1. Introduction

Feeding is one of the most important factors affecting growth, welfare and physiological performance of fish. On the other hand, under natural conditions, especially in temperate climate, many species of fish undergo natural periods of food deprivation related to low ambient temperature, spawning, migrations or reductions in their food resources and thus have evolved the capability to endure prolonged starvation (Cook et al. 2000; Park et al. 2012). In aquaculture, fish are sometimes intentionally starved, for example, during winter season, to prevent excessive production or for other purposes (Krogdahl & Bakke-Mckellep 2005).

Data concerning the effects of feeding and starvation on haematological values in fish are available but little is known about the effects of these factors on haematopoietic system. Haematological parameters are reliable indicators of physiological status of fish and are often used to evaluate fish health and immune potential. The values of red blood parameters are usually positively related to the dietary protein level (Daniels & Gallagher 2000; Abdel-Tawwab et al. 2010; Habte-Tsion et al. 2013), but an inverse relationship was also reported (Baruah et al. 2009). Protein source seems to be less important than total protein content (Kikuchi 1999). White blood parameters are usually insensitive to the dietary protein content (Kumar et al. 2005; Baruah et al. 2009; Habte-Tsion et al. 2013; Qiang et al. 2013). On the other hand, dietary deficiencies may adversely affect haematological parameters in fish causing anaemia and immunosuppression (Lim & Klesius 1997).

Fish are highly resistant to starvation. According to McCue (2010), the ability of ectotherms to endure long periods of starvation results from their relatively low energy requirements which are 10–20% of those required by endotherms of comparable size at the same conditions. In addition, haematological parameters of fish seem to be quite stable during starvation but sensitivity of various species is different. Unchanged values of red blood parameters during starvation periods from 1 to 6 months were reported by various authors (Rios et al. 2002, 2005; Caruso et al. 2010, 2011; Cho et al. 2010; Park et al. 2012) in various fish species. However, other authors (Borah and Yadav 1996; Abdel-Tawwab et al. 2006; Morshedi et al. 2011; Rios et al. 2011; Falahatkar 2012) found that starvation of 8 days–5 weeks induced alterations in the values of these parameters in other fish species. The effects of starvation on the immune response of fish also differ among the species. Caruso et al. (2010, 2011) reported different changes in the innate immune mechanisms, Rios et al. (2005) observed lymphocytopenia and thrombocytopenia, and Morshedi et al. (2011) found leukopenia in starved fish. These authors concluded that even short-term starvation periods might weaken the immunological system of fish. Shoemaker et al. (2003) observed significantly higher mortality of starved Ictalurus
punctatus caused by bacterial infection compared to the fed fish, which may indicate immunosuppression caused by food deprivation.

However, direct impact of malnutrition or starvation on composition and activity of fish haematopoietic tissue is unknown. The only available and indirect (based on peripheral blood analysis) data on the effects of starvation on haematopoietic potential in fish revealed that Hoplias malabaricus subjected to long-term food deprivation showed significantly decreased count of immature erythrocytes (Rios et al. 2005). The authors concluded that senescent red blood cells were not replaced during starvation.

Common carp under extensive aquaculture conditions are often fed grain, and naturally cease feeding at low temperatures or under adverse water quality conditions (Markovic et al. 2012; Masilko et al. 2014). Therefore, it is important to evaluate not only haematological effects but also haematopoietic effects of feeding diets of different nutritional value and starvation to predict possible consequences as anaemia or immunosuppression.

The aim of the present study was to evaluate the effects of long-term starvation and feeding two diets of different nutritional value: complete commercial carp feed and low-protein grain feed on both peripheral blood parameters and haematopoietic tissue cellular composition and activity in common carp.

2. Materials and methods

Six-month-old common carp Cyprinus carpio L. with a mean weight of 58.6 ± 8.3 g were harvested from the rearing pond of the Samoklęski Fish Farm in Kamionka, Poland, and transported to the laboratory of the Department of Animal Physiology, University of Natural Sciences and Humanities in Siedlce. The fish were acclimated for 1 month to the laboratory conditions in the 250 L flow-through aerated tank. During acclimation period, all fish were fed Aller Classic feed (4.5 mm diameter, Aller Aqua, Poland). After acclimation, 36 fish were randomly stocked in three glass aquaria of 100 L volume, 12 fish in each (n = 12). The control group was fed Aller Aqua Classic 4.5 mm (A), another group – ground barley Polgreen, Latowicz, Poland (B), and third group was starved (S). Both feeds were supplied daily in amounts of 1% of initial fish mass. Barley portions were weighed dry, and then they were cooked for 10 min. Chemical composition of both feeds is shown in Table 1.

Water was constantly aerated and three quarters of the water was renewed daily without disturbing fish: water level was lowered by gentle siphoning out through plastic tube, faeces and remains of food were removed from the bottom, and then new clean non-chlorinated tap water was supplied. Water quality parameters (temperature, pH, dissolved oxygen (DO) concentration, concentrations of NH₄ and NO₂) were measured every three days before water renewal. Temperature and DO levels were measured using oxygen meter Hanna Instruments USA, pH with the pH-meter N5123 Elwo Poland and nitrogenous metabolites using colorimetric Visocolor kits (Visocolor Eco Ammonium 3 and Visocolor Eco Nitrite) by Macherey Nagel, Germany. The results of water quality measurements are shown in Table 2, all the values being within the tolerance ranges of common carp.

The experiment lasted 15 weeks. Blood was sampled 3 times (after 3, 7 and 15 weeks) in the morning before feeding, by heart puncture with chilled heparinized needles in chilled heparinized plastic Eppendorf tubes, in amount of about 100 µL from each fish. Fish were not anesthetized and blood collection from each fish lasted about 30 s. All fish survived the experiment and after the end of it they were returned to the separate flow-through tank and refed Aller Aqua Classic once a day to satiation.

The values of basic haematological parameters were evaluated according to standard methods described by Svobodova et al. (1991). Briefly, the microhematocrit method was used for the measurement of hematocrit (Ht) values, haemoglobin concentration (Hb) was measured using spectrophotometric cyanmethemoglobin method, erythrocyte (RBC) and leukocyte (WBC) counts were evaluated using Burkner hemocytometer in blood diluted 100 times with Hayem solution. Mean cell volume (MCV), mean corpuscular haemoglobin (MCH) mass and mean corpuscular haemoglobin concentration (MCHC) were calculated using Ht, RBC and Hb values. Spontaneous oxidative metabolic activity of blood phagocytes using nitrotetrazolium blue test (NBT) was measured according to the method described by Studnicka et al. (1985). Blood smears were made and stained with May Grunwald and Giemsa solutions to evaluate erythrocyte morphology (300 cells were evaluated in each smear), differential leukocyte count (per 100 cells) and to estimate thrombocyte count (calculated indirectly from the WBC values and number of thrombocytes accompanying 100 leukocytes). After 15 weeks of feeding different feeds or starvation O₂ consumption rates were measured. Seven fish from each group (n = 7) were individually placed in 2.3 L glass aquaria filled with clean oxygenated water at 21.1°C. Initial O₂ concentrations were measured before introduction of fish, and then the tanks with fish were sealed for 30 min. After this time, O₂ concentrations were measured again, and all fish were weighed. DO levels were measured using oxygen meter Hanna Instruments USA. Oxygen consumption rates were calculated for each fish per unit of body mass and time. At the end of the experiment, Seven fish from each group (n = 7) were sacrificed and head kidney tissue smears were made to evaluate

| Component       | Aller Aqua Classic (A) | Ground barley (B) |
|-----------------|------------------------|-------------------|
| Protein (%)     | 30                     | 12                |
| Lipid (%)       | 7                      | 2.4               |
| Carbohydrate (%)| 43                     | 57                |
| Ash (%)         | 7                      | 2.3               |
| Fiber (%)       | 5                      | 7                 |
| Calorific value (kcal/100 g) | 433           | ~360              |

| Parameter | A       | B       | S       |
|-----------|---------|---------|---------|
| O₂ (mg/L) | 8.8 ± 0.7 | 8.9 ± 0.5 | 9.0 ± 0.4 |
| NH₄⁺ (mg/L) | 0.7 ± 0.3 | 0.6 ± 0.4 | 0.3 ± 0.3 |
| NO₂⁻ (mg/L) | 0.2 ± 0.3 | 0.2 ± 0.3 | 0.2 ± 0.3 |
| Temperature (°C) | 19.4 ± 0.8 | 19.4 ± 0.8 | 19.4 ± 0.8 |
| pH        | 7.3 ± 0.1 | 7.3 ± 0.1 | 7.2 ± 0.1 |
cellular composition of haematopoietic tissue (stained with May Grunwald and Giemsa solutions), and for calculation of percentage of proliferating (stained for proliferating cell nuclear antigen [PCNA]) and apoptotic cells (stained for Caspase 3). Haematopoietic cells were identified according to Fijan (2002a, 2002b) and Kondera (2011). Percentages of precursors of each cell line were calculated (per 500 cells in each smear). Immunocytochemical procedures (PCNA and Cas 3 detection) were performed according to Kondera and Witeska (2013). The results were subjected to statistical analysis using Statistica 9 software. Most variables showed non-normal distribution (indicated by the results of Shapiro–Wilk test), therefore significance of differences among experimental groups was evaluated using non-parametric Kruskal–Wallis test, and Spearman’s rank correlation coefficient was calculated for haematopoietic activity parameters and oxygen consumption rate, assuming significance level $p \leq 0.05$.

3. Results

During 15 weeks of feeding carp juveniles diets of different nutrient content or starvation, no mortalities or symptoms of disease occurred. No significant differences were found in the values of red blood parameters among experimental groups (Table 3), except for significantly reduced erythroblast frequency in starved fish (S3, S7 and S15) compared to the control group fed Aller Classic feed (A3, A7 and A15, respectively), and at the end of experiment also compared to the fish fed ground barley (S15 vs. B15). Significant differences within groups were also observed: fish from A group showed a decrease in RBC accompanied by an increase in MCV and MCH between the 7th and 15th week, and an MCV increase was observed at the same time in group B.

No significant differences in leukocyte count (WBC) among experimental groups occurred (Table 3). Differential leukocyte count revealed that in all groups lymphocytes comprised 96.8–100.0% of leukocytes, neutrophils 0.0–2.6% and monocytes 0.0–0.6% of leukocytes. No significant differences among or within groups were observed. However, at the end of the experiment, starved fish (S15) showed the highest percentage of neutrophils and monocytes compared to the control group fed Aller Classic feed (A3, A7 and A15, respectively), and at the end of experiment also compared to group S15 vs. B15. Significant differences were also observed in the WBC count between the 3rd and 15th week.

Oxidative metabolic activity of phagocytes (NBT) did not significantly differ among the groups. However, in all groups the value of NBT gradually decreased, and significant differences occurred among the groups. A significant decrease in PLT took place in group A7 compared to A3. The analysis of head kidney haematopoietic tissue at the end of the experiment also revealed some significant differences (Table 4). The fish fed Aller Classic (A) showed significantly lower frequency of early blast cells and erythroid cells compared to the groups B and S. Neutrophilic lineage was the least frequent in group S, and the most abundant in the A group (the Table 3. The effects of different feeding and starvation on haematologic parameters of carp (values with different uppercase letters significantly differ among groups at the same time, lowercase letters – within the group at various sampling times, Kruskal–Wallis test, $p \leq 0.05$, n = 12).

| Parameter          | A3     | A7     | A15    | B3     | B7     | B15    | S3     | S7     | S15    |
|--------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Ht (%)             | 28.7 ± 1.6 | 32.0 ± 1.8 $^a$ | 30.8 ± 2.3 | 25.9 ± 2.0 | 29.5 ± 3.7 | 25.2 ± 3.3 | 22.7 ± 3.3 $^a$ | 29.4 ± 7.9 $^b$ |
| Hb (g/L)           | 77.6 ± 11.0 | 99.7 ± 10.9 $^a$ | 79.7 ± 9.3 $^a$ | 88.4 ± 30.1 | 70.0 ± 15.4 | 75.3 ± 10.2 | 66.5 ± 9.6 | 75.7 ± 20.7 | 65.6 ± 9.2 |
| RBC $(10^6/µL)$    | 1.71 ± 0.34 | 2.68 ± 0.48 $^a$ | 1.27 ± 0.14 $^a$ | 1.82 ± 0.48 | 2.04 ± 0.53 | 1.54 ± 0.24 | 1.26 ± 0.33 | 2.00 ± 0.44 | 1.33 ± 0.29 |
| MCV (fL)           | 172.9 ± 34.7 | 1234 ± 27.5 $^a$ | 245.8 ± 42.2 $^a$ | 149.5 ± 33.4 | 153.6 ± 45.6 | 184.4 ± 31.1 | 210.5 ± 56.0 | 118.9 ± 30.6 $^a$ | 222.6 ± 52.4 $^b$ |
| MCH (pg)           | 46.0 ± 7.1 | 383 ± 8.9 $^a$ | 637 ± 128 $^a$ | 50.3 ± 18.8 | 50.4 ± 19.9 | 54.6 ± 10.7 | 38.5 ± 9.6 | 50.3 ± 8.3 |
| Erythroblasts (%)  | 14.2 ± 1.2 $^a$ | 150 ± 1.3 $^a$ | 140 ± 0.9 $^a$ | 11.1 ± 1.2 | 10.1 ± 0.6 | 11.9 ± 1.9 $^a$ | 8.3 ± 0.8 $^a$ | 7.8 ± 0.7 $^a$ | 1.33 ± 0.29 |
| WBC $(10^3/µL)$    | 81.3 ± 33.3 $^a$ | 312 ± 8.1 $^a$ | 560 ± 13.1 $^a$ | 66.3 ± 30.2 | 52.7 ± 13.1 | 62.0 ± 26.0 | 79.1 ± 24.2 | 743 ± 19.2 | 39.9 ± 14.0 |
| PLT $(10^3/µL)$    | 99.8 ± 33.9 $^a$ | 411 ± 9.8 $^a$ | 769 ± 24.0 | 771 ± 33.7 | 752 ± 21.0 $^a$ | 843 ± 31.9 | 850 ± 31.8 | 768 ± 19.7 $^a$ | 52.1 ± 21.7 |
| NBT (g/L)          | 1.23 ± 0.17 $^a$ | 1.07 ± 0.11 $^a$ | 0.67 ± 0.14 $^a$ | 1.24 ± 0.31 $^a$ | 0.93 ± 0.27 | 0.59 ± 0.14 $^a$ | 1.00 ± 0.28 | 0.96 ± 0.19 | 0.44 ± 0.08 |
difference between these groups was significant). Basophilic cells were the most abundant in A group, and the least in B group, while the frequency of eosinophils showed an inverse pattern. Frequency of lymphoid cells (the most abundant cell lineage in the haematopoietic tissue), monocytoid cells and thrombocytes did not significantly differ among the groups.

Proliferative activity measured as percentage of haematopoietic precursors undergoing mitosis (PCNA-positive) was the highest in the control group (A15), and significantly lower in group fed barley (B15). It was also considerably lower in the starved fish (S15) compared to the control but the difference was insignificant. On the other hand, there were no differences in apoptotic activity (measured as percentage of caspase 3-positive cells). Haematopoietic activity measured as a ratio of percentages of PCNA-positive cells to caspase 3-positive cells was significantly higher in the control compared to the groups fed low-protein feed or starved.

Oxygen consumption rates measured at the end of the experiment, after 15 weeks of different feeding or starvation were: A – 0.35 ± 0.06 mg/g/h, B – 0.26 ± 0.12 mg/g/h and S – 0.16 ± 0.05 mg/g/h, significantly lower in the S compared to groups A and B. Frequency of erythroblasts in peripheral blood and haematopoietic activity (ratio of proliferating to apoptotic cells) significantly positively correlated with oxygen consumption rate \(r = 0.59\) and \(r = 0.58\), respectively, while significant negative correlation occurred between the frequency of early blast cells and oxygen consumption rate \(r = -0.60\). Final body mass of fish was significantly lower in the group S (52.1 ± 9.0 g) compared to the groups A and B (71.7 ± 16.5 and 70.9 ± 10.8 g, respectively).

4. Discussion

The obtained results revealed that the values of most red blood parameters of carp juveniles were not significantly affected by diet of low nutritional value or starvation for 15 weeks. The available literature data show that the values of red blood parameters are usually higher in fish fed diets of higher protein level. According to Daniels and Gallagher (2000), RBC and Ht values of Paralichthys dentatus were directly related to the protein content in feed (37–56%). However, the authors stated that all the values were within the reference range for this fish species, and thus low-protein diet did not induce anaemia. Habte-Tsion et al. (2013) reported that RBC, Hb and Ht values in Megalobrama amblycephala increased significantly with the increase in dietary protein content (28–36%). Similar relationship was observed by Abdel-Tawwab et al. (2010) for Oreochromis niloticus; RBC, Hb and Ht were directly related to the dietary protein level (25–45%). Da Silva Nunes et al. (2013) reported slightly but significantly higher RBC value in Piaractus mesopotamicus fed diet containing 32% protein compared to the fish fed 24% protein feed but no significant differences in Hb occurred and the fish fed low-protein diet showed higher MCV and MCH values. Kumar et al. (2005) observed no effects of different dietary protein content (28% or 35%), different form of starch and amylase addition on RBC and Hb levels in Labeo rohita. According to Cho et al. (2010), Paralichthys olivaceus showed no alterations in Ht values when fed for 8 weeks three various diets of different protein content (50.4–58.4%) and different calorific value. No effects of protein level in feed (25–50%) on Ht and RBC values in O. niloticus were also reported by Qiang et al. (2013). An inverse relationship between dietary protein level and red blood parameters was also reported: L. rohita fed diet containing 25% of protein showed higher Hb and Ht compared to the fish fed 35% protein feed (Baruah et al. 2009). These data suggest that the relationship between the values of red blood parameters and dietary protein level in fish may be different in various species.

High resistance of red blood values to starvation was reported by Rios et al. (2002, 2005) who observed no reduction in Ht and RBC in H. malabaricus during long time of food deprivation, and significant decrease in the values of these parameters occurred after 150 and 240 days, respectively. Caruso et al. (2010) observed no significant changes in Hb and Ht of Anguilla anguilla subjected to starvation lasting 58 days, and Caruso et al. (2011) reported no Ht changes in Dicentarchus labrax and Pagellus bogaraveo starved for 31 days. Park et al. (2012) found no differences in Ht, Hb, RBC, MCV, MCH and MCHC values between fed and starved P. olivaceus over a 12-week experimental period. Morshed et al. (2011) reported no change in RBC of Huso huso starved for 8 days but observed an increase in Ht accompanied by an MCHC decrease, which suggests erythrocyte swelling. The same species subjected to 6 weeks of starvation showed a decrease in Hb and MCHC accompanied by an increase in Ht (Falakhtkar 2012). In Heteropneustes fossilis starved for 30 days Borah and Yadav (1996) reported fluctuations of RBC and Hb values – initial significant increase after 20 days of starvation, and subsequent significant decrease at the end of the experiment. According to Abdel-Tawwab et al. (2006), O. niloticus starved for 1–4 weeks showed reduced RBC and Hb values. During first four weeks of starvation, red blood parameters of Prochilodus lineatus were stable but after 5 weeks, when energy reserves were depleted, the fish became anaemic (Rios et al. 2011). No significant differences in white blood parameters: WBC, NBT or differential leukocyte count occurred during the experiment. However, at the end of it starved carp showed the lowest WBC and NBT, and the highest percentage of phagocyte cells in blood (neutrophils and monocytes). This was accompanied by the lowest frequency of neutrophilic lineage cells in head kidney, which indicates migration of these cells to the blood.

Baruah et al. (2009) reported no significant differences in WBC and NBT values of L. rohita fed diets containing 25% or 35% of protein. Qiang et al. (2013) found no effect of dietary

### Table 4

| Cell lineage | A15 | B15 | S15 |
|-------------|-----|-----|-----|
| Blast (%)   | 3.8 ± 0.6a | 9.1 ± 1.2b | 7.6 ± 2.5b |
| Erythroid (%)| 5.8 ± 1.2a  | 11.9 ± 4.1b | 10.7 ± 2.0b|
| Lymphoid (%)| 48.4 ± 3.5a | 48.0 ± 5.9a | 52.7 ± 3.2a|
| Neutrophilic (%)| 18.4 ± 1.4a | 12.1 ± 4.3ab | 10.8 ± 4.2b|
| Monocytoid (%)| 1.2 ± 0.5a  | 0.9 ± 0.7a | 1.0 ± 0.5a|
| Basophilic (%)| 1.7 ± 0.4a  | 0.6 ± 0.4ab | 1.2 ± 0.3ab|
| Eosinophilic (%)| 0.1 ± 0.1a  | 0.3 ± 0.1a | 0.2 ± 0.2a|
| Thrombocytoid (%)| 20.3 ± 3.6a | 17.1 ± 3.2a | 15.8 ± 3.7a|
| PCNA-positive (%)| 198.8 ± 7.4a | 79.9 ± 2.9a | 100.0 ± 2.7ab|
| Cas3-positive (%)| 3.1 ± 1.6a  | 2.7 ± 1.1a | 3.1 ± 0.8a|
| PCNA/Cas3 | 7.2 ± 2.7a | 3.5 ± 1.7b | 3.2 ± 0.6b|
protein level (25–50%) on the WBC value of *O. niloticus*. Similar results were obtained by Kumar et al. (2005) who fed the same species of fish diets containing 28% or 35% of protein. According to Caruso et al. (2011), *D. labrax* and *P. bogaraveo* showed different reaction of oxidative activity of phagocytes (respiratory burst) to starvation: in *D. labrax* reduction was observed, while in *P. bogaraveo* the value did not change. However, both species showed significantly reduced respiratory burst activity compared to the fed fish when phagocytes were stimulated with zymosan. Rios et al. (2005) reported leukopenia (lymphocytopenia) and thrombocytopenia in starved *H. malabaricus*. According to Morshedi et al. (2011), *H. huso* subjected to a short-term starvation showed significant leukopenia. These data show that innate immune mechanisms in fish are insensitive to dietary protein level but may be compromised by starvation.

Starved carp showed significantly reduced frequency of erythroblasts in peripheral blood compared to the control, while erythroblast percentage in fish fed barley was slightly lower compared to the control but higher than in the starved group. These results indicate that starvation significantly reduced erythrocyte turnover, and diet of low nutritional value probably might cause such an effect if used for longer time. According to Rios et al. (2005), *H. malabaricus* subjected to long-term starvation (30–240 days) showed significantly decreased erythropoiesis (count of immature erythrocytes in peripheral blood) during food deprivation. The results of the present study revealed also that fish fed barley or starved showed higher frequency of erythroid cells in haematopoietic tissue compared to the control. This suggests a lower rate of release of erythrocytes to the blood stream, and thus lower rate of erythrocyte turnover in starved fish (and in a lesser extent also in those fed barley) compared to the control. Frequency of erythroblasts in peripheral blood of carp significantly correlated with oxygen consumption rate, which indicates that erythrocyte turnover rate was proportional to the metabolic rate of fish. In addition, activity of carp head kidney haematopoietic tissue measured as a ratio of proliferating (PCNA-positive) to apoptotic (caspase 3-positive) cells significantly positively correlated with oxygen consumption rate. Higher frequency of early blast cells in haematopoietic tissue of carp fed barley and starved carp, together with lower frequency of PCNA-positive cells compared to the control may indicate that differentiation of blood cells was inhibited or arrested at early stage, and might have been an adaptive energy-saving reaction.

During starvation, fish gradually utilize energy reserves and reduce their locomotor and metabolic activity. According to Rios et al. (2002), hypometabolic state in response to food deprivation contributes to energy conservation. In the present experiment, carp starved for 15 weeks showed significantly lower oxygen consumption rate compared to the control group. According to Moon and Johnston (1980), starvation in place was associated with a decrease in spontaneous activity and metabolic capacity of skeletal muscles, and an enhanced potential for liver gluconeogenesis. Cook et al. (2000) reported a gradual decrease in oxygen consumption rate in *Salmo salar* starved for 8 weeks to levels significantly lower compared to the fed fish. *H. malabaricus* starved for 240 days also showed a significant decrease in the oxygen uptake (Rios et al. 2002). Park et al. (2012) observed reduced oxygen consumption and ventilation frequency in *P. olivaceus* over 12 weeks of starvation compared to the fed fish.

The results of present study indicate that common carp were resistant to feeding diet of low nutritional value and starvation in terms of haematological values – no anaemia or significant immunodeficiency occurred. However, starvation caused a reduction of erythropoietic rate by inhibition of blood cell differentiation within the haematopoietic tissue. The results showed also that reduction of haematopoiesis was related to a decrease in metabolic rate caused by starvation or malnutrition and might have contributed to the adaptive energy-conserving response.

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**References**

Abdel-Tawwab M, Ahmad MH, Khattab YAE, Shalaby AME. 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture. 298:267–274.

Abdel-Tawwab M, Khattab YAE, Ahmad MH, Shalaby AME. 2006. Compensatory growth, feed utilization, whole-body composition, and hematological changes in starved juvenile Nile tilapia, *Oreochromis niloticus* (L.). J Appl Aquacult. 18:17–36.

Borah S, Yadav RNS. 1996. Biochemical and haematological responses to starvation in an air breathing fresh water teleost *Heteropneustes fossilis* (Bloch). Ind J Fish. 43:307–311.

Caruso G, Denaro MG, Caruso R, Mancari F, Genovese L, Maricchiolo G. 2011. Response to short term starvation of growth, haematological, biochemical, and non-specific immune parameters in European sea bass (*Dicentrarchus labrax*) and blackspot sea bream (*Pagellus bogaraveo*). Mar Environ Res. 72:46–52.

Caruso G, Maricchiolo G, Micale V, Genovese L, Caruso R, Denaro MG. 2010. Physiological responses to starvation in the European eel (*Anguilla anguilla*), effects on haematological, biochemical, non-specific immune parameters and skin structures. Fish Physiol Biochem. 36:71–83.

Cho SH, Kim CJ, Park IS, Song YC, Park C, Kim KD. 2010. Effect of dietary nutrient composition on the growth of olive flounder (*Paralichthys olivaceus*) with different feeding regimes. Fish Physiol Biochem. 36:377–385.

Cook JT, Sutterlin AM, McVinen MA. 2000. Effect of food deprivation on oxygen consumption and body composition of growth-enhanced transgenic Atlantic salmon *Salmo salar*. Aquaculture. 188:47–63.

Da Silva NC, Moraes G, Fabrizzi F, Hackbart A, Arbelaez Rojas GA. 2013. Growth and hematology of pacu subjected to sustained swimming and fed different protein levels. Pesq Agropec Bras. 48:645–650.

Daniels H, Gallagher M. 2000. Effect of dietary protein level on growth and blood parameters in summer flounder, *Paralichthys dentatus*. J Appl Aquacult. 10:45–52.

Falahatkar B. 2012. The metabolic effects of feeding and fasting in beluga *Huso huso*. Mar Environ Res. 82:69–75.

Fijan N. 2002a. Morphogenesis of blood cell lineages in channel catfish. J Fish Biol. 60:999–1014.

Fijan N. 2002b. Composition of main haematopoietic compartments in normal and bled channel catfish. J Fish Biol. 60:1142–1154.

Habte-Tsion HM, Liu B, Ge X, Pan L, Chen R. 2013. Effects of dietary protein level on growth performance, muscle composition, blood composition,
and digestive enzyme activity of wuchang bream (*Megalobrama amblycephala*) fry. Isr J Aquicult – Bamidgeh. 65:9.

Kikuchi K. 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder *Paralichthys olivaceus*. Aquaculture. 179:3–11.

Kondera E. 2011. Haematopoiesis in the head kidney of common carp (*Cyprinus carpio* L.), a morphological study. Fish Physiol Biochem. 37:355–362.

Kondera E, Witeska M. 2013. Cadmium and copper reduce hematopoietic potential in common carp (*Cyprinus carpio* L.) head kidney. Fish Physiol Biochem. 39:755–764.

Krogdahl A, Bakke-McKellep AM. 2005. Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). Comp Biochem Physiol A. 141:450–460.

Kumar S, Sahu NP, Pal AK, Choudhury D, Yengkokpam S, Mukherjee SC. 2005. Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in *L. rohita* juveniles. Fish Shellfish Immunol. 19:331–344.

Lim C, Klesius PH. 1997. Responses of channel catfish (*Ictalurus punctatus*) fed iron-deficient and replete diets to *Edwardsiella ictaluri* challenge. Aquaculture. 157:83–93.

Markovic Z, Poleksic V, Lakic N, Zivic Z, Dulic Z, Spasic M, Raskovic B, Sorensen M. 2012. Evaluation of growth and histology of liver and intestine in juvenile carp (*Cyprinus carpio*, L.) fed extruded diets with or without fish meal. Turk J Fish Aquat Sci. 12:301–308.

Masilko J, Hartvich P, Rost M, Urbanek M, Hlavac D, Dvorak P. 2014. Potential for improvement of common carp production efficiency by mechanical processing of cereal diet. Turk J Fish Aquat Sci. 14:143–153.

McCue MD. 2010. Starvation physiology, reviewing the different strategies animals use to survive a common challenge. Comp Biochem Physiol A. 156:1–18.

Moon TW, Johnston IA. 1980. Starvation and the activities of glycolytic and gluconeogenic enzymes in skeletal muscles and liver of the plaice, *Pleuronectes platessa*. J Comp Physiol A. 136:31–38.

Morshedi V, Ashouri G, Kochanian P, Yavari V, Bahmani M, Pourdehghani M, Yazdani MA, Porali Fashtami HR, Azodi M. 2011. Effects of short-term starvation on hematological parameters in cultured juvenile Beluga. J Vet Res. 66:363–368.

Park S, Hur JW, Choi JW. 2012. Hematological responses, survival, and respiratory exchange in the olive flounder, *Paralichthys olivaceus*, during starvation. Asian-Australas J Anim Sci. 25:1276–1284.

Qiang J, Yang H, Wang H, Kpundeh MD, Xu P. 2013. Interacting effects of water temperature and dietary protein level on hematological parameters in Nile tilapia juveniles, *Oreochromis niloticus* (L.) and mortality under *Streptococcus iniae* infection. Fish Shellfish Immunol. 34:8–16.

Rios FS, Carvalho CS, Pinheiro GHD, Donatti L, Fernandes MN and Rantin FT. 2011. Utilization of endogenous reserves and effects of starvation on the health of *Prochilodus lineatus* (Prochilodontidae). Environ Biol Fish. 91:87–94.

Rios FS, Kalinin AL, Rantin FT. 2002. The effects of long-term food deprivation on respiration and haematology of the neotropical fish *Hoplias malabaricus*. J Fish Biol. 61:85–95.

Rios FS, Oba ET, Fernandes MN, Kalinin AL, Rantin FT. 2005. Erythrocyte senescence and haematological changes induced by starvation in the neotropical fish traira, *Hoplias malabaricus* (Characiformes, Erythrinidae). Comp Biochem Physiol A. 140:281–287.

Shoemaker CA, Klesius PH, Lim C, Yildirim M. 2003. Feed deprivation of channel catfish, *Ictalurus punctatus* (Rafinesque), influences organosomatic indices, chemical composition and susceptibility to *Flavobacterium columnare*. J Fish Dis. 26:553–561.

Studnicka M, Siwicki AK, Ryka B. 1985. Phagocytic ability of neutrophils in carp (*Cyprinus carpio* L.). Isr J Aquicult – Bamidgeh. 37:123–128.

Svobodova Z, Pravda D, Palackova J. 1991. Unified methods of haematological examination of fish. Vodnany: Research Institute of Fish Culture and Hydrobiology. (Methods no 20).