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Influence of astaxanthin on growth rate, condition, and some blood indices of rainbow trout, *Oncorhynchus mykiss*

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Abstract

A trial was conducted in flow-through fibre-glass tanks at a water temperature of 11.1 ± 3.0°C, at a dissolved oxygen content of 9.3 ± 1.0 mg/l, and at an oxygen saturation level of 84.2 ± 4.9% (mean $\pm$ SD) in rainbow trout with average starting weight of 178 ± 23 g for the effect of astaxanthin on growth, condition, and some hematological and blood biochemical indices of the fish. Using correlation and regression analysis methods, it was found that the fish fed a diet containing 49.8 mg astaxanthin/kg for 84 days had similar growth (the relative gain was 19.4 vs. 18.4%) and significantly ($P < 0.05$) lower red blood cell count (RBC: 1.06 vs. 1.15 T/l), hematocrit (PCV: 0.386 vs. 0.422), hemoglobin (Hb: 71.8 vs. 76.5 g/l) and mean cell hemoglobin concentration (MCHC: 0.19 vs. 0.18) when compared to fish fed a diet without astaxanthin supplement. The fish fed the astaxanthin-containing diet had lower ($P < 0.05$) plasma triacylglycerol (TGL: 3.69 vs. 4.85 mmol/l), lower ($P < 0.01$) plasma calcium (Ca^{2+}: 2.75 vs. 2.92 mmol/l) and significant ($P < 0.01$) negative correlation between the levels of plasma cholesterol and plasma uric acid ($r = -0.960$ vs. $r = -0.489$). These changes in blood remained within the interval of physiological values on our laboratory's summation curve for rainbow trout under Czech conditions. The different relationships between some biochemical indices (lactate dehydrogenase and cholesterol) and the parameters of the oxygen in water (quantity of dissolved oxygen

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and the oxygen saturation of water), and/or the water temperature, are discussed.

Keywords: Rainbow trout; *Oncorhynchus mykiss*; Astaxanthin; Hematological indices; Biochemical indices of blood plasma; Condition parameters

1. Introduction

The production of rainbow trout depends on the responses of the consumer market. Consumers require fish with better flesh quality and appearance. Carotenoids, which belong to the natural constituents of salmonid fish feed, may help meet the latter requirement. Bearing in mind the need for hygienic safety of foods, research in this area has focused on the replacement of the currently used canthaxanthin by the natural astaxanthin. Both products are used as dietary supplements in salmonid fish feeds to produce the desired flesh colour. Generally, astaxanthin is more efficiently utilised than canthaxanthin by rainbow trout (Foss et al., 1984, 1987; Torrissen, 1986). Interactions of astaxanthin and canthaxanthin, as influencing pigment deposition in rainbow trout, have been studied by Torrissen (1989). Fish with individual body weight below 90 g deposited relatively low amounts of carotenoids compared to fish heavier than 90 g. The carotenoid concentration in the flesh increased with increasing growth but leveled off for fish with a large absolute growth. March et al. (1990) found that intensity of flesh pigmentation in coho ranging in weight from 30 to 400 g and fed a diet supplemented with astaxanthin, was significantly correlated with body weight. Flesh pigmentation of rainbow trout fed astaxanthin or canthaxanthin at different rates in freshwater and saltwater was studied by Storebakken and Choubert (1991). No and Storebakken (1991) defined the effect of water temperature on the uptake, deposition, and metabolism of astaxanthin in rainbow trout. Instrumentally measured colour values of the trout flesh and their correlations with carotenoid concentration and fat content of the flesh were also evaluated. Astaxanthin has also been shown to increase egg survival and percentage of fertilized eggs, to protect eggs against extreme conditions (Craik, 1985) and to stimulate growth (Torrissen, 1984). As for astaxanthin, mainly, its influence on the reproduction cycle of the fish has been discussed intensively, but it is too early to draw any final conclusions. A number of studies on astaxanthin were published by Christiansen who examined the issue from the viewpoint of the effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (Christiansen and Torrissen, 1997), growth and survival of Atlantic salmon juveniles (Christiansen and Torrissen, 1996), and first-feeding fry (Christiansen et al., 1995a), antioxidant status and immunity in Atlantic salmon (Christiansen et al., 1995b) and effects of astaxanthin and vitamin A on growth and survival during the first feeding of Atlantic salmon (Christiansen et al., 1994). Besides pigmentation, carotenoids are involved in certain physiological functions, as pointed out by Nakano et al. (1995) who studied the biochemical characteristics of the liver and blood in rainbow trout fed a diet supplemented by red yeast (*Phaffia rhodozyma*) containing astaxanthin as its principal carotenoid pigment or synthetic astaxanthin.

It follows from the literature survey that there is little knowledge of the effect of astaxanthin on physiological functions of the salmonids, including those responsible for the performance characteristics and state of health of the fish. The purpose of this study was to test the effect of this pigment on the growth and condition of the rainbow trout and on a wide spectrum of the blood indices of the fish during an 85-day period of administration of granules with an admixture of astaxanthin, contained in the commercial product sold as Carophyll Pink 8%.

2. Materials and methods

2.1. Feeding experiment

The experiments were conducted in fibre-glass tanks of flow-through type with a continuous supply of new fresh water on a trout farm (652 m above sea level) from 17 June to 9 September. The average water temperature was 11.1 ± 3.0°C (mean $\pm$ SD) during the tests, the water contained 9.3 ± 1.0 mg/l dissolved oxygen and the O_2 saturation level of the water was 84.2 ± 4.9%. The values of the remaining hydrochemical parameters were within the following ranges: pH 7.0–7.4, alkalinity 0.55–0.70 mmol/l, HCO_3 33.5–42.7 mg/l, total hardness 3.1–4.8°N, COD_{Mn} 2.2–3.9 mg/l, NH_4^+–N 0.12–0.70 mg/l, NO_2^-–N 0.014–0.048 mg/l,NO_3^-–N 5.70–10.40 mg/l.

The rainbow trout (*Oncorhynchus mykiss*) were all of the same origin, in good condition, and in a good state of health. Two test groups and two control groups were formed, each containing 300 fish. The average starting weight of the fish was 178 ± 23 g. Pellets 5 mm in size were used as the experimental feed; their formulation and chemical composition are shown in Table 1. The fish were either fed an astaxanthin-free diet (control diet, denoted as C) or a diet containing 49.8 mg astaxanthin per kg (experimental diet, denoted as A): astaxanthin was added to the vitamin and micromineral premix of the same composition. The premix was supplied by the Belgian firm Vitamex and the amount of astaxanthin corresponded to a 0.166% concentration, which is recommended by the firm to achieve flesh colouring in 10 weeks time. The fish were fed twice daily, the size of the daily feeding rates corresponding to 2–4% of body weight per day. During the 2-week acclimation period, the fish were fed a commercial dry pelletted feed free of astaxanthin.

2.2. Growth

Random selection and returning were used to study the growth of the fish. In an interval of 21 days within an 84-day period (from June 19 to September 11), 30 fish out of the total stock of 300 fish were caught in each test group and control group and were individually weighed and measured at an accuracy of 1 g and 1 mm. Before weighing and measuring, the fish were starved for 24 h and were anesthetised with Menocain (0.067 g/l) to enable easy handling. The body conformation traits studied included total body length, body length, body height and body width.

Table 1
Formulation and chemical composition of the control diet

	per kg
Ingredients (g)	
Fish meal[a]	150
Meat and bone meal[b]	370
Soya bean meal (free of fat)	230
Wheat flour	140
Hay meal	50
Milk powder	30
Vitamin and micromineral premix[c]	30
Chemical analysis (g)	
Dry matter	900
Crude protein (N × 6.25)	400
Crude fat	70
Ash	160
Crude fibre	30
Nitrogen free extract	230

[a] "Köster" Fishmeal — 64% protein, Germany.
[b] "Viandor" — 60% protein, France.
[c] Supplied per kg of diet: Vitamin A, 27, 000 IU; vitamin D_3, 2, 250 IU; α-tocopherol, 108 mg; vitamin K_3, 8.1 mg; thiamin, 12.75 mg; riboflavin, 8.25 mg; niacin, 30 mg; Ca-pantothenate, 42 mg; pyridoxine, 10.8 mg; vitamin B_{12}, 0.018 mg; ascorbic acid, 64.5 mg; folic acid, 1.08 mg; biotin, 0.24 mg; Fe, 48 mg; Cu, 4.5 mg; Co, 0.99 mg; Se, 0.18 mg; Zn, 27 mg; Mn, 30 mg; I, 1.20 mg; choline chloride, 840 mg; antioxidant Endox.

As to the condition parameters, the total weight of the fish and the weight of the fish without viscera were recorded at an accuracy of 1 g, and the absolute weights of the liver (AWL) and of the spleen (AWS) were determined at an accuracy of 0.01 g. These data were used to calculate the liver somatic index (LSI: liver weight as % of the body weight), the spleen somatic index (SSI: spleen weight as % of the body weight), and the condition coefficient after Fulton (FQ) (body weight in $g \times 100/\text{body length}^3$ in cm) and coefficient after Clark (CQ) (body weight without viscera in $g \times 100/\text{body length}^3$ in cm).

2.3. Preparation of the blood samples

Blood was sampled from the 10 fish caught at random. The sampling was performed 20 hours after the last feeding during the forenoon, the samples were taken by puncturing the caudal veins immediately after catching and stunning. EDTA and an aqueous solution of heparin were used as anticoagulants, the former being used for the hematological examination and the latter for the biochemical analyses of the blood plasma. We believe that the knowledge of water temperature and oxygen level in the water at the moment of blood sampling is an integral part of any blood analysis: the oxygen levels recorded during the experiment are therefore shown in Fig. 1. Of the

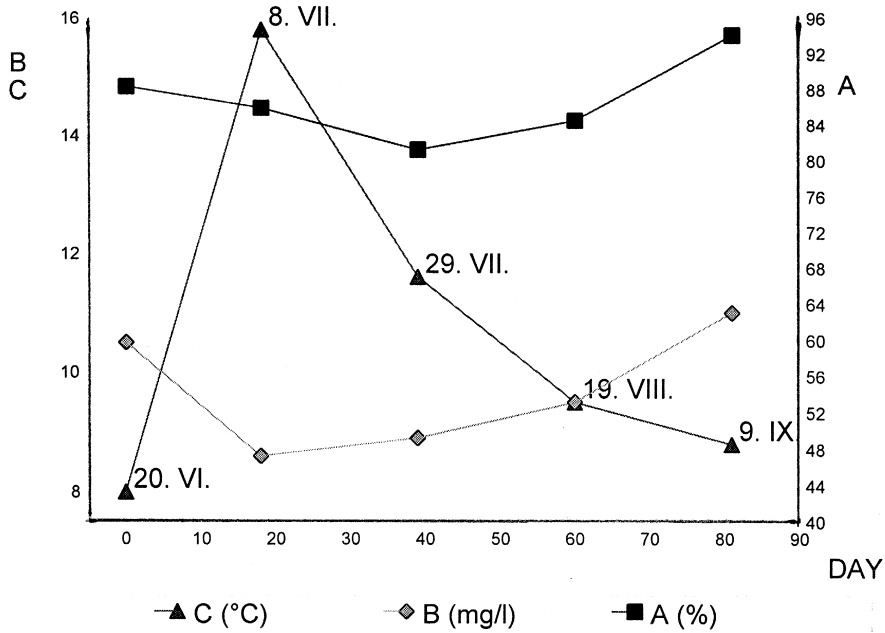


Fig. 1. O_2 saturation of water (A), dissolved O_2 content (B), and water temperature (C) at the moment of blood sampling from rainbow trout.

whole complex of physical and hydrochemical characteristics, it is the water temperature and oxygen levels that represent, in our view, the essential minimum needed for the objective interpretation of the values of hematological indicators in the given context.

2.4. Hematology and clinical chemistry

For this purpose, we used standard human methods with the following details in certain indices. The red blood cell count (RBC T/l) was determined in a 1:20 dilution of the blood sample in the Hayem solution with a Bürker hemocytometer. Hematocrit (PCV) was analysed in microhematocrit-heparinised capillaries within 40 min after blood sampling, using a microhematocrit centrifuge (13,000 rpm for 3 min). Hemoglobin (Hb g/l) was determined by the cyanhemoglobin method. The derived blood indices of mean cell volume (MCV in fl), mean cell hemoglobin (MCH in pg), and mean cell hemoglobin concentration (MCHC) were calculated from the hematological figures. The biochemical indices of the blood plasma were determined within 24 h of storage at 4°C; a Hitachi 704C instrument was used for the determinations. These included urea (BUN mmol/l), creatinine (CREA μmol/l), uric acid (UA μmol/l), total protein (TP g/l), glucose (GL mmol/l), cholesterol (CHOL mmol/l), triacylglycerol (TGL mmol/l), activities of alanine aminotransferase (ALT μkat/l), aspartate aminotransferase (AST μkat/l), alkaline phosphatase (ALP μkat/l), lactate dehydrogenase (LD μkat/l) and

hydroxybutyryl dehydrogenase (HBD μkat/l). Inorganic phosphate (P mmol/l) was determined spectrophotometrically in the UV area and the contents of calcium (Ca^{2+} mmol/l), sodium (Na^{+} mmol/l), and potassium (K^{+} mmol/l) were determined by flame emission photometry.

2.5. Statistical analysis

The following basic numerical characteristics were used during the statistical processing of the results: arithmetic mean, SD, and variance coefficient. The confidence interval of the arithmetic means of the basic sets was determined at a significance level of $P = 0.05$ (Reisenauer, 1965). The normality of the selected sets was verified by means of the coefficient of skewness $\alpha_{x,3}$ and coefficient of kurtosis $\beta_{x,4}$. Statistical significance of the differences between the arithmetic means of the selected sets was examined by Student's *t*-test. Correlation and regression analyses were used in the study of the dynamics of weight growth and of the growth of conformation traits. The "t" time variable was chosen to serve as the independent variable. Linear-type regression lines $y' = a + bx_i$, $y' = a_{yt} + b_{yt}$, respectively, were used to evaluate the trends of development. The relative weight gain and relative increment of the conformation characteristics were calculated from the proportion of $(b_{yt}/\overline{y_i}) \times 100$, the regression parameter.

3. Results

3.1. Growth

As follows from the regression lines of the experimental group $A(y' = 121.2 + 56.0\,x)$ and control group $C(y' = 129.1 + 53.3\,x)$ whose parameters were used to calculate theoretical values at the absolute level and to calculate the relative balance values, the pigmenting substance tested did not influence the growth of rainbow trout. This is best shown by the values of the relative increment in the b_{yt} parameter, which was 19.38% in the experimental group of astaxanthin-treated fish and 18.44% in the astaxanthin-free control group. As to the conformation parameters, the body height and body width were also higher in the astaxanthin-treated fish (8.21% and 7.94% vs. 7.88% and 7.06%, respectively). The absolute and relative balanced (theoretical) values of body weight (401 g vs. 396 g and 227% vs. 218%), body height (78 mm vs. 77 mm and 139% vs. 138%), and body width (39 mm vs. 38 mm and 134% vs. 131%) also correspond with these results.

3.2. Hematological, biochemical, and condition indices

The results of the hematological and biochemical examination of the blood plasma involved analyses of 22 parameters, whose development was checked in 3-week intervals between 20 June and 9 September. The dynamics of the RBCs and biochemical indices of the blood plasma depending on time during the 81 days of investigation can be regarded as being split into five intervals, as shown in (Figs. 2 to 23). The data in the

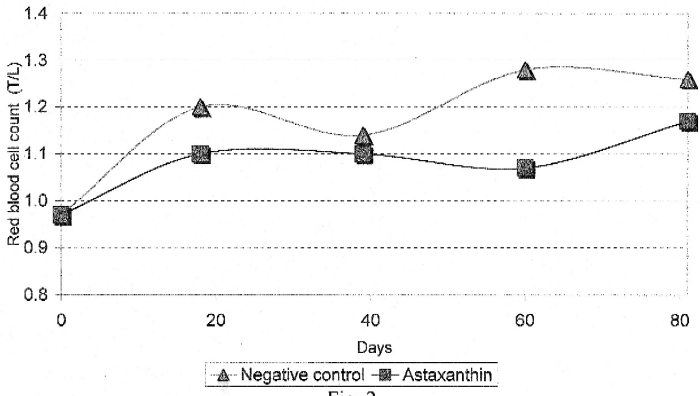
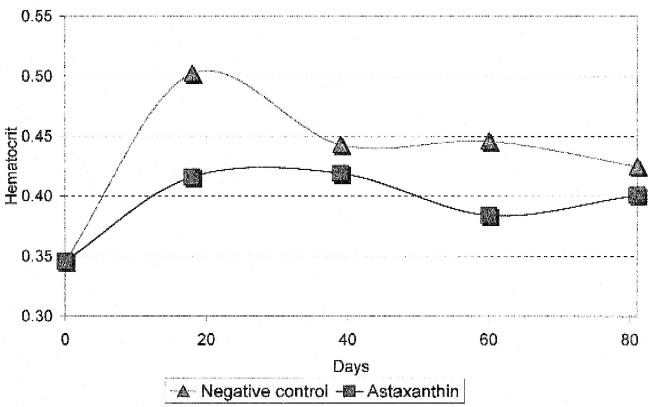


Fig. 2



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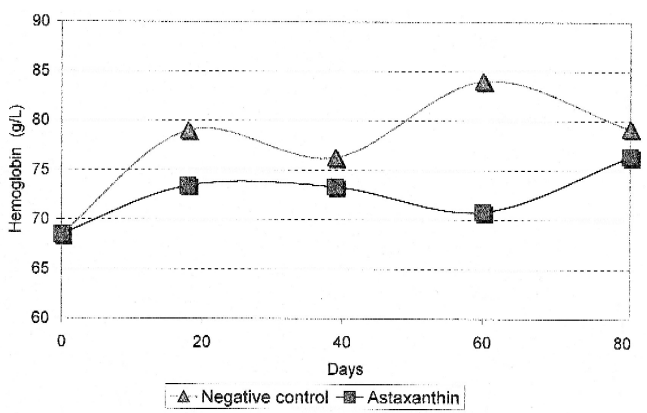
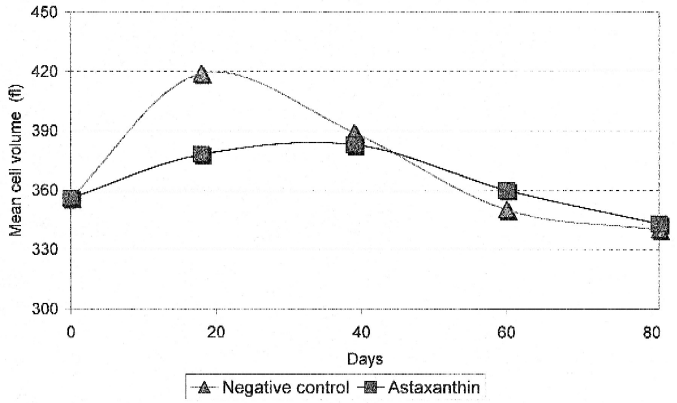


Fig. 4

Figs. 2–23. Dynamics of the blood indices during the course of the experiment with rainbow trout fed an astaxanthin-containing diet against an astaxanthin control.



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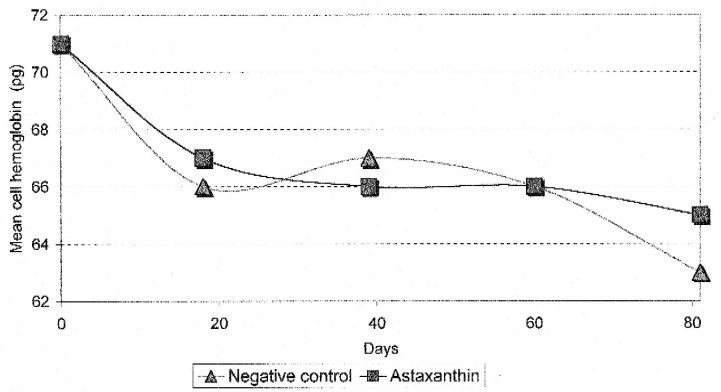

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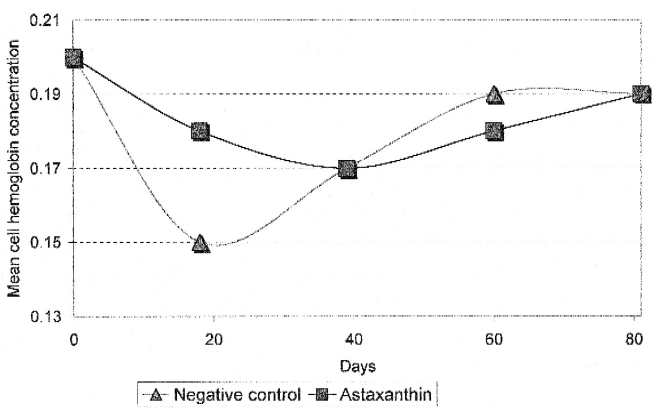

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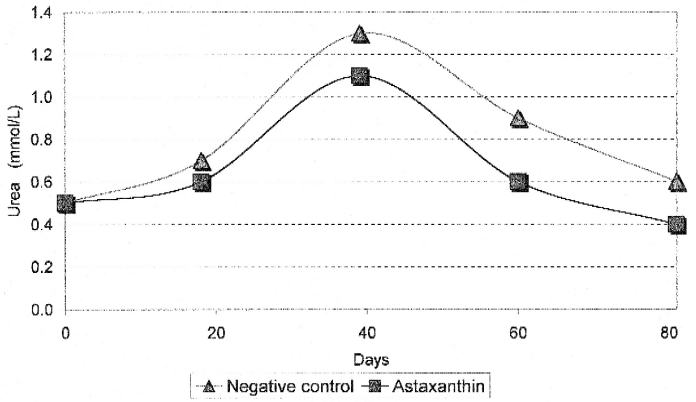


Fig. 8

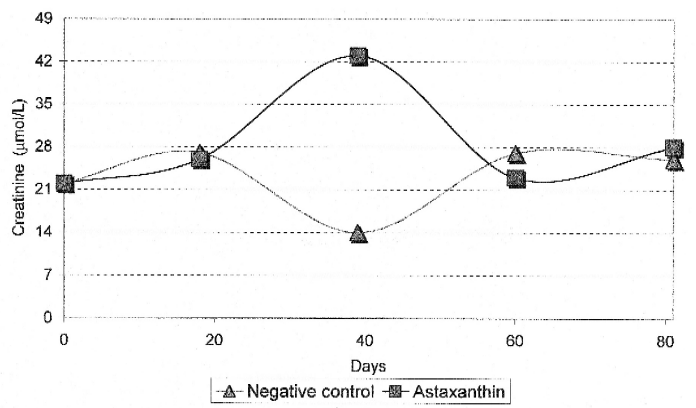
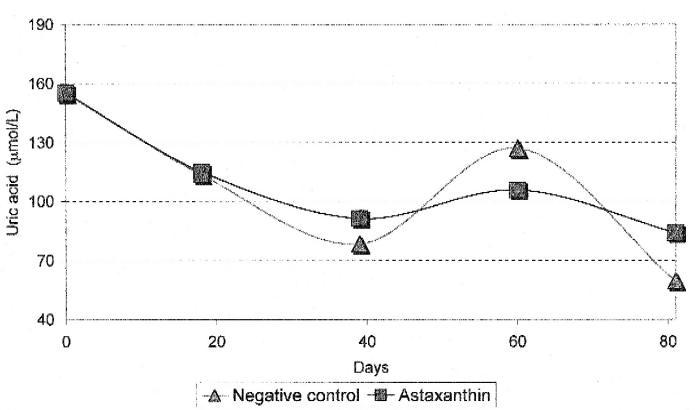


Fig. 9



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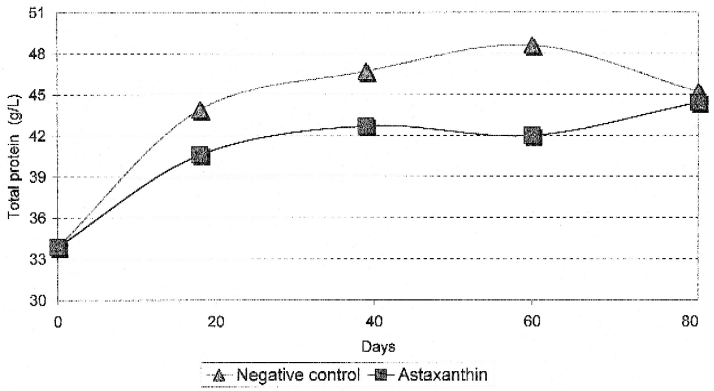

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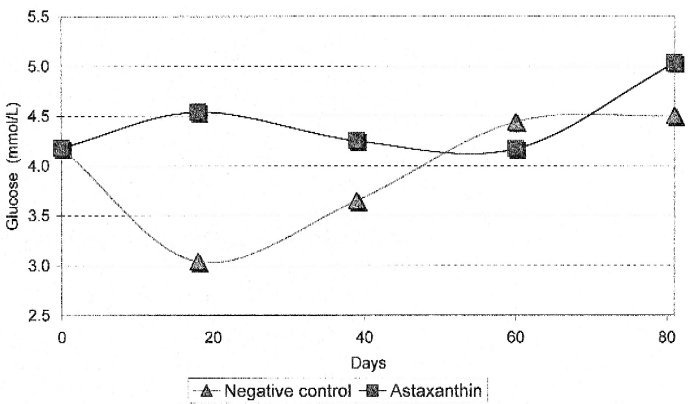


Fig. 12

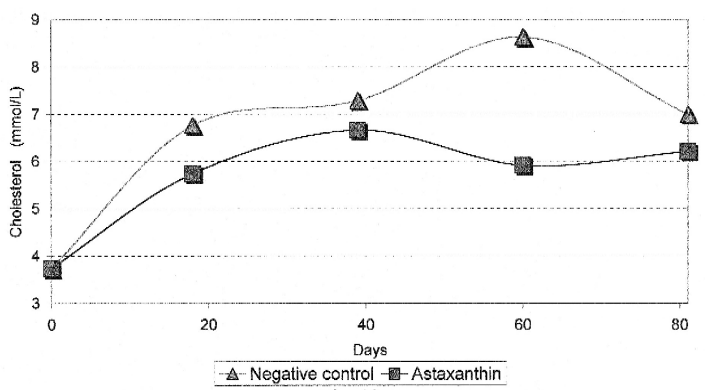
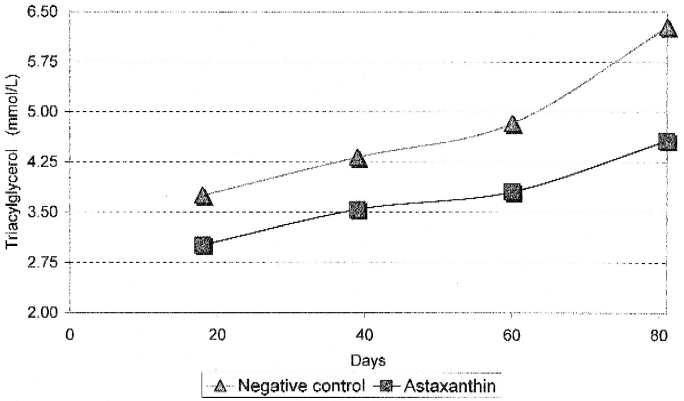


Fig. 13



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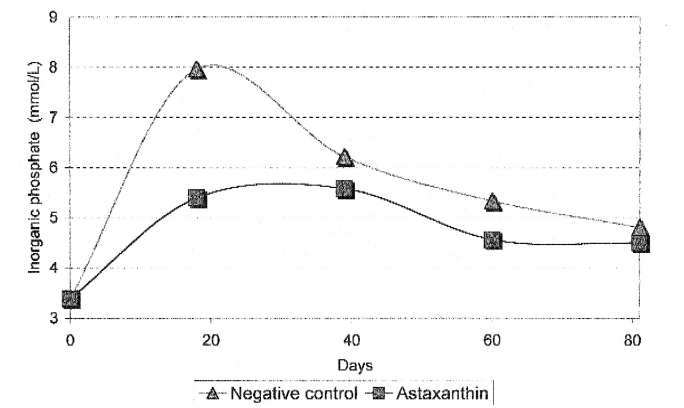

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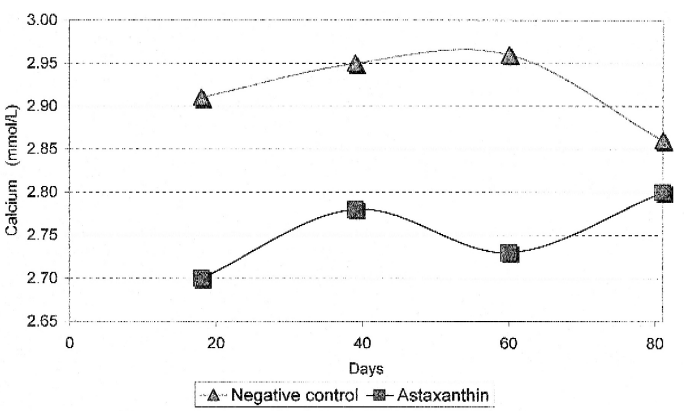


Fig. 16

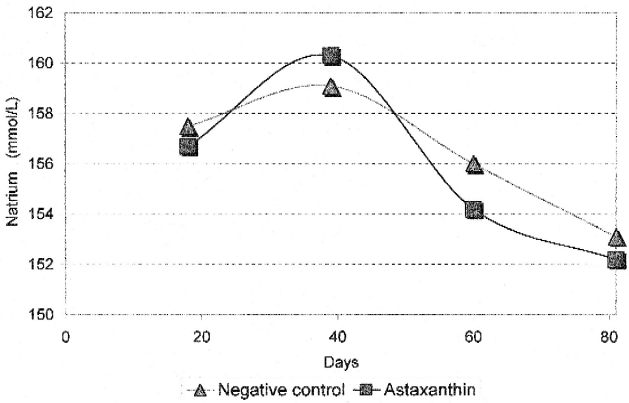
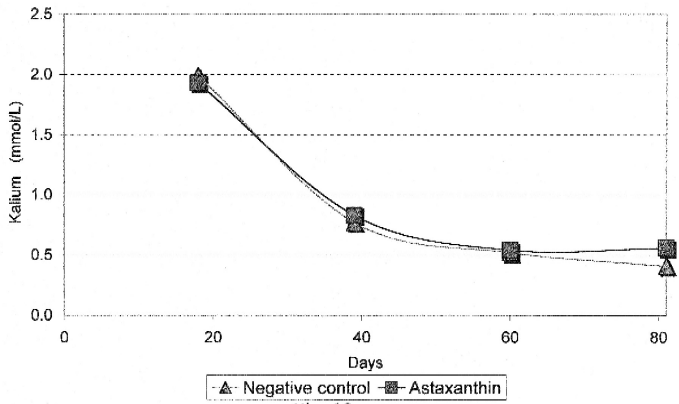


Fig. 17



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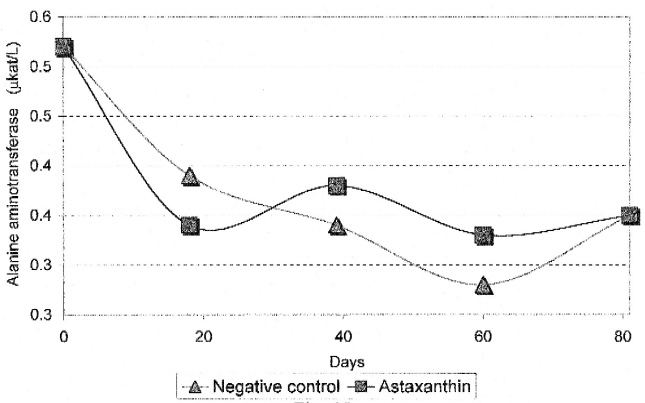

Fig. 19

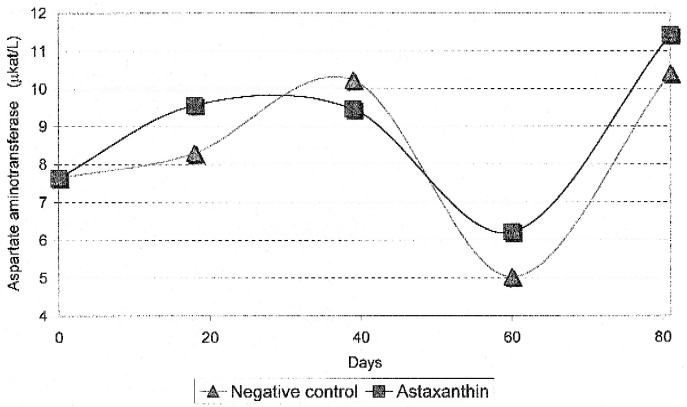

Fig. 20

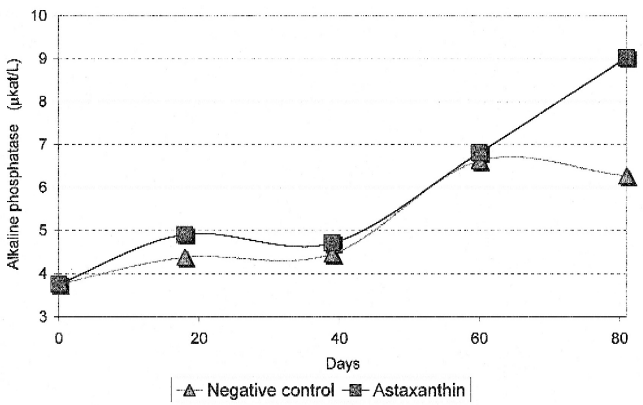

Fig. 21

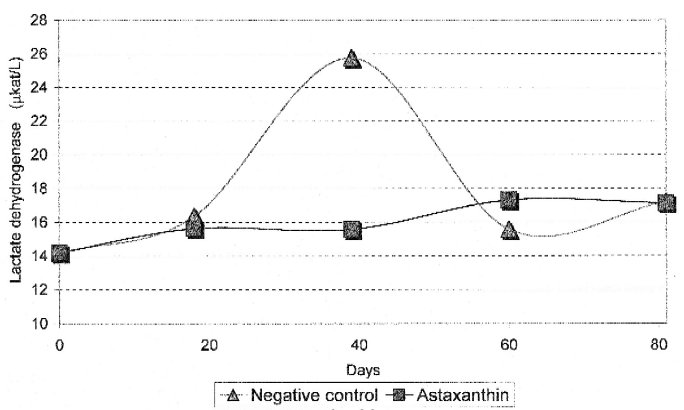

Fig. 22

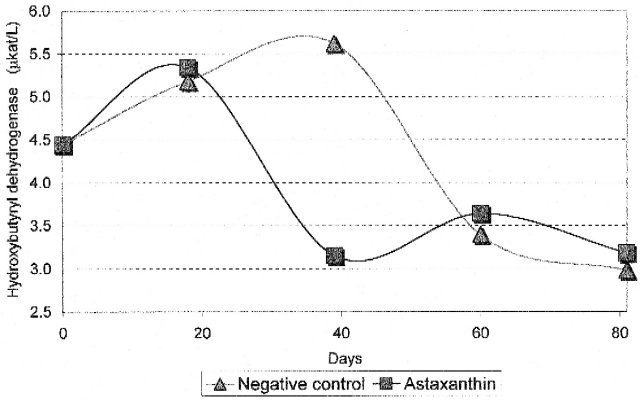

Fig. 23

figures indicate that a significant ($P < 0.05$) decline of the PCV (0.416 vs. 0.502) and a highly significant ($P < 0.01$) increase of MCHC (0.18 vs. 0.15) occurred in the astaxanthin-treated fish, compared with the control group, as early as after 18 days. After 60 days of the experiment, a significant ($P < 0.05$) reduction was recorded in the RBC level (1.07 vs. 1.28). As to the biochemical parameters, there was a significant ($P < 0.05$) decline in P (5.39 vs. 7.96) on the 18th day of the trial; later on, on the 39th day, we recorded an increased ($P < 0.05$) CREA level (43 vs. 14) and reductions ($P < 0.05$) of LD (15.58 vs. 25.76) and the HBD isoenzyme (3.15 vs. 5.62). In the later stage of the trial, after 60 days, a marked and significant ($P < 0.05$) decline was recorded in the level of CHOL (5.91 vs. 8.63). The different developmental trends of some of the studied indices of the hematological and biochemical profile are clearly illustrated by the diagrams. These include, in particular, RBC (Fig. 2), PCV (Fig. 3) and Hb (Fig. 4) and two of the investigated biochemical parameters: TGL and Ca^{2+} (Figs. 14 and 16) which exhibited a tendency to stay at lower values during the experiment. This finding was confirmed by a summarising overview of the individual values of each of the parameters during the whole trial; the results are shown in Table 2. Also, there was a close dependence of LD on the oxygen saturation of water ($I_{yx} = 0.908$) in the control group of fish, compared with the astaxanthin-treated experimental group ($I_{yx} = 0.444$), which can be expressed by the quadratic regression equations: $y' = 1121.623 - 25.839x + 0.151x^2$ vs. $y' = 199.837 - 4.249x + 0.024x^2$. Another important finding is the dependence of CHOL on the content of oxygen in water ($I_{yx} = 0.993$, $y' = -175.336 + 40.660x - 2.248x^2$ vs. $I_{yx} = 0.494$, $y' = 66.553 - 12.123x + 0.597x^2$) and the dependence of LD on water temperature ($I_{yx} = 0.883$, $y' = -63.357 + 14.056x - 566x^2$ vs. $I_{yx} = 0.405$, $y' = 5.531 + 1.884x - 0.080x^2$).

The condition indices (Figs. 24–29), LSI was, for the most part, lower in the astaxanthin-fed group over the period of the experiment, compared with the control group of fish (Fig. 27). The decline of the LSI figures was significant ($P < 0.05$) on the 60th day (1.52 vs. 1.82).

Table 2
Comparison of the values of blood and condition indices of the rainbow trout for the whole experimental period (81 days)
Note: the upper (u) and lower (l) boundary of the confidence interval for the arithmetic mean for the base set at a significance level of $P = 0.05$; V = variance in %.

Indices	Experimental group										Statistical significance
	A					C					
	n	Mean	SD	V	u–l	*n*	Mean	SD	V	u–l	
RBC (T/l)	28	1.06	(0.120)	11.32	1.01–1.11	30	1.15	(0.162)	14.09	1.09–1.21	$P < 0.05$
PCV	28	0.386	(0.0488)	12.64	0.367–0.405	30	0.422	(0.0671)	15.90	0.397–0.447	$P < 0.05$
Hb (g/l)	28	71.8	(7.40)	10.31	68.9–74.7	30	76.5	(7.9)	10.39	73.5–79.5	$P < 0.05$
MCV (fl)	28	364	(34.3)	9.42	350–378	30	368	(41.0)	11.14	352–384	
MCH (pg)	28	68	(5.5)	8.09	66–70	30	67	(5.7)	8.51	65–69	
MCHC	28	0.19	(0.015)	7.89	0.18–0.20	30	0.18	(0.019)	10.56	0.17–0.19	$P < 0.05$
BUN (mmol/l)	29	0.6	(0.27)	45.0	0.5–0.7	30	0.7	(0.42)	60.0	0.5–0.9	
CREA (μmol/l)	29	27	(11.3)	41.85	23–31	28	23	(8.1)	35.22	20–26	
UA (μmol/l)	29	118.9	(49.82)	41.90	99.6–138.2	29	114.9	(53.31)	47.27	94.3–135.5	
TP (g/l)	29	39.4	(5.91)	15.0	37.1–41.7	29	41.0	(6.94)	16.13	38.4–43.7	
GL (mmol/l)	29	4.37	(0.460)	10.53	4.19–4.55	29	4.03	(0.948)	23.52	3.96–4.40	
CHOL (mmol/l)	29	5.30	(1.518)	28.64	4.71–5.89	29	6.17	(1.924)	31.18	5.43–6.31	
TGL (mmol/l)	19	3.69	(1.007)	27.29	3.19–4.19	19	4.85	(1.628)	33.57	4.04–5.66	$P < 0.05$
P (mmol/l)	29	4.47	(1.164)	26.04	4.02–4.56	28	4.98	(1.716)	34.46	4.30–5.66	
Ca^{2+} (mmol/l)	19	2.75	(0.183)	6.65	2.66–2.84	19	2.92	(0.180)	6.16	2.83–3.01	$P > 0.01$
Natrium (mmol/l)	19	156.1	(3.89)	2.49	154.2–158.0	19	156.4	(3.281)	2.10	154.8–158.0	
Kalium (mmol/l)	18	0.95	(0.652)	68.63	0.62–1.28	15	0.88	(0.648)	73.64	0.51–1.25	
ALT (μkat/l)	27	0.39	(0.248)	63.59	0.29–0.49	27	0.40	(0.231)	57.75	0.31–0.49	
ASP (μkat/l)	27	8.43	(3.0)	35.59	7.22–9.64	26	7.96	(2.661)	33.43	6.89–9.06	
ALP (μkat/l)	29	5.37	(2.610)	48.60	4.36–6.38	29	4.89	(1.667)	34.09	4.24–5.54	
LD (μkat/l)	27	15.03	(3.974)	26.44	13.43–16.51	29	17.76	(6.729)	37.89	15.16–20.36	
HBD (μkat/l)	29	3.99	(1.537)	38.52	3.40–4.58	29	4.68	(2.260)	48.29	3.81–5.55	
FQ coefficient	24	1.55	(0.136)	8.77	1.49–1.61	24	1.58	(0.128)	8.10	1.52–1.64	
CQ coefficient	24	1.31	(0.101)	7.71	1.27–1.35	26	1.33	(0.096)	7.22	1.29–1.37	
AWL (g)	29	4.46	(1.505)	33.74	3.88–5.04	31	5.11	(1.808)	35.38	4.44–5.78	
LSI (%)	29	1.50	(0.349)	23.27	1.36–1.64	31	1.65	(0.411)	24.91	1.50–1.80	
AWS (g)	24	0.43	(0.147)	34.19	0.37–0.49	26	0.45	(0.142)	31.56	0.39–0.509	
SSI (%)	29	0.15	(0.045)	21.00	0.13–0.17	31	0.15	(0.044)	29.33	0.13–0.17	

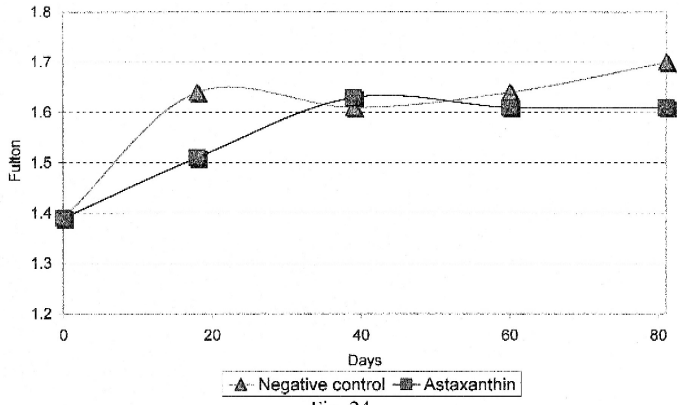

Fig. 24

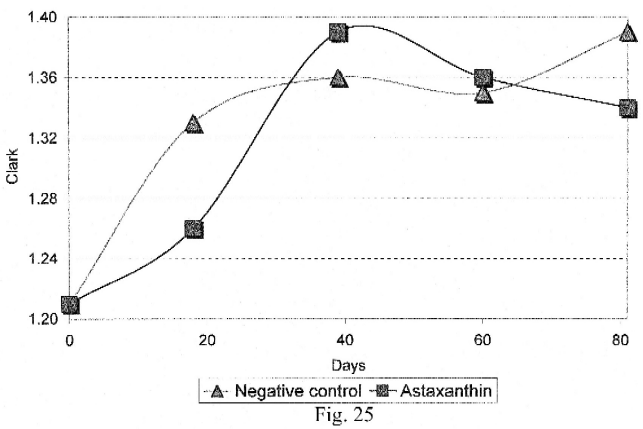

Fig. 25

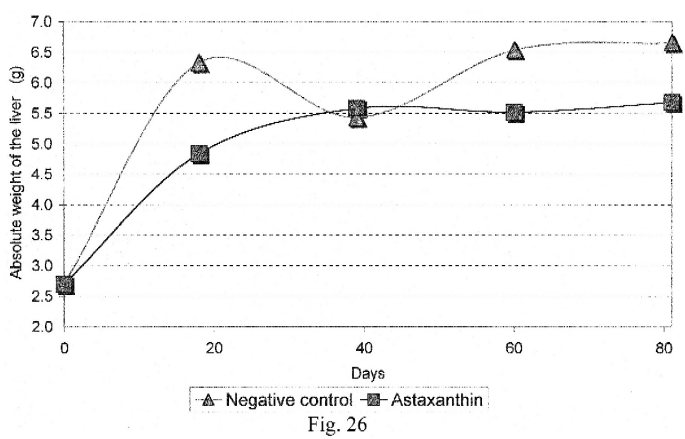

Fig. 26

Figs. 24–29. Dynamics of the condition indices during the course of the experiment with rainbow trout fed an astaxanthin-containing diet against an astaxanthin control.

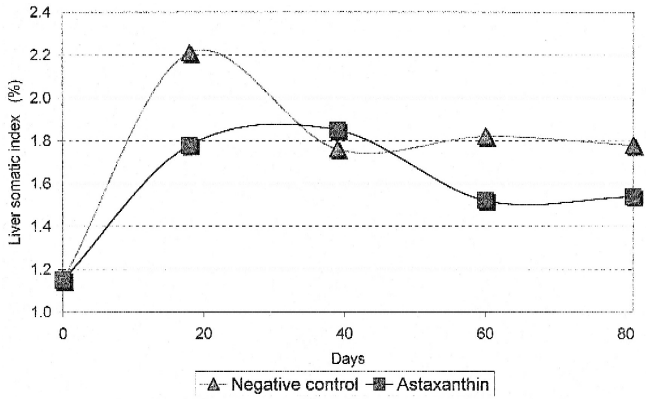

Fig. 27

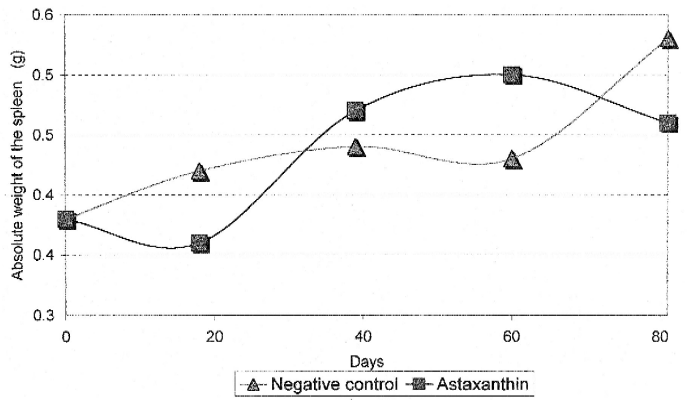

Fig. 28

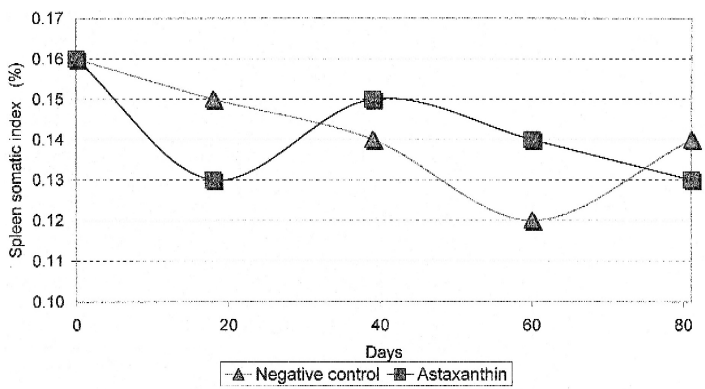

Fig. 29

4. Discussion

The physiological condition of the fish is among the key factors underlying the attainment of the required performance levels. This is why monitoring the physiological state of the rainbow trout has become an integral part of the routine examination of the health of the fish, and won a permanent position within the complex of methods serving to interpret the results of feeding trials, involved in the testing of the biological and production efficacy of feeds and the constituents thereof. For the rainbow trout kept under Czech conditions, this is shown by the studies conducted by Bělík et al. (1978), Dobšínská et al. (1980), Řehulka (1984) and Jirásek et al. (1989). The present results show that astaxanthin can intervene, to a certain extent, with the physiological and metabolic processes.

Our results relating to the study of growth are similar to the conclusions published by Foss et al. (1984) who, in trials with rainbow trout weighting 0.35 kg, found no differences in growth and mortality when testing astaxanthin and canthaxanthin. Storebakken and Choubert (1991), who examined flesh pigmentation of rainbow trout fed astaxanthin and canthaxanthin at different feeding rates in freshwater and saltwater, found no significant differences in growth and feed conversion due to pigments. In trials focused on the pigmentation of rainbow trout and sea trout with astaxanthin and astaxanthin dipalmitate, in comparison with canthaxanthin, Foss et al. (1987) achieved a better growth in the rainbow trout (initial weight of 93 g), compared with sea trout (initial weight of 48 g). More convincing results were obtained by Torrissen (1984) who tested the effect of carotenoids in eggs and start-feeding diet on the survival and growth rate of the fry of the Atlantic salmon. Two experimental diets were prepared by adding 30 mg/kg of synthetic canthaxanthin and 30 mg/kg of synthetic astaxanthin, respectively, to the basic diet. There was no significant difference between the groups fed astaxanthin and those fed canthaxanthin in Torrissen's tests; however, these groups grew faster than did the control groups, and the difference was significant after 3 weeks of feeding. Interesting results were reported by No and Storebakken (1991) who tested the pigmentation of rainbow trout given astaxanthin at different water temperatures. The effect of water temperature (5°C and 15°C) on growth rate of rainbow trout (initial average weight of 507 g) was investigated in trials with fish fed a test diet supplemented with 57 mg of astaxanthin per kg of the feed, compared with fish given a control diet containing no astaxanthin supplement. The growth rate was higher in the fish reared at 15°C than at 5°C throughout the experiment. Our results are also in keeping with the data of the growth trial conducted by Řehulka and Žák (1986) to study the effect of canthaxanthin on rainbow trout (initial weight of 75 g): in that trial with fish fed a diet with 400 mg canthaxanthin per kg, the feed conversion ratio (FCR) was improved by 5.9–6.5%.

By analysing a wide range of hematological and biochemical parameters of peripheral blood, we followed up with the results of Nakano et al. (1995), who had studied the effects of red yeast containing astaxanthin and synthetic astaxanthin on the serum enzyme activities GPT (ALT) and GOT (AST). As the author asserts, the levels of both enzymes in the fish fed a diet containing red yeast or synthetic astaxanthin were

significantly lower than those of the fish fed a control diet. The mean amount of serum lipid peroxide in the fish given a diet containing red yeast and synthetic astaxanthin was also lower than that of the control fish. In addition, the decline in the HSI (LSI) in experimental fish has demonstrated that the dietary red yeast and synthetic astaxanthin have the potential to improve the health of fish, e.g. provide a better function of the liver. Like Nakano et al. (1995), we found an increased ALT and AST activation in the blood serum of the fish fed a diet with oxidized fat; however, this was so in our previous trials (Řehulka, 1990). In these experiments, the AST and ALT levels of the fish fed dry pellets with oxidized fat were up to four times higher than in the control fish (AST: 11.7 vs. 2.6 μkat/l, ALT: 0.96 vs. 0.23 μkat/l). Considering the results of the hematological examination, we believe it is important to point out the significantly lower levels of RBC, PCV, and Hb and the two biochemical parameters, TGL and Ca^{2+}, in the plasma of the fish fed the astaxanthin-containing diet. These findings show that astaxanthin can influence the blood components. These results confirm that rainbow trout can respond sensitively to environmental changes which we saw in our previous studies in relation to the physical and chemical properties of water (Řehulka, 1997), to the quality and nutrient structure of food (Řehulka, 1984, 1989, 1990, 1994a,b), and infectious diseases (Řehulka, 1998). The close and significant ($P < 0.01$) negative correlation between CHOL and UA in the fish given astaxanthin-containing diet ($r = -0.960$), as distinct from the control fish ($r = -0.489$). A similar finding was obtained in the tests conducted to examine the NEOX antioxidant (Řehulka, 1994a) in the same age category of fish and at the same physical and hydrochemical conditions of the water ($r = -0.883$), and also in other trials in which the relation between these parameters was expressed by the parametric dependence of ($I_{yx} = 0.908$) (Řehulka, 1994b). The concentration of UA in the plasma was discussed (Řehulka, 1994a) because it increased in the plasma of the fish showing the higher production performance. The idea that UA may have a priority position in the hierarchy of the methods of biochemical examination of the rainbow trout was suggested by Koudela (1983), who believed that the dynamics of the biosynthesis and concentration of plasma UA was a valuable parameter of the modern physiology of poultry. The level of UA biosynthesis, as Koudela suggests, is an important indicator of the amino acid demand and biological value of feed.

Hematological and biochemical examination showed that astaxanthin exerted a certain influence on some of the blood indices studied, but the deviations caused by this influence remained within the physiological range of the reference values for the rainbow trout as derived from the summarising distribution curve recorded in our laboratory. In conclusion, it can be said that in addition to its importance for producing an attractive colour of muscle, astaxanthin in the diet for the rainbow trout may also significantly influence certain physiological functions, especially hematopoiesis and the metabolism of lipids and calcium. This finding, along with the results published by Nakano et al. (1995), encourage efforts to expand the knowledge in this area in order to be able to determine the best astaxanthin dose in the feed to maintain the organism's internal environment in an optimum state. Our results have again the justification of using the methods of clinical hematology and biochemistry within the complex of methods employed in assessing the biological and productive effectiveness of feeds.

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