

EFFECTS OF DIETARY VITAMIN C AND PHYSICAL STRESS
ON HEAD KIDNEY AND LIVER ASCORBIC ACID, SERUM
CORTISOL, GLUCOSE AND HAEMATOLOGY IN
ATLANTIC SALMON (*SALMO SALAR*).

By

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ABSTRACT

Circulating levels of cortisol were increased in Atlantic salmon (*Salmo salar*) following moderate physical stress, but were not significantly influenced by the ascorbic acid (AA) status of the salmon. The experimental fish had been fed practical diets for four weeks without supplementation (group A) or supplemented with Ca ascorbate-2-monophosphate (AmP) equivalent to 500 mg AA/kg dry feed (group B). The concentration of AA in the head kidney reflected the dietary intake of AmP, but was not significantly influenced by the stress applied to the fish. The secondary stress response in terms of serum glucose level and the haematological parameters haemoglobin (Hb), haematocrit (Hct) and mean cell haemoglobin concentration (MCHC) were not significantly affected by dietary composition.

INTRODUCTION

High concentrations of ascorbic acid (AA) are generally found in adrenal tissues. In fish, the adrenal functions are located to the head kidney (or anterior kidney) as reviewed by Butler (1973). This organ contains among the highest AA levels reported in fish (Halver *et al.* 1975; Lovell and Lim, 1978). Cortisol, a steroid hormone which plays an important role in stress reactions, is synthesised in the head kidney. An association of AA with steroidogenesis has long been recognized, whereas relationships between different dietary levels of AA and physiological stress responses in fish are not known.

Recently studies have focused on the role of dietary AA levels in increasing the resistance to diseases and improving general health (Navarre and Halver, 1989; Hardie *et al.*, 1990). Stress increases the susceptibility to infections in fish (Maule *et al.*, 1989), and modern fish farming implies several forms of

handling such as netting, grading, vaccination and transport. High stocking density, pollution and poor water quality are also factors known to impose stress reactions in fish.

The response to a wide variety of stressors is controlled by adrenocorticotrophic hormone (ACTH) from the pituitary which triggers the release of corticosteroids and catecholamines from the interrenal and chromaffin tissues in the head kidney (primary response). Among the physiological effects caused by the release of these hormones (secondary response) in fish are dilation of gill filament arteries, an increase in stroke volume of the heart, increased glycogen metabolism, and depression of the immune response (Mazeaud and Mazeaud, 1981; Gratzek and Reinert, 1984; Wiik *et al.*, 1989).

Ascorbic acid is involved in the metabolism of corticosteroids as demonstrated in swine by the application of exogenous ACTH, resulting in changes in AA levels of the body similar to those occurring in states of stress (Dvorak, 1984). This was also demonstrated in salmonid fishes by Wedemeyer (1969), who found a reduction in head kidney AA after different forms of stress or ACTH injection. Ascorbic acid is also involved in the metabolism of catecholamines as shown by Levine *et al.* (1985) who demonstrated that AA doubled the rate of norepinephrine formation from dopamine in isolated chromaffin granules from bovine adrenal medulla, suggesting that AA provides reducing equivalents for the hydroxylation of dopamine.

The present experiment was conducted to study primary (cortisol) and secondary (serum glucose) stress responses in Atlantic salmon (*Salmo salar*) with different AA status. Haematological parameters in fish are affected by dietary AA (Sandnes *et al.*, 1990) as well as by stress (Wedemeyer and Yasutake, 1977), and analyses of haemoglobin (Hb), haematocrit (Hct) and mean cell haemoglobin concentration (MCHC) were included to support interpretation of the data.

MATERIALS AND METHODS

Atlantic salmon (mean weight about 600 g), partly depleted of AA, were fed conventional fish meal based diets in duplicate groups for four weeks (32 fish in each 1,5 x 1,5 m tank). One group was fed a diet without supplementation of any form of vitamin C and was compared to fish given Ca ascorbate-2-monophosphate (AmP) (F. Hoffmann-La Roche & Co.), equivalent to 500 mg crystalline AA per kg diet. The mean water temperature and salinity were 10,6 °C and 16 g/L throughout the feeding period.

Eight fish were sampled before the experiment was started and five fish from each tank at the end of the feeding experiment. Individual fish were carefully caught and killed immediately by a blow to the head. Blood was

withdrawn and treated as described by Sandnes *et al.* (1988). The liver and the head kidney were dissected and quickly frozen on dry ice. They were kept at -80°C until analysed for AA.

After sampling of resting fish by the end of the feeding period, the duplicates from each dietary group were transferred to one tank and stressed by emptying the water three successive times in the course of five minutes. Five fish were then sampled from each group immediately, and after 0.5, 1.0, 1.5, 2.0, 3.0, 6.0, 12.0, 24.0 and 72.0 hours.

Ascorbic acid (including dehydro AA) was analysed in the head kidney and in the liver by an automated fluorometric method described by Roy *et al.* (1976).

Haemoglobin (Hb) and haematocrit (Hct) were analysed according to Sandnes *et al.* (1988) and serum glucose as described by Hemre *et al.* (1989). Serum cortisol was determined using a (^{125}I) cortisol radioimmunoassay kit (GammaCoat, Travenol Gentech Diagnostics).

As there were no significant differences between the duplicate groups in any of the parameters reported, the mean values are given.

RESULTS AND DISCUSSION

The fish fed without AmP supplementation in the diet (group A) showed no macroscopic or clinical signs of vitamin C deficiency. A liver concentration of $27\ \mu\text{g AA/g}$ wet weight (Table 1) in resting fish indicated a poor, but not

Table 1. Ascorbic acid (AA + DHA) in liver and head kidney ($\mu\text{g/g w.w}$) of Atlantic salmon with low (A) and high (B) vitamin C status prior to and after handling stress.

| | Liver | | Head kidney | |
|--------------------|------------|-------------|-------------|-------------|
| | A | B | A | B |
| Resting fish | 27 ± 8 | 171 ± 25 | <5 | 196 ± 17 |
| Handling stress + | | | | |
| 0.0 h | 24 ± 4 | 194 ± 28 | <5 | 207 ± 19 |
| 0.5 h | 25 ± 3 | 143 ± 19 | <5 | 187 ± 12 |
| 1.0 h | 33 ± 11 | 168 ± 45 | 17 ± 24 | 220 ± 37 |
| 1.5 h | 31 ± 5 | 155 ± 18 | 14 ± 9 | 199 ± 5 |
| 3.0 h | 37 ± 7 | 175 ± 58 | 16 ± 18 | 227 ± 20 |
| 6.0 h | 36 ± 16 | 152 ± 39 | 28 ± 17 | 220 ± 16 |
| 12.0 h | 32 ± 6 | 181 ± 28 | 9 ± 11 | 228 ± 18 |
| 24.0 h | 35 ± 7 | 175 ± 79 | 10 ± 18 | 246 ± 57 |
| 72.0 h | 29 ± 10 | 148 ± 18 | 44 ± 75 | 212 ± 11 |

marginal, physiological status of this vitamin as reported in salmonids (Hilton *et al.*, 1977; Sandnes, 1982). The corresponding value in fish given dietary AmP (group B) was 171 μg AA/g liver, showing a good physiological status (Sandnes *et al.*, 1990).

To our knowledge, no data are available on AA in the head kidney of Atlantic salmon. The resting values for the groups A and B (<5 and 196 μg AA/g, respectively) indicate that the AA concentration in this organ reflects the dietary intake of AmP (Sandnes and Waagbø, 1991), and can be used to evaluate the AA status in this species as has been suggested for rainbow trout (*Oncorhynchus mykiss*) by Halver *et al.* (1975).

The changes found in the concentration of AA in the organs following stress were not significant, except from an increased level in the head kidney in group B concomitant with the peak levels of cortisol and glucose in serum (Fig 1).

There were no significant differences in serum cortisol and glucose levels due to dietary treatments (Fig. 1). Significant increases following stress were,

Fig. 1. The concentrations of cortisol and glucose in serum of Atlantic salmon in the course of 72 hours after exposure to stress. The fish had been fed diets without (A) and supplemented with Ca ascorbate-2-monophosphate equivalent to 500 mg ascorbic acid/kg (B) prior to the experiment.

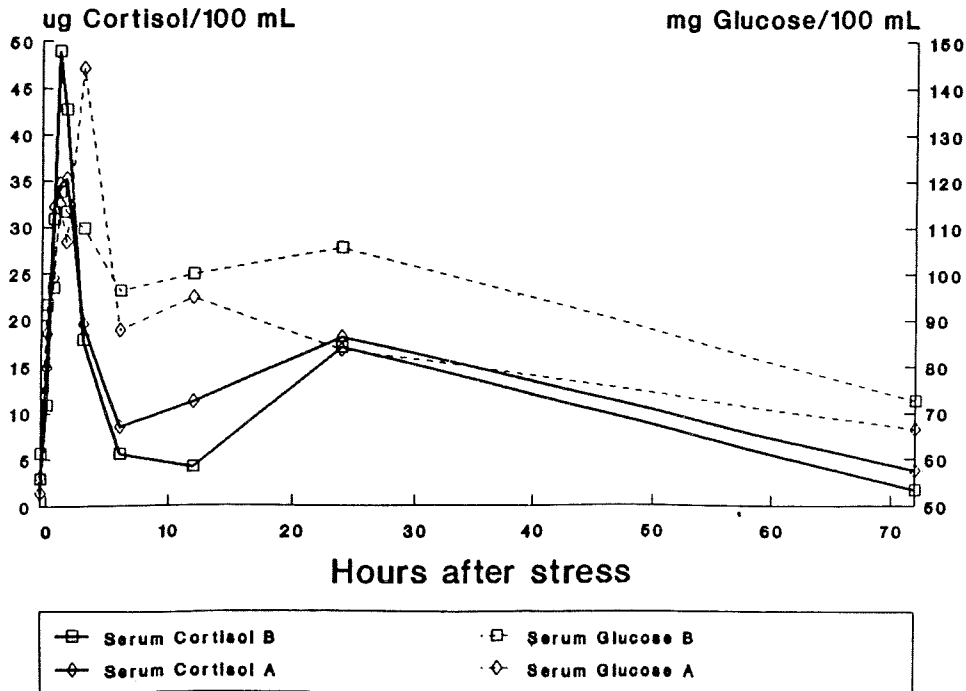


Table 2. Blood haematocrit (%), haemoglobin (g/100 ml) and mean cell haemoglobin concentration (MCHC) in Atlantic salmon with low (A) and high (B) vitamin C status prior to handling stress.

| | Haematocrit | | Haemoglobin | | MCHC | |
|--------------------|-------------|-------|-------------|---------|----------|----------|
| | A | B | A | B | A | B |
| Resting fish | 41±5 | 39±6 | 8.2±0.9 | 8.0±1.1 | 20.2±1.1 | 20.7±1.4 |
| Handling stress + | | | | | | |
| 0.0 h | 45±3 | 46±7 | 8.7±0.4 | 8.7±1.3 | 19.5±1.4 | 19.0±0.7 |
| 0.5 h | 45±2 | 44±5 | 9.0±1.0 | 8.9±0.8 | 19.4±2.5 | 20.4±1.3 |
| 1.0 h | 48±3 | 45±5 | 9.5±0.7 | 8.8±0.7 | 19.6±0.9 | 19.5±0.6 |
| 1.5 h | 42±6 | 45±4 | 8.7±0.8 | 8.9±0.7 | 20.9±1.2 | 19.9±0.5 |
| 3.0 h | 41±3 | 40±7 | 8.6±1.0 | 7.8±1.1 | 21.1±1.5 | 19.4±1.1 |
| 6.0 h | 38±5 | 36±3 | 8.2±1.5 | 8.1±1.0 | 21.2±1.3 | 22.7±1.9 |
| 12.0 h | 36±9 | 42±4 | 8.0±1.1 | 8.6±1.2 | 23.3±5.8 | 20.6±1.9 |
| 24.0 h | 43±5 | 40±11 | 8.2±1.1 | 8.7±2.1 | 19.0±2.6 | 21.9±1.6 |
| 72.0 h | 38±4 | 44±5 | 8.6±0.9 | 8.7±0.5 | 22.6±1.3 | 19.8±2.2 |

however, demonstrated in both groups, with the highest levels found after 1.0 – 1.5 hours. These parameters showed considerable individual variations, which are in accordance with other studies in salmonids (Strange *et al.*, 1977).

In both groups the haematological values at the end of the feeding period (Table 2) were slightly below normal ranges found in adult Atlantic salmon fed in net pens in the sea (Sandnes *et al.*, 1988), but somewhat higher than values reported in Atlantic salmon smolt with good vitamin C status held in tanks (Sandnes *et al.*, 1990). The latter study also demonstrated that blood Hb and Hct were reduced in vitamin C deprived fish (Sandnes *et al.* 1990). Neither dietary AmP nor stress affected Hb and Hct significantly in the present study. Reports on haematological responses to different stressors have shown increased levels of Hb and Hct in several fish species (Fletcher, 1975; Giles *et al.*, 1984; Wells *et al.*, 1984; Jones *et al.*, 1987), while other authors have found no effects (Flos *et al.*, 1988).

Many studies have been published on blood chemistry following stress in a wide variety of fish species, but data on Atlantic salmon are scarce. A compilation of literature data on salmonid fishes by Schreck (1981) seems to indicate that in the present investigation the fish were subjected to light or moderate stress as judged from the cortisol values. The fact that no fish died in the course of the experiment also points to a moderate stress.

Adrenal AA depletion has been found useful in monitoring the effects of stress in terrestrial animals (Dvorak, 1984) as well as in fish. In coho salmon

(*Oncorhynchus kisutch*) and rainbow trout, Wedemeyer (1969) reported a ten-fold increase in the serum cortisol level one hour following handling stress, and a decrease in the head kidney AA concentration during the first 20 minutes followed by a sustained rise to approximately initial levels by the end of 2 hours. The present results showed an even greater increase in serum cortisol concentration, but no reduction of AA in the head kidney.

In group A the head kidney content of AA was initially below the detection limit of the analytical method used, and thus a possible AA depletion could not be detected. The increase from below 5 to 17 μg AA/g found during the period from 0.5 to 1.0 hour after stress at which serum cortisol reached its highest level may indicate, however, a compensatory mechanism following exhaustion of head kidney AA reserves. The release of cortisol from adrenal tissues is followed by mobilization of AA body stores to restore head kidney AA (Wedemeyer, 1969). The AA influx observed in the present study may possibly be attributed to an overcompensation or an extra need for AA to support the release or synthesis of cortisol. Ascorbic acid may have been mobilized from the total body stores including the liver. Due to the differences in organ weights, a possible transfer of AA from the liver to the head kidney would hardly be detected by the liver AA analyses.

Group B fish had available AA in the head kidney and significant stores in the liver. Circulating cortisol levels were not different between the groups A and B, nor were the secondary response in terms of serum glucose concentrations. Assuming that the turnover of cortisol was similar in fish with different AA status, the biosynthesis and release of this hormone were also the same. This indicates that the natural physiological response to stress as regards these parameters was not affected by the vitamin C status of the fish. According to Dvorak (1984) a high adrenal AA concentration generally may be regarded as a sign of adrenal capacity to produce corticosteroids, but the present results showed no differences in circulating cortisol concentrations following stress among fish having $<5 \mu\text{g}$ AA/g or 171 μg AA/g in head kidney tissue.

The effects of AA on steroidogenesis are complex, and although an association of AA with steroidogenesis has long been recognized, the biochemical mechanisms involved are not known. Ascorbic acid may have regulatory functions related to the release of steroid hormones, or act specifically in hydroxylation reactions in the synthesis from cholesterol. Ascorbic acid has been shown to play a role in subcellular compartmentization related to specific steroid biosynthetic conversions (Pintauro and Bergan, 1982). Acting as a prooxidant it may regulate the release of adrenal steroid hormones by inhibiting the interaction of ACTH with the cell membrane (Kitabchi and West, 1975). One of the physiological reactions to stress in fish is related to the water-mineral balance, which is affected by a breakdown of barriers to water

and electrolyte flux, as stressed fish become hydrated in fresh water and dehydrated in sea water (Mazeaud *et al.*, 1977; Schreck, 1981). In mullet (*Mugil cephalus* L), Thomas (1984) showed that the gill AA concentration increased twofold after exposure to a hypoosmotic medium. The present experiment was carried out in brackish water at a salinity of 16 g/L with an anadrome species as test animal. This salinity is closer to the physiological salinity of the fish (9 g/L) than full salt or fresh water. The hematological analyses and lack of mortality may indicate that brackish water is beneficial to Atlantic salmon subjected to stress, in accordance with literature cited by Schreck (1981).

The present study indicates that the synthesis and/or release of cortisol following moderate physical stress do not require a high adrenal AA status in Atlantic salmon. Ascorbic acid is also involved in catecholamine metabolism, but the impact of AA status on these hormones were not studied. Immunosuppression caused by stress in fish is related to the endocrine system (Ellis, 1981; Anderson, D. P., 1982; Wiik *et al.*, 1989). The interactions between dietary vitamin C, stress and immunocompetence await further studies.

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