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ERYTHRODERMATITIS OF CARP, CYPRINUS CARPIO (L.): AN ELECTROPHORETIC STUDY OF BLOOD SERUM PROTEIN FRACTION LEVELS

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Abstract

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Paper electrophoresis was used during the growing season from May to October to analyze the dynamics of total proteinaemia of blood serum and its fractions in clinically healthy carp, Cyprinus carpio L., and in carp diseased with erythroderma titis (CE). Four basic fractions were detected including γ-globulins (2 subfractions), β-globulins (3 subfractions) and albumins. The physiological state of carp after overwintering was found to be optimum at a minimum total protein level of 25 g/l, with a relative albumin proportion of 0.25 and at an A/G quotient of 0.35. When fish were subsequently given favourable conditions and full-value food, the physiological state was stabilised at total protein levels above 33 g/l, albumins 8 to 12 g/l (relatively 0.25 to 0.35), and at an A/G quotient of 0.35 to 0.50. The severe form of CE, in fish reared under conditions without supplementary feeding, was accompanied by a decline in total protein (10 to 16 vs 32 to 34 g/l), by a relative and absolute decline in albumins (0.121 to 0.197 vs 0.276 to 0.293 and 1.21 to 3.16 vs 8.84 to 9.97 g/l), and by a low A/G ratio (0.15 to 0.25 vs 0.38 to 0.41).

Erythrodermatitis, carp, paper electrophoresis, total serum protein, serum protein fraction levels, experimental conditions, growing season

In order to rear carp, *Cyprinus carpio (L.)*, intensively with minimal losses, it is necessary to be aware of the state of the health of the fish. Better diagnostics are required to enhance our understanding of pathogenesis and to control the course of diseases, evaluate the therapy and state the prognoses. To improve the diagnosis of carp diseases, it was decided to study the total protein of the blood serum of clinically healthy and diseased fish by electrophoretic analysis in order to distinguish changes from normal conditions in the different fractions and to derive criteria for evaluation of the state of health. These changes depend on a number of external and internal factors, especially nutrition, environmental conditions, method of rearing, stock weight and seasonal effects (Sorvachev 1957; Łysak and Wójcik 1960; Sadykhov and Petrenko 1969; Vlasov 1974; Rónyai et al. 1982). It has, however, also been demonstrated that a significant contribution to changes in the fractions that constitute the protein spectrum can be made by pathological state (Ranke and Ranke 1955; Offhaus et al. 1955; Flemming 1958; Liebmann et al. 1960; Riedmüller 1965, 1966; Gulyaev et al. 1966; Reichenbach-Klinke 1966, 1973; Kulow 1969; Ivasik and Karpenko 1971; Golovnev et al. 1983) including parasitoses (Jara- and Szerow 1981; Jara et al. 1981). The majority of authors, quoted above, used paper electrophoresis, so this technique was adopted in the first stage of our research. In later experiments it is proposed to follow up the results obtained by those authors who has used more advanced techniqued of separation in electrophoretic analysis, including the use of agar (Ivasik and Karpenko 1971), cellulose acetate paper (Hattingh 1972; Boon et al. 1986), polyacrylamide gels (Rónyai et al. 1982) and cellogel microelectrophoresis (Fašaić and Paláčková 1990).

The present paper reports on experiments carried out under field conditions. The results represent the initial stage of long-term studies on the biochemistry of the common carp.

Materials and Methods

Fish

The trials were conducted in the Fish Culture and Hydrobiology Research Institute's experimental pond at Klimkovice for 154 days from May to October. The experimental fish were scaly

(2+) common carp, *Cyprinus calpio (L.)*, from one of the State Fishery's wintering ponds. These fish were subjected to a veterinary check-up at the end of hibernation and were found to be pres disposed to erythrodermatitis (CE). Early in May, 180 fish were picked at random and put in group- of 30 into 6 test cages 2.5 × 5 m in size, installed onto the bottom of a pond 1 m deep. During the experimental period the carp were given feed pellets. In the first stage of the experiment (61 days from May 7 to July 8) the amount of the feed corresponded to a maintenance ration. Thereafter (93 days from July 8 to October 9) the ration was a production diet. The growth dynamics of the fish during the experiment are shown in Fig. 1.

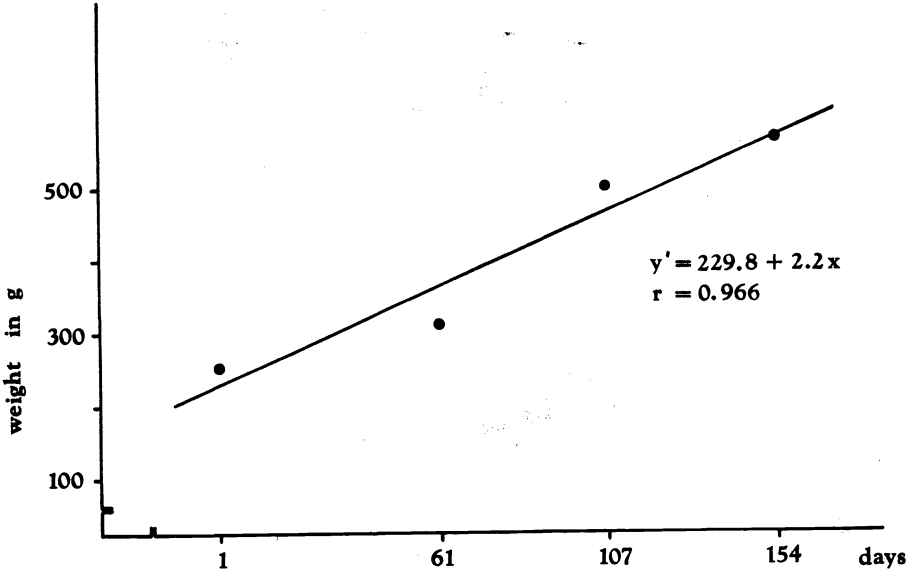


Fig. 1. Live weight growth dynamics of carp during the experimental period, as depending on time (expressed by linear regression).

The state of health of the fish was evaluated post-mortem by dissection and parasitological, histological and bacteriological examination. Tissue was embedded in 10 % neutral buffered formalin for histological examination and the paraffin slices were stained with haematoxylin and eosin (H & E). The objective of the bacteriological and mycological examination of the fish was to detect the causative agents of skin lesions. Tissue smears were prepared and stained and nutrient media were inoculated. The cultivation media included the blood, Ordal, Mueller-Hinton, Sabouraud and sweet-wort agars. The incubation temperature for examination of the growth and biochemistry of the causative microorganism was 20 to 25°C.

Serum protein

Four blood samples were taken during the experiment (on May 7, July 8, August 23 and October 9) from twenty fish on each date (7 to 10 fish with signs of CE and 7 to 10 fish without signs of CE). Blood was collected from the severed caudal vein in the morning immediately after removing the fish from the experimental cages. The fish were stunned before the blood was collected. The blood serum was separated after centrifugation at 5 800 rpm for 15 min at 4°C and stored for future analysis at 4° C. Total protein was determined by the Biuret method, photometrically, at a wavelength of 545 nm, using the BM Standard. This was done within 4 hours of blood collection. The physicochemical properties of the water at dates of blood sampling are presented in Table 1.

Paper electrophoresis

Four hours after blood collection, serum proteins were separated by two-dimensional paper electrophoresis. Barbital acetate buffer was used at a pH of 9 and ionic strength of 0.06. The protein separation time lasted 16 hours at a temperature of 20 to 22° C at a current intensity of 10 mA (2 mA per one paper strip) and at a voltage of 120 V. Serum amounts of 0.02 ml were applied

Table 1

The physical and hydrochemical characteristic of the water at time of blood samplings

	May 7	Jul 8	Aug 23	Oct 9
Water temperature (°C)	18.8	24.3	25	9
pH	9.2	7.4	7.9	7.8
Dissolved O_2 (mg/l)	13.4	11.5	7.6	8.5
O_2 saturation of water (%)	144	135.8	90.8	73.1
Mn oxidability (mg/l)	14.6	12.5	13.3	10.6
NH_3-N (mg/l)	0.054	0.014	0.086	0.004

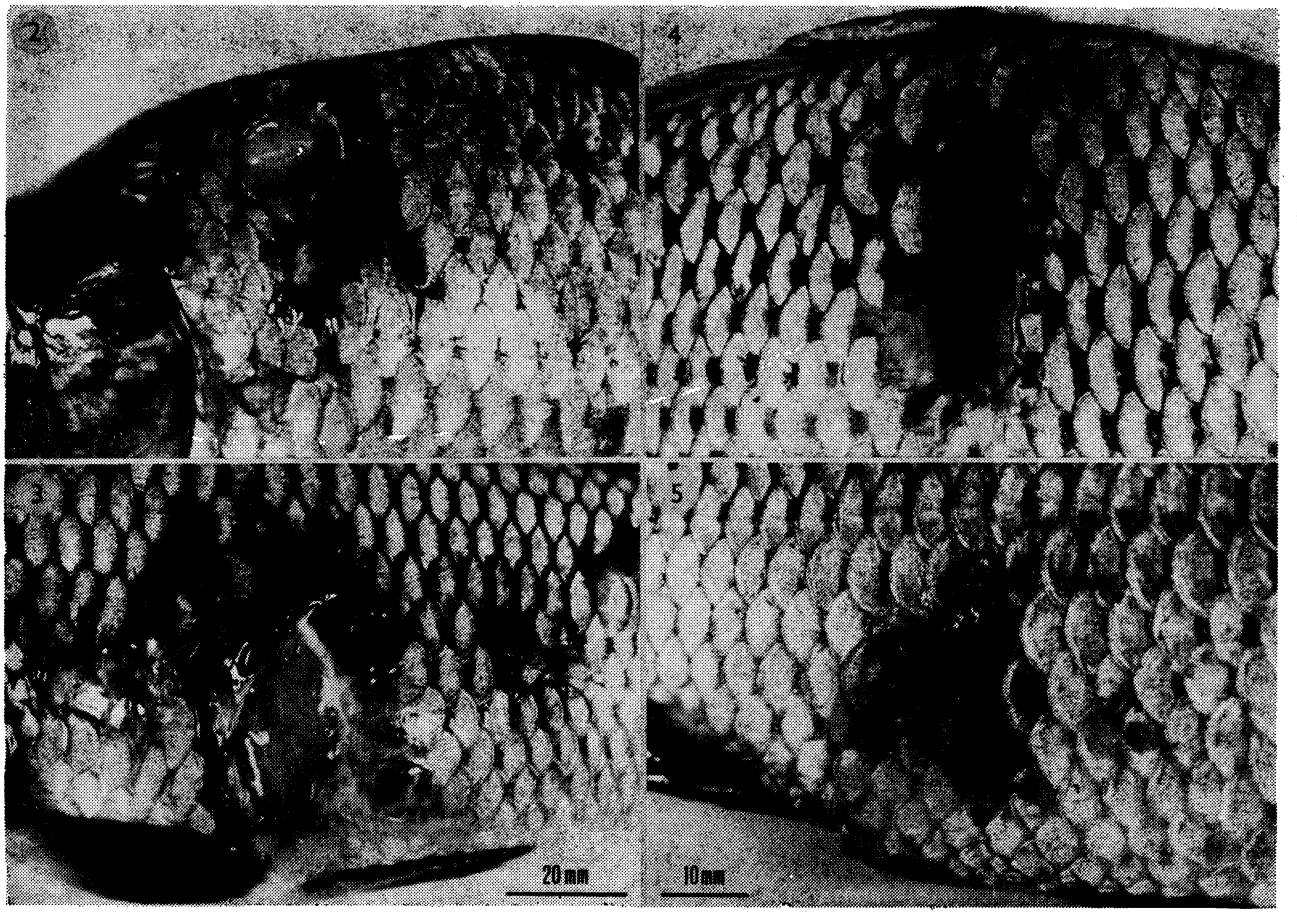
onto FN−1 paper strips 38 cm long and 4.5 cm wide. Electropherograms were stabilized at 105° C for ten minutes and stained with phenylene blue. They were evaluated by the Zeiss ERI densitometer and integrative curves were constructed to estimate the values for each fraction. In Figs 6 to 11, components of the serum protein spectrum are designated by lower-case letters; they were classified, for the purposes of comparison with the authors cited above, as gamma globulins ($h = \gamma_2$, $g = \gamma_1$), beta globulins ($f = \beta_3$, $e = \beta_2$, $d = \beta_1$), alpha globulins ($c = \alpha_2$, $b = \alpha_1$) and albumins (a).

Statistical significance of the differences between the means for the selected sets was verified by the t − test at the significance levels of $P = 0.05$ and $P = 0.01$.

Results

Early in May, 20 days after being taken from the storage pond, the clinically healthy experimental fish, free of patho-anatomical findings, had a total protein level of 28 g/l of blood serum; however, there were also fish showing cachexia, local anemic foci at the bases of gill filaments, and a slightly icteric hepatopancreas. Parasitological examination revealed sporadic *Trichodina* sp. and *Apiosoma* sp. on the skin; in 30% of hearts there was sporadic *Sanguinicola inermis*, and in 50% of intestines there was a moderate to severe infestation of the wall by *Goussia subepithelialis*. Histology revealed necrosis of the gill lamellae, hyperaemia of the spleen, and hyperplastic reticular cells. Within 25 days, in 30% of carp emaciation gradually increased, visceral oedema was registered, and inflammatory lesions began appearing on the skin signalling the onset of CE. *Aeromonas hydrophila* subsp. *hydrophila*, *Flavobacterium* sp., *Schewanella putrefaciens* and *Pseudomonas maltophila* were isolated from erythrodermatitis lesions. All these species were isolated in the cases of CE as described by Schulz (1980) and some of them were experimentally confirmed to be causative agents of CE. This latter study supports our interpretation of the bacterial aetiology of the skin lesions, as described, which occurred in our experimental fish.

In the first ten-day period of July, classical alterations due to CE, in the form of epidermal necrosis and ulcerations in muscle tissue, were registered in 80% of fish (Figs. 2, 3). The lesions ranged from 1 to 10 cm^2 in size. In 80% of affected cases there was mild peritonitis and an increase in water retention by organs, while in 20% of cases, severe peritonitis and moderate water retention were recorded. Histologically, there were inflammatory alterations in the interstitial tissue of muscles, sometimes even degenerative changes in muscle fibres. In cases where the epidermis tended to re-epithelize, the oedematous skin was intensively invaded by a cellular inflammatory reaction which penetrated the subcutis and epidermis. Histology of the hepatopancreas indicated increased activity of RES, presence of inflammatory elements in portal fields, and hyperaemia in some regions.



Figs 2 to 5. Development of erythrodermatitis lesions in (2+) carp during the growing season: severe form of erythrodermatitis in summer (2, 3) and residues of erythrodermatitis lesions in a stage of satisfactory regeneration (4) and with visible bases of new scales (5) in October.

In the third ten-day period of August, the lesions had resolved in 60% of fish. The remaining 40% possessed both progressing and regressing lesions. In the former case, there were ulcerations up to 4.5 cm^2 in dimension; in the latter case, there were flat, shallow, dark pigmented scars up to 23 cm^2, almost devoid of scales. Severe peritonitis was registered in both groups.

The final examination in October showed advanced regeneration manifested by a dark pigmentation of the epidermis and, occasionally, even by minute bases of new scales (Figs. 4, 5). At this stage, histology illustrated regenerated epidermis with residual signs of inflammation. In most carp, considerable amounts of glycogen were evident in hepatocytes.

According to the level of total proteins and the absolute and relative changes in protein fractions which occurred in the course of the experiment, the state of health of the common carp was classified as follows: fish in good condition, manifesting no clinical symptoms 14 days after taking them out of the storage pond, had total serum protein ranging from 25 to 28 g/l (Fig. 6); in carp which were emaciated and displayed a clear exudate in the body cavity and inflammation foci on the skin, the values were substantially lower, 14 to 20 g/l ($P < 0.01$) (Fig. 7). This reduction in total protein in diseased fish was associated with an increased d-fraction ($\beta_1 = 0.13$ to 0.21) and with a decrease in relative and absolute levels of albumin (0.11 to 0.205 and 1.9 to 3.9 g/l, respectively) ($P < 0.05$ and $P < 0.01$). In healthy carp, the d-fraction amounted to 0.053 to 0.112 ($P < 0.05$) and the mean relative and absolute levels of albumin were 0.293 (0.24 to 0.34) ($P < 0.05$) and 8 g/l (7.6 to 8.5 g/l) ($P < 0.01$), respectively.

In carp severely affected by CE — which occurred in its most intensive form in July under conditions without supplementary feeding — the decrease in total protein to 10 to 16 vs 32 to 34 g/l ($P < 0.01$) (Figs. 8, 9) was particularly evident. This state was connected with a prolonged increase of relative β_1 -globulin (0.150 to 0.189) and a gradual decrease of relative and absolute albumin (0.121 to 0.197 vs 0.276 to 0.293 and 1.21 to 3.16 vs 8.84 to 9.97 g/l) ($P < 0.01$), respectively.

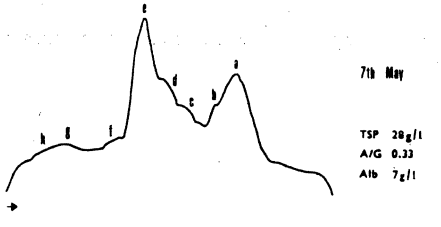


Fig. 6.: Electropherogram from (2+) carp in good condition and with no pathological-anatomical findings.

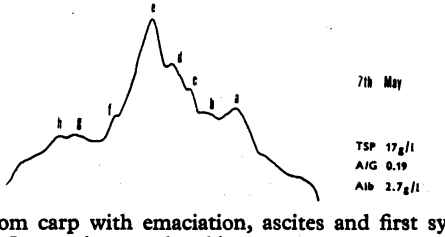
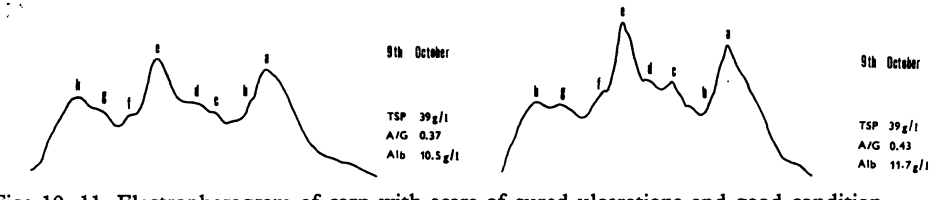


Fig. 7. Electropherogram from carp with emaciation, ascites and first symptoms of erythrodermatitis in form of local inflammation on the skin

Abundant feeding with pelleted food containing 15 to 19% of crude protein resulted in a substantial increase in total protein within one month. Total protein values subsequently remained at the level of 39 g/l till the final examination in October and were accompanied, besides an increased level of albumin and the albumin/globulin quotient (A/G) (Fig. 12), by a satisfactory recuperation from CE. Electropherograms from these carp (Figs. 10, 11) illustrate that albumins outbalanced, both relatively and absolutely (rel. 0.276 to 0.299 and 10.5 to 11.7 g/l) the e-fraction (β_2, rel. 0.200 to 0.230 and 7.8 to 8.9 g/l). In fish suffering with CE the relation was reverse, as demonstrated by the albumin/β_2-globulin ratio (A/β_2): in fish in good condition the A/β_2 values ranged from 1.2 to 1.6 while in sick fish it was significantly ($P < 0.01$) lower, (0.57 to 0.87) (Figs 7, 8, 10, 11).

Fig. 8. Electropherogram of carp with circumscript inflammation on skin and imbued inner organs.

Fig. 9. Electropherogram of carp with severe erythrodermatitis manifested by ulceration and denuded muscle tissue.



Figs 10, 11. Electropherogram of carp with scars of cured ulcerations and good condition.

Fish of the October sample also typically showed a decline in beta globulins (fractions f, e, d) (0.36 to 0.41), compared with the fish with symptoms of CE in the summer and spring seasons (0.48 to 0.55); gama globulins, represented by two subfractions (h, g) had a comparatively increasing tendency during the year (in relative terms), with fluctuating values in fish having CE in July (0.16 to 0.22) and with a stabilised culmination in October (0.20 to 0.21), compared with fish examined in May (0.14 to 0.15).

Discussion

Our results are comparable to findings by some of the quoted authors, particularly Sadykhov and Petrenko (1969) and Sorvachev (1957), confirming the correlations between total protein and protein fractions on the one hand and quality of nutrition and density of population on the other. In addition to this, our results show that the albumin fraction, in absolute terms or relative to the level of globulins, presents a valuable indicator of nutritional and physiological status in the carp.

In agreement with Sadykhov and Petrenko (1969) we found that if a physiological minimum associated with a normal protein synthesis is to be maintained, it is necessary to provide sufficient food with adequate available crude protein. The fish in our experiments responded to the production ration, provided after July 8, and reached a total proteinaemia, an absolute level of albumin and A/G ratio, with a simultaneous slight increase in γ-globulin. Like Vlasov (1974), we found that α-globulins are the labile fraction in relative terms and that γ-globulins increase in autumn.

Sorvachev (1957) found that maintaining the optimum level of total protein in the blood serum of carp yearlings in spring (March and April) is essential to ensure a good state of health in the subsequent period and to avoid losses. This is in good agreement with our results. A decrease in total protein to under 20 g/l (10 to 16 g/l) together with a decline in the absolute and relative levels of albumin (1.21 to 3.16 g/l and 0.121 to 0.197, respectively) did not maintain a good physiological state in the carp. The optimum levels of total protein which we determined in spring and autumn (>25 and >33 g/l, respectively) are in agreement with values published by Kulow (1967 as cited by Ivasik and Karpenko 1971). We also agree with the results published by Pravda (1985) who considers 25 g/l

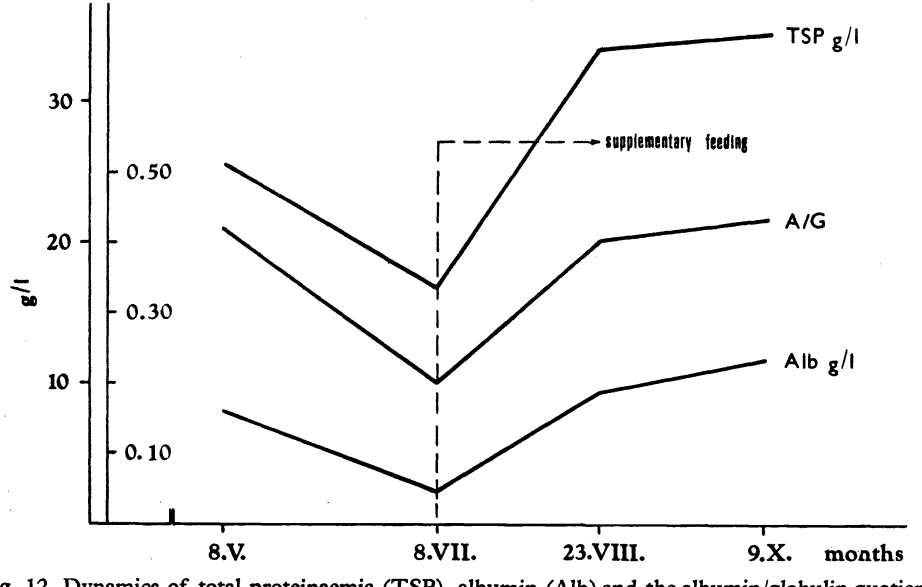


Fig. 12. Dynamics of total proteinaemia (TSP), albumin (Alb) and the albumin/globulin quotient (A/G) in carp during the period of investigation.

as the normal proteinaemia for carp fry. Pravda (1985) also draws attention to the results obtained by Červinka (1971) and to unpublished results by Adámek (1977), who were the first in the Czech Republic to emphasize the importance of plasma proteins for maintaining an optimum development of carp fry. Spurný and Mareš (1989) provided convincing evidence on the importance of spring feeding of carp fry for increasing the total protein level of blood plasma. Our results, intended primarily for evaluation of the state of ehalth of carp, can be compared with findings published by pioneers in the investigation of this problem, including the following German authors: Ranke and Ranke (1955), Offhaus et al. (1955), Flemming (1958), Liebmann et al. (1960), Riedmüller (1965 and 1966) and Kulow (1966 and 1969). As to infectious ascites and CE, Ranke and Ranke (1955) and Flemming (1958) agree that both diseases are accompanied by marked decrease in total protein and level of albumin, which is in keeping with our findings. High importance is attached to the system worked out by Offhaus (quoted by Riedmüller 1966) which takes into account the total protein level, albumin level and A/G ratio in order to classify the state of health of carp into four categories.

Rónyai et al. (1982) conducted experiments with 3-year-old carp and interpreted the results of electrophoretic analysis on polyacrylamide gels. Unlike us, they recorded comparatively high levels of albumin (15.7 to 20.5 g/l) and reported that the A/G ratio (0.7 to 1.18) increased with stocking density. Ivasik and Karpenko (1971), who carried out electrophoresis on agar, put forward an interesting interpretation of values for relative albumin and A/G ratio. Comparing these conclusions with our results makes it advisable to try different carriers, for example, agarose and cellulose paper.

Among other factors affecting the onset of CE in carp — whose aetiology is understood in the Czech Republic in the same way as described by Fijan (1982) — a considerable role must be attributed to optimum supply, not only of proteins, but also of fatty acids (Csengeri et al. 1983).

From the practical point of view, our results, supported by the cited literature, indicate that if total protein drops below 25 g/l after overwintering, the physiological state of carp will be suboptimum and is likely to be severely impaired by the traditional handling and transferring in the spring season. From many years of experience we know that CE, which affects carp of any age, is an extremely dangerous disease and that the particularly predisposed fish are those in poor conditions prior to storage or those weakened in its course.

The levels of protein and its fractions which provide the best prognosis after overwintering are at least 28 g/l for total protein and blood serum, a relative albumin over 0.25 (8 g/l) and an A/G ratio of 0.35. If fish are not offered appropriate rearing and feeding conditions during the period atfer transfer, the disease aggravates and combines mostly with spring viremia. If there is vigorous competition for food, as indicated by a decreased total protein (10 to 16 g/l) and if albumin is depressed below 3 g/l whilst β-globulins show a slight relative increase, CE develops in a severely progressive form. With adequate environmental conditions, the physiological state of carp may be stabilized at a total protein over 33 g/l, albumin 8 to 12 g/l (relative level 0.25 to 0.35), and an A/G ratio 0.35 to 0.5. These values represent significant criteria of the state of health.

It is concluded that, in order to stabilize the state of health and to prevent losses in carp, the diagnostic criteria should take albumin level and an A/G ratio into consideration in addition to total serum protein.

Elektroforetická studie bílkovinných frakcí krevního séra kapra, *Cyprinus carpio* L., při erytrodermatitidě

Metodou dvousměrné elektroforézy na papíře byla v průběhu vegetačního období od května do října sledována dynamika celkového proteinu a její frakce u kapří násady klinicky zdravé a nemocné erytrodermatitidou. Za použití barbital-acetátového pufru o pH 9, iontové síle 0,06, době dělení 16 hodin, intenzitě proudu 10 mA a napětí 120 V byly v krevním séru detekovány čtyři frakce, které jsme zařadili mezi gamma globulíny (2 podfrakce), beta globulíny (3 podfrakce), alfa globulíny (3 podfrakce) a albumíny. Bylo zjištěno, že fyziologický stav kaprů po přezimování je v optimu při minimální hladině celkového proteinu 25 g/l, relativním zastoupení albuminů 0,250 a A/G koeficientu 0,35. Nabídnou-li se rybě v následujícím období příznivé podmínky odchovu s dostatkem potravy, je fyziologický stav ryb konsolidován při hodnotách celkové bílkoviny nad 33 g/l, relativním množství albuminů 0,25 až 0,35 a A/G koeficientu 0,35 až 0,50. Těžká forma erytrodermatitidy u ryb odchovávaných v podmínkách na přirozené potravě bez přikrmování byla provázena výrazným poklesem celkového proteinu (10—16 proti 32—34 g/l), relativním a absolutním snížením albuminů (0,121 až 0,197 proti 0,276—0,293 a 1,21—3,16 proti 8,84—9,97 g/l) a nízkým A/G (0,15 až 0,25 proti 0,38—0,41). Zvýšení celkového proteinu na 39 g/l v důsledku přikrmování urychluje regenerační proces erytrodermatitidy za současného relativního a absolutního převýšení hladiny albuminů (0,276—0,299 a 10,5—11,7 g/l) nad frakcí e (β_2-globulín) (0,20—0,23 a 7,8—8,9 g/l). Při tomto stavu je typická elevace a/e koeficientu (A/β_2-globulín) na 1,2 až 1,6 ve srovnání s rybami s erytrodermatitidou, kde tento dosahuje nižších hodnot (0,57—0,87).

Электрофоретический анализ белковых фракций кровяной сыворотки карпа, *Cyprinus carpio L.*, при эритродерматите

Методом двустороннего электрофореза на бумаге в вегетационный период с мая по октябрь проводили исследования динамики общего протеина и ее фракций на клинически здоровом и заболевшем эритродерматитом посадочном материале карпа. При применении барбитал-ацетатового буферного раствора pH 9, ионной силы 0,06, периода деления 16 часов, интенсивности тока 10 мА и напряжения 120 В определяли в кровяной сыворотке четыре фракции, отнесенные к гамма-глобулинам (2 подфракции), бета-глобулинам (3 подфракции), альфа-глобулинам (3 подфракции), и альбуминам. Установили, что физиологическое состояние карпов после зимовки оптимально при минимальном уровне общего протеина 25 г/л, относительном наличии альбуминов 0,25 и частном А/Г 0,35. Если в последующий период создать для рыбы благоприятные условия содержания с достаточным количеством пищи, то ее физиологическое состояние консолидировано при общих белках более 33 г/л, относительном количестве альбуминов 0,25 - 0,35 и частном А/Г 0,35 - 0,50. Тяжелая форма эритродерматита рыб, содержимых в условиях естественной пищи без подкорма, сопровождалась существенным понижением общего протеина (10 - 16 по сравнению с 32 - 43 г/л), относительным и абсолютным понижением альбуминов (0,121 - 0,197 по сравнению с 0,276 - 0,293 и 1,21 - 3,16 по

сравнению с 8,84 - 9,97 г/л) и низким А/Г (0,15 - 0,25 по сравнению с 0,38 - 0,41). Увеличение общего протеина до 39 г/л в результате подкормки ускоряет процесс регенерации эритродерматита при одновременном относительном и абсолютном повышении уровня альбуминов (0,276 - 0,299 и 10,5 - 11,7 г/л) над фракцией е (β_2 - глобулин) (0,20 - 0,23 и 7,8 - 8,9 г/л). При наличии данного состояния характерно увеличение а/е частного (А/β_2 - глобулин) до 1,2 - 1,6 по сравнению с рыбой, заболевшей эритродерматитом, у которой оно достигает более низких величин (0,57 - 0,87).

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