Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Effect of protein and fat content in artificial feeds on productive and biological parameters of *Tinca tinca*

R. Konopelskyi*, H. Kurinenko*, O. Vishchur**, Y. Tuchapskyi*,
O. Krasnopolska*, M. Simon*, B. Hrishyn*, N. Rudyk-Leuska***

**Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine*
***Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine, Lviv, Ukraine*
****National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine*

Article info

Received 06.09.2025
Received in revised form 12.10.2025
Accepted 08.11.2025

Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, Kyiv, 03164, Ukraine. Tel.: +38-067-318-15-10. E-mail: annazakharenko@ukr.net

Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine, Lviv, 79034, Ukraine. Tel.: +38-068-046-26-89. E-mail: vishchur_oleg@ukr.net

National University of Life and Environmental Sciences of Ukraine, Kyiv, 03041, Ukraine. Tel.: +38-067-274-09-17. E-mail: rudyk-leuska@ukr.net

Konopelskyi, R., Kurinenko, H., Vishchur, O., Tuchapskyi, Y., Krasnopolska, O., Simon, M., Hrishyn, B., & Rudyk-Leuska, N. (2025). Effect of protein and fat content in artificial feeds on productive and biological parameters of Tinca tinca. Regulatory Mechanisms in Biosystems, 16(4), e25181. doi:10.15421/0225181

The variety of technologies for growing tench (*Tinca tinca* Linnaeus, 1758) is currently limited by the low intensity of pond aquaculture. However, the demand for it is growing and there is a need to study the physiological parameters that affect the well-being of tench during its more intensive cultivation. This study analyzed the growth intensity and the effect of different formulations of artificial feeds when growing age-1+ tench in a recirculating aquaculture system facility. Three 0.96 m^3 tanks were used for the study. The duration of cultivation was 225 days. The stocking density of age-1+ fish was 600 ind./m^3. Three experiments were formed in which the artificial feed differed in protein and fat contents. Experiment I – artificial feed with 44% protein and 9% fat content; Experiment II – artificial feed with 46% protein and 10% fat content; Experiment III – artificial feed with 54% protein and 15% fat content. The experiments showed that the highest yield and maximum body weight were obtained in the experiment using artificial feed with 46% protein and 10% fat content. According to the obtained survival rates and average body weight, the yield of age-1+ tench was 35.5–41.6 kg/m^3, and the fish productivity of the tanks was within 30.6–36.7 kg/m^3, the highest productivity being in experiment with a medium protein and fat content. The specific growth rate of age-1+ tench was maximum in the first two months of cultivation, and gradually decreased as they grew in October. The superiority of age-1+ fish fed with artificial feed with 46% protein content in terms of growth rate was noted only in April and June-July. Age-1+ tench of this experimental group also had a higher count of erythrocytes, neutrophils, basophils, platelets and hematocrit, as well as the relative content of neutrophils, monocytes and basophils. The analysis of blood serum showed that the content of urea, creatinine and the activity of transaminases and alkaline phosphatase in the experimental groups of tench did not have a direct dependence on the protein content in the diet of tench, but was defined by the generalized effect of the composition of each feed on protein metabolism. According to the results of the studies, it was found that the physiological needs of age-1+ tench for growing in conditions of recirculating aquaculture systems require artificial feeds with a protein content above 45% and below 10% fat.

Keywords: tench weight; fish survivability; specific growth rate of age-1+ fish; fish weight gain; leukogram of tench; serum of age-1+ fish, recirculating aquaculture system.

Introduction

Currently, tench is a popular fish species in pond aquaculture (FAO, 2022; Kucharczyk et al., 2024), but it has not been the subject of significant scientific research. Since it is considered an object of pond aquaculture, a significant part of the professional literature is devoted to biological features and the basics of its growing in ponds (Kocour, 2010; Zarski, 2011; Simic, 2013). Studies of this species gained popularity in European countries only in recent years and they proved that tench can fully adapt to artificial systems (Kamler, 2006; Celada et al., 2007; Celada et al., 2009; Targonska et al., 2012).

Scientific studies that highlighted the impact of stocking density when using intensive tench farming technologies were mainly conducted in experimental conditions in small aquariums and cannot be directly used in production (Celada et al., 2007). Analysis of the available scientific literature shows that juvenile tench in artificial cultivation have a higher growth rate and better absorb artificial feed at higher stocking densities (Celada et al., 2009; Panagiotis, 2012). However, excessive stocking densities and improperly selected diets were found to cause body defects and significant variability in individual body weight of juvenile tench (Wolnicki et al., 2003, 2006), while a decrease in stocking density directly affects the cost, so studies on these issues are important and should precede widespread introduction into production.

Research also focused on studying artificial feed rations, namely the quantity and quality of feeds and the possibility of producing tench seeds for cultivation in both natural and industrial conditions (Quiros & Alvarino, 2000; De Pedro et al., 2001, Kamler et al., 2006). The main reason for conducting these studies was the inefficiency of traditional tench breeding using pond technology, which is mainly caused by high mortality in the early stages of life and low growth rate (Cileček et al., 2011). Considering the biological characteristics, in particular the low growth rate, and problems with reproduction, scientists have conducted a number of studies aimed at obtaining triploid juveniles. In some experiments, the offspring even reached sexual maturity. However, triploid individuals are inferior in both productive parameters and physiological conditions, in particular the chemical composition of muscle tissues (Buchtova et al., 2005; Flajšhans et al., 2010; Bytyutskyy & Flajšhans, 2014).

Given that tench is an interesting fish species for the market, the problem of intensive cultivation under controlled conditions becomes more urgent. The main issue remains the possibility of feeding tench with commercial feeds intended for other fish, while achieving better survival and growth rates (Sáez-Royuela et al., 2015; Carral et al., 2021). The use of artificial feeds can potentially improve the growth and survival of tench, as well as reduce the cost of production in artificial conditions in contrast to pond cultivation. Currently, there are no artificial feeds specifically designed for tench, so carp, salmon or sturgeon feeds are usually used. Therefore, the question of the impact of such feeds on the tench body remains important, and it is hematology that ensures the correctness of the indicators of the fish physiological state.

When conducting studies of feeding rations, in order to fully analyze the physiological state of the experimental individuals, it is worth considering not only productive parameters. In this case, in aquaculture, hematological parameters are often used as physiological indicators. These indicators are an effective tool for detecting changes in the functional state of the body, as well as diagnosing and predicting the health status of fish (Shah, 2006; Singewald et al., 2010; Cray, 2021; Esmaeili, 2021; Raposo de Magalhães et al., 2024).

Considering that each biological individual has specific metabolic features, which are determined by the biochemical individuality of the internal environment parameters, it is worth paying attention to which biological indicators can most fully characterize the physiological state of the body. The most sensitive indicator may be blood, since changes in hematological parameters quite clearly reflect the dynamics of the general physiological state of fish. Comparisons of biochemical indicators showed that the blood and oxygen affinity of different fish species was closely related not only to the physical and chemical parameters of the environment in which they live, but also to their age and sex (Ainsworth, 1992; Nabi et al., 2022; Witeska et al., 2022; Ortiz & Esteban, 2024).

Materials and methods

The study was conducted in 2022 on the basis of the incubation unit of "Karpatsky Vodogray" LLC using a recirculating aquaculture system. Three 0.96 m^3 tanks were used in the study (Fig. 1). The duration of cultivation was 225 days. The objects of the study were age-1+ tench obtained by the artificial method.

Experiments were conducted to study the effect of feeding ration on productive parameters and physiological condition of age-1+ tench. The stocking density was 600 ind./m^3. Three variants of artificial feeds from different manufacturers were used in the study:

– experiment I – Aller Parvo EX GR, 0.5–2.0 mm with 44% protein and 9% fat content (feed with a low protein and fat content);

– experiment II – Vital feed for carp fry, 0.5–1.2 mm with 46% protein and 10% fat content (feed with a medium protein and fat content);

– experiment III – Advance feed for sturgeon fry, 1.0 mm with 54% protein and 15% fat content (feed with a high protein and fat content).

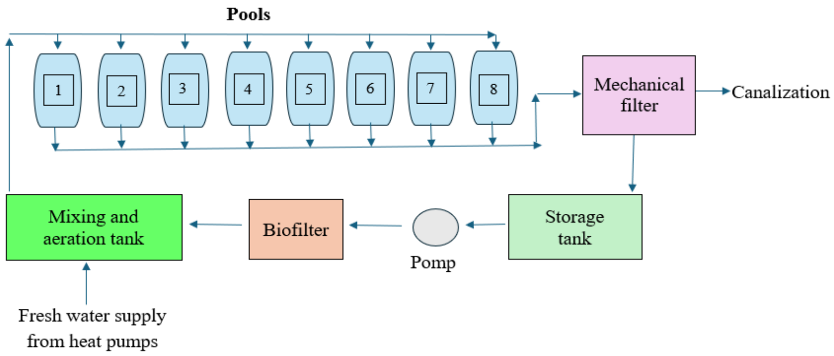

Fig. 1. Scheme of the tank module of the recirculating aquaculture system of "Karpatsky Vodogray" farm for rearing and growing fish juveniles of various species

The fish were sampled monthly to study growth dynamics. Body length measurements were performed using a centimeter tape. Weighing was performed on torsion scales AXIS – A 500 with a resolution of 0.01, up to a weight of 5 g, then Axis BDM 1.5 with a resolution of 0.05.

Daily growth rate was calculated using the following formulas:

$$GR = \frac{Wt - Wo}{t};$$

$$SGR = \frac{LnWt - LnWo}{t} \cdot 100,$$

where GR – daily weight gain (mg), SGR – specific growth rate (%/day), Wt – end body weight (mg), Wo – initial body weight (mg), t – growing period (days).

The analysis of the main hematological parameters and the determination of the leukocyte formula of the blood of age-1+ tench was carried out using the automatic biochemical analyzer "Miura 200" and the veterinary hematological analyzer "ABAXIS Vetscan HM5".

The experiments were carried out in compliance with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes".

The numerical material obtained as a result of the experiments was subjected to statistical processing using standard computer programs. The criteria for the analysis of the parameters were their average value (x) and standard error (SD). To analyze the main productive and biochemical parameters of the blood of age-1+ tench, a one-factor analysis of variance (ANOVA) was used, with P < 0.05 being considered statistically significant.

Results

As a result of 225 days of growing age-1+ tench using artificial feeds with different protein and fat contents, their average body weight reached 62.3–71.2 g. At the same time, the maximum body weight was reached by fish fed with a feed with 46% protein content and 10% fat. This experiment also showed the highest survival rate - 97.4%, which was 2.4% higher than that of the experiment with feed with 44% protein content and 4.3% than that of the experiment in which the feed with the highest protein level was used. When using a feed with a significantly higher content of both protein and fat – 54% and 15%, respectively, the weight of age-1+ tench was lower by 10% and amounted to 64.1 g, their survival rate was also the lowest – 93.1%. The average weight of age-1+ fish in the experiment when using a feed with 44% protein content and 9% fat was lower by only 1.8 g compared to the experiment with the highest protein level, but the survival rate of age-1+ tench was higher by 1.9%. The obtained survival rates and average body weight allowed the age-1+ tench to reach a weight of 35.51 to 41.61 kg/m^3 of. At the same time, the fish productivity of the tanks was within 30.65–36.74 kg/m^3, the highest productivity being in the experiment in which feed with a medium protein content was used (Table 1).

The growth rate of age-1+ tench had an increasing cumulative dynamic. During the entire growing period, age-1+ fish from the experiment in which they were fed with a feed with 46.0% protein content dominated in terms of growth rate. Already at the end of the first month of growing, their superiority in weight was 3.8–6.6%. In May, this superiority almost did not increase, but it increased to 11.4–14.0% during June and July. Starting from August, the fish growth rate in the experiment using feed with the medium protein content showed a smaller lead over their peers from the other two experiments, so at the end of October, the weight of age-1+ fish was greater than in the experiment using a feed with protein contents of 44.0% and 54.0% by 10.7–10.9%. Also, due to the more intensive growth of age-1+ fish from the experiment with the lowest protein content in September–October, their weight at the end of October was equal to those fed with a feed with 54% protein content. However, in November, the growth of age-1+ fish in the experiment with the average protein levels intensified, due to which, at the end of the study, their weight superiority was 14.2% over age-1+ fish from the experiment with a low protein level and 11.1% over those from the experiment with the highest protein level (Table 2).

Table 1
Results of growing age-1+ tench in tanks of a recirculating aquaculture system using artificial feeds with different protein and fat contents (x ± SD, n = 10)

Parameters	Feed with low protein and fat content (protein 44%, fat 9%)	Feed with medium protein and fat content (protein 46%, fat 10%)	Feed with high protein and fat content (protein 54%, fat 15%)
Water volume in the tanks, m^3	0.9	0.9	0.9
Average weight of age-1 fish, g	8.11 ± 0.37	8.11 ± 0.37	8.11 ± 0.37
Stocking density, ind./m^3	600	600	600
Yield, %	95.0	97.4	93.1
Average weight of age-1+ fish, g	62.29 ± 0.92	71.18 ± 1.23	64.05 ±1.08
Specific growth rate, %/day	0.91	0.97	0.92
Weight of fish product, kg/m^3	35.51	41.61	35.81
Fish productivity, kg/m^3	30.65	36.75	30.95
Growing period, days		225	

Table 2
Growth dynamics of age-1+ tench grown in tanks of a recirculating aquaculture system using artificial feeds with different protein and fat contents (x ± SD, n = 10)

Eperiment period	Feed with low protein and fat content (protein 44%, fat 9%)	Feed with medium protein and fat content (protein 46%, fat 10%)	Feed with high protein and fat content (protein 54%, fat 15%)
April	10.52 ± 0.61[a]	11.23 ± 0.82[a]	10.88 ± 0.53[a]
May	15.32 ± 0.30[a]	16.37 ± 0.44[a]	15.80 ± 0.56[a]
June	21.33 ± 0.58[a]	23.53 ± 0.41[b]	20.92 ± 0.52[a]
July	29.11 ± 0.45[a]	33.22 ± 0.57[b]	29.81 ± 0.58[a]
August	38.86 ± 0.73[a]	55.68 ± 0.58[b]	40.04 ± 0.69[a]
September	50.13 ± 1.03[a]	49.23 ± 1.19[b]	50.73 ± 1.03[a]
October	59.63 ± 0.69[a]	66.10 ± 1.05[b]	59.73 ± 0.97[a]
November	62.29 ± 0.92[a]	71.18 ± 1.23[b]	64.05 ± 1.08[a]

Note: different letters indicate values that were significantly different from each other within the same row of the table according to the results of comparison using the Fisher test; differences were considered significant at $P < 0.05$.

The average monthly body weight gains of age-1+ tench in the experiments were 7.2, 8.4 and 7.5 g. At the same time, they were 2.4–3.1 g during April, they almost doubled in May – to 4.8–5.2 g/day. A gradual increase in monthly gains occurred until September, in this month they reached their maximum – 10.7–11.6 g. In October, weight gains decreased by 10.4–15.9% compared to September, which may have been due to a decrease in temperature. At the same time, such a decrease was lower in the experiment with the medium protein content, apparently due to the higher weight of age-1+ fish. During the entire growing period, the highest monthly gains were recorded in the same experiment, which indicated a greater optimality of the composition of this feed for age-1+ tench (Fig. 2).

In accordance with the dynamics of monthly growth rates of age-1+ tench, a similar dynamic of average daily growth rates was recorded, which was characterized by an increasing rate – from 0.1 g/day in April to 0.36–0.39 g/day in September. In October, daily growth rates decreased to 0.29–0.34 g/day, and in November they increased in experiments with medium and high protein content, and decreased in the experiment with low protein content. Throughout the season, the maximum daily growth rate was observed in age-1+ fish from the experiment where they were fed with a feed with 46.0% protein content of (Fig. 3).

Analysis of the specific growth rate of age-1+ tench showed that its values were 9.0–24.9% higher in the experiment with the medium protein content at the beginning of cultivation in April, and by 8.0–12.0% in June–July. In other months of cultivation, the superiority of the specific growth rate in the experiment with the medium protein content was minimal, or they were inferior to other experimental variants. During the first two months of cultivation, the specific growth rate was the highest – about 1.2%/day. As the age-1+ tench grew, the specific growth rate gradually decreased to 0.53–0.56%/day in October (Fig. 4).

When conducting studies to determine the optimal diet for feeding tench with artificial feeds when growing in recirculating aquaculture systems, it is worth focusing not only on fish productive parameters, but also on the physiological state of the experimental individuals. Since blood is the main structural element of the body, which fully characterizes the physiological state, we conducted a study of hematological parameters of age-1+ tench. Given that hemoglobin is the most important element of the oxygen transport system in vertebrates, accordingly, its determination became part of our study. The analysis of the results showed that the hemoglobin level when using all feeds for feeding age-1+ fish was within 110.0–117.0 g/L, without statistically significant differences.

During a complete blood count, the content of formed elements was examined: erythrocytes, leukogram cells and platelets, and the hematocrit index, which indicates the ratio of their volume to the total blood volume. It was found that in terms of erythrocyte count and hematocrit, age-1+ tench of the second experimental group prevailed, in which the erythrocyte content was 1.95×1012/L and which was 10.2–13.4% higher than the values recorded in the third and first experimental groups, respectively. At the same time, in terms of hematocrit, the superiority was 22.9% over fish of the first experimental group and 9.4% over those of the experiment in which they were fed with a feed with the highest protein content (Table 3).

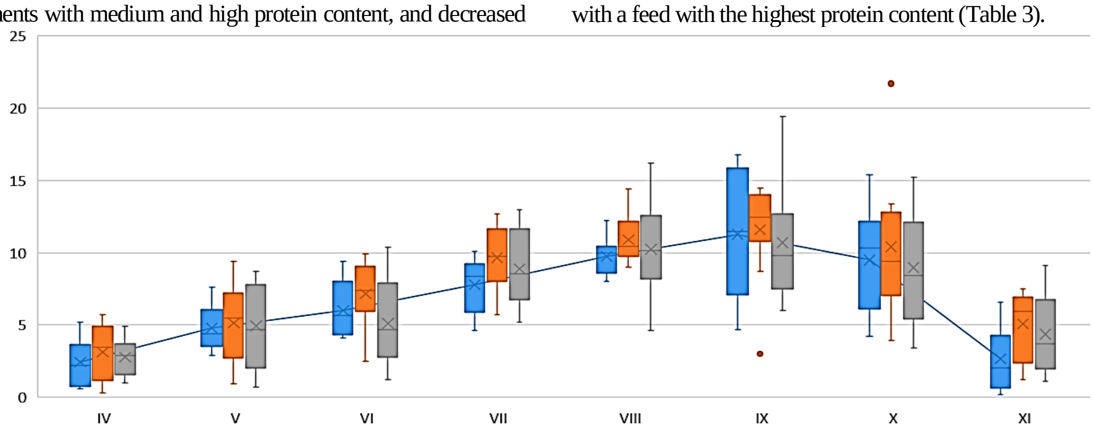

Fig. 2. Weight gain (g/month) of age-1+ tench in tanks of a recirculating aquaculture system using artificial feeds with different protein and fat content, blue marker – with 44% protein and 9% fat content; red marker – with 46% protein and 10% fat content; green marker – with 54% protein and 15% fat content: on the abscissa axis – the month of the experiment (beginning – April, end – November); on the ordinate axis – the average weight gain of age-1+ tench per month; n = 10

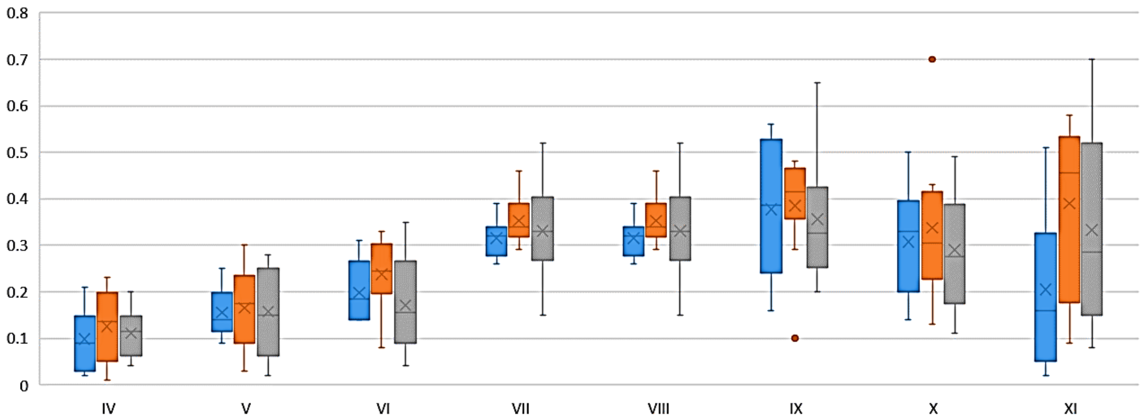

Fig. 3. Daily weight gain (g/day) of age-1+ tench in tanks of a recirculating aquaculture system using artificial feeds with different protein and fat contents, blue marker – with 44% protein and 9% fat content; red marker – with 46% protein and 10% fat content; green marker – with 54% protein and 15% fat content: on the abscissa axis – the month of the experiment (beginning – April, end – November); on the ordinate axis – the average value of the average daily weight gain of age-1+ tench, n = 10

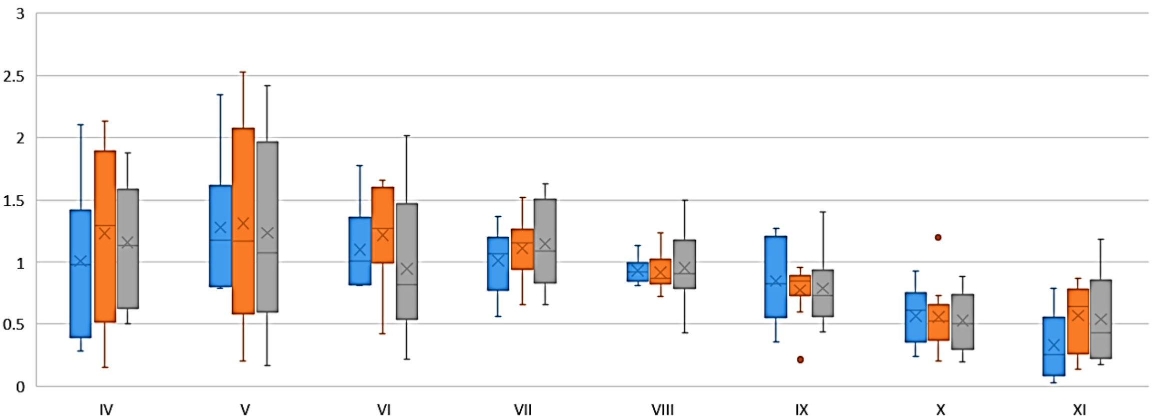

Fig. 4. Specific growth rate (%/day) of age-1+ tench in tanks of a recirculating aquaculture system, using artificial feeds with different protein and fat content, blue marker – with 44% protein and 9% fat content; red marker – with 46% protein and 10% fat content; green marker – with 54% protein and 15% fat content: on the abscissa axis – the month of the experiment (beginning – April, end – November); on the ordinate axis – the average value of the specific growth rate of age-1+ tench, n = 10

The content of leukocytes, which support the immune system in an optimal state and play an important role in protecting the body from infections and inflammatory processes, was within quite wide limits – from 11.3 to 17.3 × 10^9/L. The maximum leukocyte content was recorded in the experimental group with the maximum protein feed, the superiority of which over experimental fish fed with a low-protein feed was 17.6%, and over fish fed with a feed with the medium protein content – 39.5%.

At the same time, the share of lymphocytes in the leukogram was 51.5–68.3% of all leukocytes with the superiority of age-1+ fish fed with a feed with the lowest protein content, by 20.7–32.6%. A more significant difference was recorded among the absolute values of the lymphocyte content – their lowest content was observed in age-1+ fish fed with a feed with the medium protein content – 5.81 × 10^9/L, and in the other two experimental groups it was higher by 79.3% and 70.7%, respectively.

The share of monocytes in the total leukogram among the experimental groups was within 1.1–5.6% with their content of 0.18–0.63 × 10^9/L. The maximum absolute and relative values of monocyte content were recorded in age-1+ fish from the experiment, in which a feed with 46% protein content was used and they were 64.6% higher than in individuals from the experiment with the lowest protein content and 71.9% higher than in individuals from the experiment in which a feed with the maximum protein content was used (Table 3).

Among the forms of leukocytes in age-1+ tench, neutrophils were in second place. They protect the animal body from microbial infection and microorganism toxins. Their share was 29.7% in the experiment with a low protein content, while it was equally higher in individuals fed with a feed with the medium and high protein content – 42.0–42.4%. Higher absolute and relative values of neutrophil content in age-1+ fish of these experimental groups indicate a higher level of their immune system, while the presence of basophils in age-1+ tench grown on a feed with 46.0% protein content is a sufficiently convincing indicator of their high immune status. Fish of this experimental group also had a statistically significant highest platelet count – 52.7 × 10^9/L. They also had a higher average platelet volume – 8.47 fL, which indicates a predominance of juvenile platelets in the population, and the highest degree of platelet size difference – 34.6% versus 26.7% and 29.1% in age-1+ of the other two experiments, respectively.

Biochemical parameters of blood serum of fish are important for assessing their physiological status. The results of the conducted studies indicate that feeding of age-1+ tench with feeds of different manufacturers and formulas with different contents of essential nutrients when growing them in conditions of a recirculation aquaculture system affects the biochemical composition of their blood. For example, the total protein content in blood serum of age-1+ tench was somewhat lower in the experiment when using a feed with the highest content of protein and fat. The fractional analysis of blood serum showed that the content of both albumins and globulins was minimal in this variant of the study. At the same time, the content of albumins was highest in age-1+ tench fed with a feed with the maximum level of protein, and globulins – with the minimum. Along with the minimum content of globulins and albumins in the blood serum of age-1+ fish fed with a feed with the highest protein content, the maximum level of urea nitrogen was recorded – 4.8 mg%, which was also quite high in the experiment with the minimum protein content – 4.2 mg% and probably lower in the experiment with the medium protein level – 3.2 mg%. The creatinine content was minimal in age-1+ fish in the experiment with the lowest protein level in the feed and higher by 30.3–50.0% in individuals from both other experiments.

Table 3
Morphological and biochemical blood parameters of age-1+ tench grown in tanks of a recirculating aquaculture system with the use of artificial feeds with different protein and fat contents (x ± SD, n = 5)

Parameters	Feed with low protein and fat content (protein 44%, fat 9%)	Feed with medium protein and fat content (protein 46%, fat 10%)	Feed with high protein and fat content (protein 54%, fat 15%)
Hemoglobin, g/	117.002 ± 3.960^a	111.004 ± 1.600^a	110.301 ± 3.802^a
Erythrocytes, 10^{12}/L	1.724 ± 0.084^a	1.946 ± 0.034^a	1.768 ± 0.078^a
Hematocrit, %	21.10 ± 0.65^a	25.93 ± 1.85^a	23.69 ± 0.78^a
Mean corpuscular volume, fL	122.3 ± 1.8^a	139.6 ± 13.1^b	134.6 ± 8.4^{ab}
Mean corpuscular hemoglobin, pg	68.1 ± 0.9^a	61.5 ± 8.5^a	62.3 ± 0.8^a
Mean corpuscular hemoglobin concentration, g/L	555.6 ± 5.9^a	435.3 ± 28.8^b	467.0 ± 23.9^{ab}
Red blood cell distribution width c, %	17.37 ± 0.44^a	20.77 ± 2.75^a	18.17 ± 0.50^a
Red cell distribution width s, fL	41.2 ± 1.8^a	63.0 ± 4.6^b	52.9 ± 5.4^{ab}
Leukocytes, 10^9/L	15.21 ± 1.18^a	11.29 ± 1.34^a	17.27 ± 1.67^a
Leukogram:			
Lymphocytes, 10^9/L	10.39 ± 0.93^a	5.81 ± 0.68^b	9.98 ± 1.67^a
Lymphocytes, %	68.27 ± 2.19^a	51.50 ± 1.01^b	56.60 ± 4.45^a
Monocytes, 10^9/L	0.22 ± 0.02^a	0,63 ± 0.09^b	0.18 ± 0.09^a
Monocytes, %	1.50 ± 0.17^a	5.60 ± 0.49^b	1.07 ± 0.54^a
Neutrophils, 10^9/L	4.52 ± 0.42^a	4.80 ± 0.59^a	7.06 ± 0.31^b
Neutrophils, %	29.73 ± 2.08^a	42.43 ± 0.48^b	42.00 ± 4.61^b
Eosinophils, 10^9/L	0.07 ± 0.01^a	0.05 ± 0.01^a	0.06 ± 0.02^a
Eosinophils, %	0.47 ± 0.07^a	0.43 ± 0.03^a	0.37 ± 0.07^a
Basophils, %	0.00 ± 0.00^a	0.03 ± 0.03^a	0.00 ± 0.00^a
Platelets, 10^9/L	28.33 ± 2.37^a	52.67 ± 22.63^b	31.00 ± 6.55^a
Mean platelet volume, fL	7.23 ± 0.28^a	8.47 ± 0.88^a	6.83 ± 0.14^a
Procalcitonin, %	0.020 ± 0.001^a	0.047 ± 0.026^a	0.020 ± 0.005^a
Platelet distribution width c, %	29.10 ± 1.22^a	34.57 ± 3.48^a	26.70 ± 0.83^a
Platelet distribution width s, fL	7.47 ± 0.63^a	12.20 ± 3.56^b	6.10 ± 0.25^a

Note: different letters indicate values that were significantly different from each other within the same row of the table according to the results of comparison using the Fisher test; differences were considered statistically significant at $P < 0.05$.

The activity of transaminases (alanine, aspartate aminotransferase) in blood serum and their ratio are important indicators of the functional state of the liver of animals. It was found that the activity of aspartate aminotransferase in age-1+ fish of all experimental groups was close, and the activity of alanine aminotransferase was significantly higher in age-1+ fish fed with a feed with the medium protein content. The ratio of AST/ALT (aspartate aminotransferase / alanine aminotransferase) in age-1+ tench of this experimental group was 3.61, and it was significantly higher by 76.7–88.9%, respectively, in fish from the other two experiments (Table 4).

Table 4
Biochemical parameters of blood serum of age-1+ tench grown in tanks of a recirculating aquaculture system with the use of artificial feeds with different protein and fat contents (x ± SD, n = 5)

Parameters	Feed with low protein and fat content (protein 44%, fat 9%)	Feed with medium protein and fat content (protein 46%, fat 10%)	Feed with high protein and fat content (protein 54%, fat 15%)
Total protein, g/L	51.8 ± 2.3^a	47.2 ± 2.1^a	44.2 ± 1.9^a
Albumins, g/L	13.95 ± 0.63^a	14.05 ± 0.63^a	11.04 ± 0.49^a
Globulins, g/L	37.9 ± 1.7^a	33.1 ± 1.5^a	33.1 ± 1.5^a
Urea, mmol/L	2.193 ± 0.099^a	1.706 ± 0.076^b	2.508 ± 0.112^a
Urea nitrogen, mg%	4.19 ± 0.19^a	3.21 ± 0.14^b	4.82 ± 0.22^a
Creatinine, mmol/L	57.8 ± 2.6^a	135.5 ± 6.1^b	136.5 ± 6.1^b
Aspartate aminotransferase (AST), U/L	352 ± 16^a	408 ± 18^a	423 ± 19^a
Alanine aminotransferase (ALT), U/L	54.817 ± 2.464^a	113.377 ± 5.063^b	62.207 ± 2.778^a
Aspartate aminotransferase / alanine aminotransferase	6.379 ± 0.287^a	3.612 ± 0.161^b	6.823 ± 0.305^a
Alkaline phosphatase, U/L	793.7 ± 79.3^a	332.1 ± 14.8^b	658.6 ± 29.4^a
Amylase, U/L	1887.3 ± 84.8^a	1865.1 ± 83.3^a	1818.1 ± 81.2^a
Total bilirubin, mmol/L	3.588 ± 0.161^a	3.211 ± 0.14^a	4.415 ± 0.197^a
Direct bilirubin, mmol/L	1.60 ± 0.69^a	0.40 ± 0.02^b	2.81 ± 0.13^a
Indirect bilirubin, mmol/L	2.20 ± 0.35^{ab}	2.81 ± 0.13^a	1.61 ± 0.07^b
Glucose, mmol/L	8.47 ± 0.38^a	13.55 ± 0.61^b	11.64 ± 0.52^{ab}
Ca, mmol/L	3.19 ± 0.14^a	3.01 ± 0.13^a	3.01 ± 0.13^a
P, mmol/L	1.894 ± 0.085^a	2.408 ± 0.108^b	2.107 ± 0.094^{ab}
Ca/P, mmol/L	1.694 ± 0.076^a	1.304 ± 0.058^b	1.405 ± 0.063^{ab}
Cholesterol, mmol/L	4.88 ± 0.22^a	4.92 ± 0.22^a	3.91 ± 0.18^b

Note: different letters indicate values that were significantly different from each other within the same row of the table according to the results of comparison using the Fisher test; differences were considered statistically significant at $P < 0.05$.

Alkaline phosphatase activity was also significantly lower in the experiment with the medium protein content – 332.1 U/L versus 658.6–793.7 U/L in two other experimental groups. At the same time, the content of amylase, which ensures the breakdown of carbohydrates, was equally high in all variants of the experiment, however, a tendency was found to decrease in the level of amylase with an increase in the protein content in the diet. Analysis of the content of urea, creatinine and the activity of transaminases and alkaline phosphatase in the experimental groups of tench showed the absence of their direct dependence on the protein content in the diet of tench and indicated that they were the result of the generalized effect of the composition of each feed on protein metabolism. The content of total and direct bilirubin was highest in age-1+ tench fed with a feed with the maximum protein content. However, the content of indirect bilirubin was minimal, indicating a direct relationship between their concentration and the amount of protein in the feed.

The content of calcium and phosphorus in the blood serum of animals affects the rate of enzyme reactions and homeostasis of the body, therefore their determination is important in the assessment of feeds for new objects of aquaculture. In our study, the calcium content was within narrow limits – 3.01–3.19 mmol/L, with a superiority in tench fed with a feed with the lowest protein content, while the phosphorus content was significantly higher in fish fed with a feed with the medium protein content. In this regard, the Ca/P ratio was significantly lower in the experiment with the medium protein content, while its highest value was recorded in age-1+ fish in the experiment with the lowest protein content in the feed.

Use of an artificial feed with 44% to 54% protein content resulted in an increase in glucose levels with an increase in protein content. For example, in the experiment with 46% protein content, the maximum value was recorded, which was 13.55 mmol/L, while this value was minimal and statistically significantly lower by 38% in the experiment with 44% protein content. When using a feed with 54% protein content, the glucose level was 11.64 mmol/L, which was 37% higher than the minimum value.

The cholesterol level when using feeds with 44% and 46% protein contents was practically at the same level and was 4.88 and 4.92 mmol/L, respectively, while at 54% protein in the feed, the level statistically significantly decreased to 3.91 mmol/L.

Discussion

In the conditions of Ukrainian aquaculture, cultivation of tench in industrial aquaculture is practically not undertaken due to the lack of technological standards and the undeveloped market for specialized artificial feeds. The unresolved nature of these problems is the basis for conducting studies to determine the optimal technological parameters of tench cultivation, primarily stocking densities at each stage of cultivation and selection of feeding rations among the feeds available on the market for different fish species, since there are no specialized feeds for tench. When conducting such studies, it is important to assess the physiological state of fish, objective and operational information about which can be obtained from the analysis of hematological parameters.

Each fish species has characteristic features of the blood composition, expressed in the quantitative and qualitative values of red and white blood cells and the distribution of leukocyte types (Ainsworth, 1992). The leukocyte blood formula under optimal living conditions was found to have a stable structure in different fish species, but at the same time it is quite labile and depends on a number of factors, in particular – physical and chemical parameters of the environment, season, age, sex. Since each type of leukocyte performs a specific function, analysis of the structure of the leukogram allows one to establish the direction of influence of a certain factor, or their complex, on the state of the fish body (Shandilya & Banerjee, 1989; Vosylienė, 1999; Witeska, 2004).

As for the content of erythrocytes, which is also a species-specific feature, the range of their fluctuations in fish is very wide - from 0.5 - 1.5 to 3.0–4.2 million/μL. One of the main reasons for these differences is the natural mobility of the species: pelagic fish species, capable of covering significant distances, have a higher ability to oxygenate, while in low-active benthic fish species, the blood contains a smaller number of erythrocytes, and accordingly it has a lower ability to transport oxygen (Houston, 1996; Lay, 1999; Docan, 2018).

The conducted studies showed that age-1+ tench had a high hemoglobin level, typical of fish (Giardina et al., 1973; D'Avino & Di Prisco, 1988; Souza & Bonilla-Rodriguez, 2007), without statistically significant differences, but it significantly exceeded that of tench from natural water bodies (Omelkovets, 2016) and from cultivation in cages (Shalak, & Goncharik, 2017). The latter, apparently, may be associated with their constant stay in the conditions of a recirculating aquaculture system with a high content of dissolved oxygen in water - 7.5–8.0 mg O_2/dm^3. The leukocyte blood count was represented by five morphological cell types. The maximum share in the relative content were lymphocytes (51.5–68.3%), however, no statistical dependence on the composition of the feed was recorded. At the same time, in age-1+ tench fed with a feed with 46% protein content, a high level of neutrophils and monocytes and the presence of basophils were recorded, which is a fairly convincing indicator of their high immune status. At the same time, in this experiment, the highest content of platelets was established, their highest mean volume and the degree of difference in platelet size, which indicates a slower renewal of these cells than in fish of other experimental groups and an increased level of blood clotting, which is one of the main functions of erythrocytes, respectively, is a confirmation of the optimality of the feeding ration used (Hrubec et al., 2000; Ortiz & Esteban, 2024). This structure of the leukogram fully corresponds to freshwater fish species previously studied by a number of scientists from different countries (Ortega-Villaizan et al., 2022; Huu & Dung, 2023).

Analysis of serum showed no statistically confirmed dependence of albumin and globulin content on the composition of the feed in the study variants. At the same time, the content of globulins was higher than the level of albumin by 57.7–66.7%, which is typical for fish, and depends on their environmental conditions (Christiansen et al., 2015; Abdel-Tawwab et al., 2020; Alfonso et al., 2024; Campos-Sanchez et al., 2024). At the same time, the minimum content of globulins and albumins in the serum age-1+ tench fed with a feed with 46% protein content was accompanied by the maximum level of urea nitrogen and creatinine, which indicates the lowest functional activity of the kidneys. Also, the highest bilirubin level recorded in these individuals is a sign of the fastest aging of erythrocytes.

Alkaline phosphatase is the main enzyme that ensures phosphorus metabolism, which affects the process of calcium supply to bone tissue, as well as the transport and metabolism of lipids. Changes in the activity of this enzyme are widely used in biomonitoring of the ecological state of aquatic ecosystems and to assess the degree of intoxication of the fish body with toxic substances (Molina et al., 2005). Our study showed that the content of alkaline phosphatase in the blood serum of age-1+ tench fed with an artificial feed with the medium protein content was significantly lower than in individuals fed with a feed with a low and high protein content. These differences in the level of alkaline phosphatase may indicate a greater compliance of the feed used in this experiment with the physiological needs of age-1+ tench in terms of the content and source of essential nutrients, which is also confirmed by the results of cultivation.

Conclusion

The use of artificial feeds with 44% to 54% protein content and 9% to 15% fat had a positive effect on the complex of fish productive parameters and can be recommended for use in production conditions. When using feeds with different protein contents, the highest average body weight of age-1+ tench of 71.2 ± 1.23 g and the highest survival rate of 97% were obtained when feeding them with an artificial feed with 46% protein and 10% fat content and the yield was 41.61 kg/m^3. Summarizing the results of the hematological study, it can be concluded that the feed with 46% protein content and 10% fat meets the physiological needs of age-1+ tench fed exclusively with artificial feeds to a greater extent than other tested feeds (protein 44%, fat 9% and protein 54%, fat 15%), which is also confirmed by the results of cultivation. Thus, the obtained results are important for improving the theoretical foundations of tench cultivation in artificial conditions and can be used to increase the efficiency of fish breeding centers in the reproduction and cultivation of tench in aquaculture in Ukraine.

This research did not receive any special grants from organizations funded by the state, commercial or non-commercial sectors.

The authors declare that they have no known competing financial interests or personal relationships that could affect the results of this article.

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