry pellet mix (containing about 50 % fish meal) and this diet had a natural selenium content of 1.0 mg/kg dry weight (Diet 1). This level is similar to the selenium concentrations found in twelve commercial salmon dry diets available in Norway. The mean selenium values in these diets was  $1.1 \pm 0.4$  mg/kg and the range in Se concentration varied between 0.65 and 1.6 mg/kg. The selenium concentrations in fish meal vary

between 0.8 and 2.5 mg/kg (Lunde, 1973). Hence the dietary selenium requirement of Atlantic salmon and trout is likely met when fish meal constitutes a significant proportion of the diet.

The low serum zinc concentrations found in both groups initially may well have been caused by handling stress (Weinberg, 1974) and a reduced feed intake in connection with the start of the experiment. The high serum zinc concentrations ( $> 30$  mg/L) are, however, the highest serum zinc values ever reported in salmonids (Knox et al., 1982, 1984; Lanno et al., 1985; Bettger et al., 1987). The physiological significance of high blood zinc concentrations in fish has been discussed by Bettger et al. (1987). The hepatic zinc levels found in the present study were higher than those reported for juvenile rainbow trout (18-24 mg/kg wet weight) fed diets containing zinc in the range of 15 to 600 mg/kg dry feed, but similar to those found in wild adult Atlantic salmon (Poppe, 1986). The dietary concentration of zinc in the present study varied between 50 and 240 mg/kg wet weight.

The exact dietary zinc requirement for adult salmon is unknown. But studies with juvenile rainbow trout (Ogino and Yang, 1978) suggest that the requirement may be between 10 and 30 mg/kg dry diet. However, 30 mg Zn/kg dry weight may not meet the zinc requirement of salmonids when the diet contains high levels of calcium relative to zinc levels in the presence of one or more mineral binding agents (Rickardson et al., 1985).

There were no relationships found in this study between liver copper concentrations and fish weight as previously reported in small rainbow trout fed diets containing 3.5 mg Cu/kg dry feed (Julshamn et al., 1988). Moreover, there were no significant effects of dietary selenium on the liver copper concentrations. This is not in accordance with studies on juvenile rainbow trout fed diets containing a constant copper level but different selenium levels (Hilton, 1989). This discrepancy may be explained by a masking of the higher copper loading in the liver of adult fish. Nevertheless, a strong positive correlation was found between the liver selenium and copper levels in both groups studied, as well as in the wild salmon which indicates a possible selenium/copper interaction also in adult fish. The high liver copper concentrations in wild salmon can be explained by the content of this element in their natural prey. For instance crustacea contain high levels of copper (Eisler, 1981).

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