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RESEARCH ARTICLE

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Optimising growth and physiological performance of tilapia (*Oreochromis niloticus*) in a modular multitrophic aquaculture system with natural feed integration

Olena Honcharova^a , Sabine Boucetta^b , Mustafa Doğan^c  and Erkan Can^d 

^aDepartment of Water Bioresources and Aquaculture, Kherson State Agrarian and Economic University, Kherson, Ukraine;

^bDepartment of Nature and Life Sciences, University of August 20 1955, Skikda, Algeria; ^cFisheries Products Inc. Biotechnology Department, Fethiye, Turkey; ^dDepartment of Aquaculture, Faculty of Fisheries, Izmir Katip Celebi University, Izmir, Turkey

ABSTRACT

This study investigated the impact of alternative protein-enriched diets on the growth performance, haematological profile and biochemical parameters of *Oreochromis* spp. reared in a modular multitrophic aquaculture system. Four dietary treatments were evaluated: a control group (100% commercial feed) and three experimental groups supplemented with natural protein sources, including *Artemia*, *Spirulina platensis* and *Hermetia illucens* (black soldier fly larvae), alone or in combination. Results showed that all experimental diets significantly enhanced growth performance compared to the control ($p < 0.05$). The highest final body weight (301.4 ± 49.3 g), specific growth rate ($5.27 \pm 0.51\%$ day⁻¹) and lowest feed conversion ratio (1.03 ± 0.21) were recorded in group C, receiving 25% *Hermetia illucens* and 10% *Spirulina*. Haematological analyses revealed a significant increase in red blood cell count (1.98 ± 0.15 T/L), haemoglobin (Hb) concentration (88.2 ± 4.9 g/L) and mean corpuscular Hb concentration (360 ± 3.4 g/L) in fish fed the enriched diets, particularly group C ($p < 0.001$). Biochemical assays indicated a marked elevation in plasma total protein (59.1 ± 5.5 g/L) and creatinine levels (22.4 ± 1.9 µmol/L) in the enriched diet groups, suggesting improved metabolic and physiological status. These findings demonstrate the potential of integrating insect meal and microalgae as sustainable and effective protein sources in tilapia aquafeeds, promoting optimal health and growth within an ecologically responsible aquaculture model. The results demonstrate the feasibility of multitrophic aquaculture and natural protein alternatives.

HIGHLIGHTS

- Up to 16.5% increase in growth using *Spirulina*, *H. illucens*.
- Enhanced blood parameters indicating better oxygen transport and better metabolism.

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Aquaculture multitrophic; tilapia; blood biochemistry; haematological parameters; growth performance

Introduction

In today's era of increasing technogenic pressures, ensuring the quality and environmental safety of food products has become a pressing concern for scientists, practitioners and producers worldwide (FAO 2025). Environmentally friendly products often command significantly higher market prices, typically due to their superior quality and sustainability characteristics.

Within the aquaculture sector, many recent scientific and experimental efforts have focused on identifying and developing alternative sources of nutrients, proteins and energy to promote more sustainable

aquaculture practices (Gasco et al. 2020; Randazzo et al. 2021; Honcharova 2024; Honcharova and Bekh 2024; Boucetta et al. 2025).

Current priorities in aquaculture research primarily focus on optimising technological conditions for rearing and feeding practices (Austin et al. 2022). Increasing attention is being paid to the implementation of modular systems – both in monoculture and integrated forms – that enhance the efficiency of production.

At the technological level, it is now possible to design process maps in aquaculture in a way that ensures the rational use of resources at every stage of production. In this

CONTACT Olena Honcharova  anelsatori@gmail.com  tamdogan02@hotmail.com

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context, microalgae and insect-based feeds have emerged as sustainable alternatives, promoting circular resource utilisation and reducing environmental pressure on aquatic ecosystems (Abdel-Tawwab et al. 2020; Ogello et al. 2024; Tignani et al. 2025). In aquaculture production, enhancing environmental safety and reducing ecosystem pressure require the continuous optimisation and revision of technological processes. Such improvements can significantly increase the efficiency of resource utilisation, including the reuse of materials traditionally considered as production waste. For instance, the effective integration of terrestrial and aquatic ecosystem components in closed-loop systems – such as hydroponics and aquaponics – demonstrates the potential of cyclical agriculture in symbiosis with aquaculture. Recent studies have confirmed the relevance and positive outcomes of implementing these technological approaches in the broader context of aquaculture optimisation and strategic development (Can et al. 2025). Both fisheries and aquaculture present promising opportunities for adapting environmentally sustainable technologies, either individually or in combination. These systems are typically based on the cultivation of aquatic organisms in ponds, tanks, recirculating aquaculture systems (RASs), cages, lakes and reservoirs (Pivovarov et al. 2017; Honcharova et al. 2021; Can and Seyhaneyildiz Can 2023).

In Ukraine, as in many other countries, the development of organic practices in the agricultural sector – including aquaculture and water resource management – is a national priority. This direction plays a particularly important role in shaping and advancing strategies for agricultural sector reform.

The introduction of multitrophic modular systems can create synergies between different production components, leading to more efficient and sustainable use of available resources across sectors.

The aim of this study is to evaluate the functional status of tilapia cultured under multitrophic aquaculture conditions using alternative, natural feed components such as microalgae, artemia and insect-based proteins. The research seeks to assess the impact of these technological innovations on the physiological, haematological and biochemical parameters of tilapia, as well as their growth performance and metabolic activity. The study also aims to demonstrate the potential of multitrophic modular systems to promote resource efficiency and environmental sustainability in aquaculture.

Materials and methods

Experimental site

The experimental study was conducted at the Scientific Research Laboratory and the 'Physiological

and Biochemical Research' Laboratory of Kherson State Agrarian and Economic University (KSAEU), located in southern Ukraine. All experimental procedures were carried out in accordance with ethical and methodological standards as outlined by Yevtushenko and Khyzhniak (2019) and Dehtiarov et al. (2001). The work strictly complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), as well as the Animal Ethics Committee of KSAEU and the Ukrainian Ministry of Health Order No. 281 (dated 1 November 2000). All research involving animals must have been reviewed and approved by an ethics committee prior to commencing the study and performed in accordance with relevant institutional and national guidelines and regulations (Excerpt from protocol No. 4 of 2024 meeting of the commission on ethics and academic integrity of the Kherson State Agrarian and Economic University) (ASAB Ethical Committee/ABS Animal Care Committee 2023).

Experimental organism

The study focused on hybrid red tilapia (Florida Red strain, *Oreochromis* spp.) – a cross between ♂ *Oreochromis niloticus* and ♀ *Oreochromis mossambicus* (Popma and Lovshin 1995). At the start of the experiment, 240 juvenile tilapia (initial weight: 2.637 ± 0.286 g) were stocked at a density of 60 individuals per 250 dm³ tank. To minimise the influence of potential blocking factors, fish were first stratified into blocks according to their initial weight, and then randomly assigned to treatment groups within each block. All experimental fish originated from the same incubation batch. Measurements were conducted on a per-tank basis, not pooled. As fish grew, tank volumes were progressively increased to 500 dm³ and then to 1000 dm³ to maintain appropriate rearing conditions.

Rearing and feeding conditions

Fish were raised under RAS conditions, which included standard water filtration, temperature regulation and oxygenation systems. The experimental setup incorporated a modular integrated multitrophic aquaculture (IMTA) system, which also featured the cultivation of natural feed components (Figure 1).

The modular system was built on a mobile platform equipped with an environmental monitoring unit and microcrystalline solar panels for supplementary energy. Each module was functionally organised to allow resource recycling, energy efficiency and synergistic

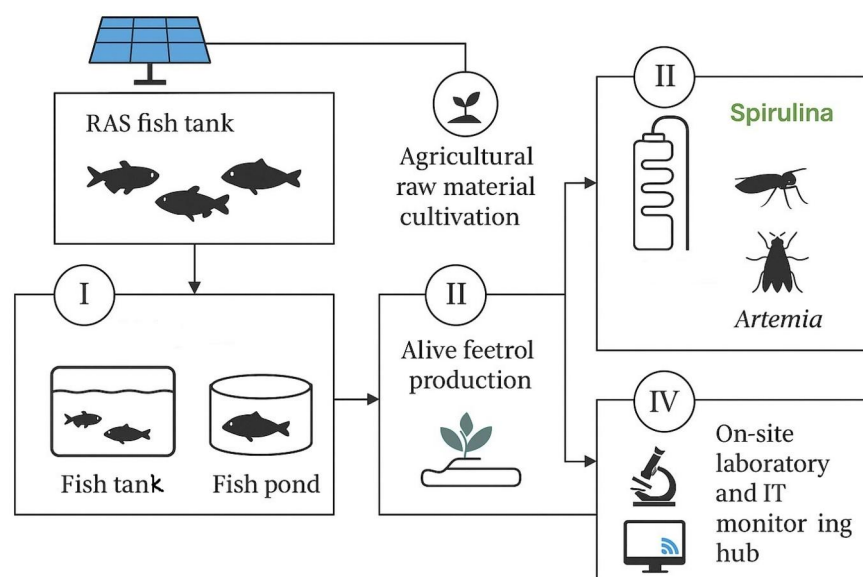


Figure 1. Schematic representation of the modular multitrophic aquaculture system (explanation provided in text). (I) RAS tanks (1a) and open fish ponds (1b), combined with elements of biological filtration; (II) cultivation units for agricultural raw materials used in feed production; (III) cultivation chambers for *Hermetia illucens* (black soldier fly), *Artemia* nauplii and *Spirulina*; (IV) technological management units for equipment and process control; mini-laboratories for hydrochemical and process diagnostics.

interactions among aquatic and agricultural components.

Experimental design

Fish were randomly divided into four dietary groups, each with three replicates:

- *Control group*: Commercial feed only;
- *Group A*: *Spirulina*-enriched diet (25%);
- *Group B*: *Artemia* (10%) + *Spirulina* (25%);
- *Group C*: *Spirulina* (10%) + *Hermetia illucens* (25%).

Fish were hand-fed to apparent satiation, and feeding behaviour was monitored closely. During the initial stages, some reluctance was observed towards natural supplements (*Artemia*, *Spirulina* and *H. illucens*), which normalised after an adaptation period. Physiological adaptations typically occurred between days 10 and 14. Feeding rates were adjusted based on body weight and water temperature (26.2–28.2 °C), following the schedule presented in Table 1 and Table 2 (Popma and Lovshin 1995). Water quality was monitored throughout the trial using standard methods. Temperature and dissolved oxygen were measured daily using a YSI ProODO multiparameter probe. pH was determined using a calibrated digital pH metre (HANNA Instruments, Woonsocket, RI, model HI98107). Ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) concentrations were measured weekly using spectrophotometric methods (APHA 2017).

Table 1. Feeding rate, % body weight/day.

Weight, g	Feeding rate, % body weight/day
1–5	7–10
5–20	4–6
20–100	3–4
100–200	2–3
200–400	1.5

Feeding rate is expressed as percentage of live body weight per day.

Table 2. Water quality.

Parameters	Measurement results	
	Average	min/max
Dissolved oxygen, mg/L	7.0	6.5–7.2
Temperature, °C	27.2	26.2–28.2 °C
pH	7.5	7.4–7.6
NH_4^+ , mg/L ^a	0.16	0.12–0.20
NO_3^- , mg/L ^b	15.0	14.8–15.2
NO_2^- , mg/L ^c	0.038	0.035–0.040

^aconcentration of ammonium ions

^bconcentration of nitrate ions

^cconcentration of nitrite ions

Feed ingredients and cultivation

- *Hermetia illucens*: Cultured using cereal bran and fruit/vegetable residues. Cereal bran was selected as the optimal substrate based on prior trials. Rearing conditions (temperature, humidity and light) followed standard recommendations. The section for cultivating black soldier fish was located in the same unit cultivating *Artemia* and *Spirulina*. Composition (%): moisture – 68.4; protein – 43.8; fat – 25.9; ash – 6.5.
- *Spirulina*: Grown in a closed-loop bioreactor using energy-saving technology developed by the authors.

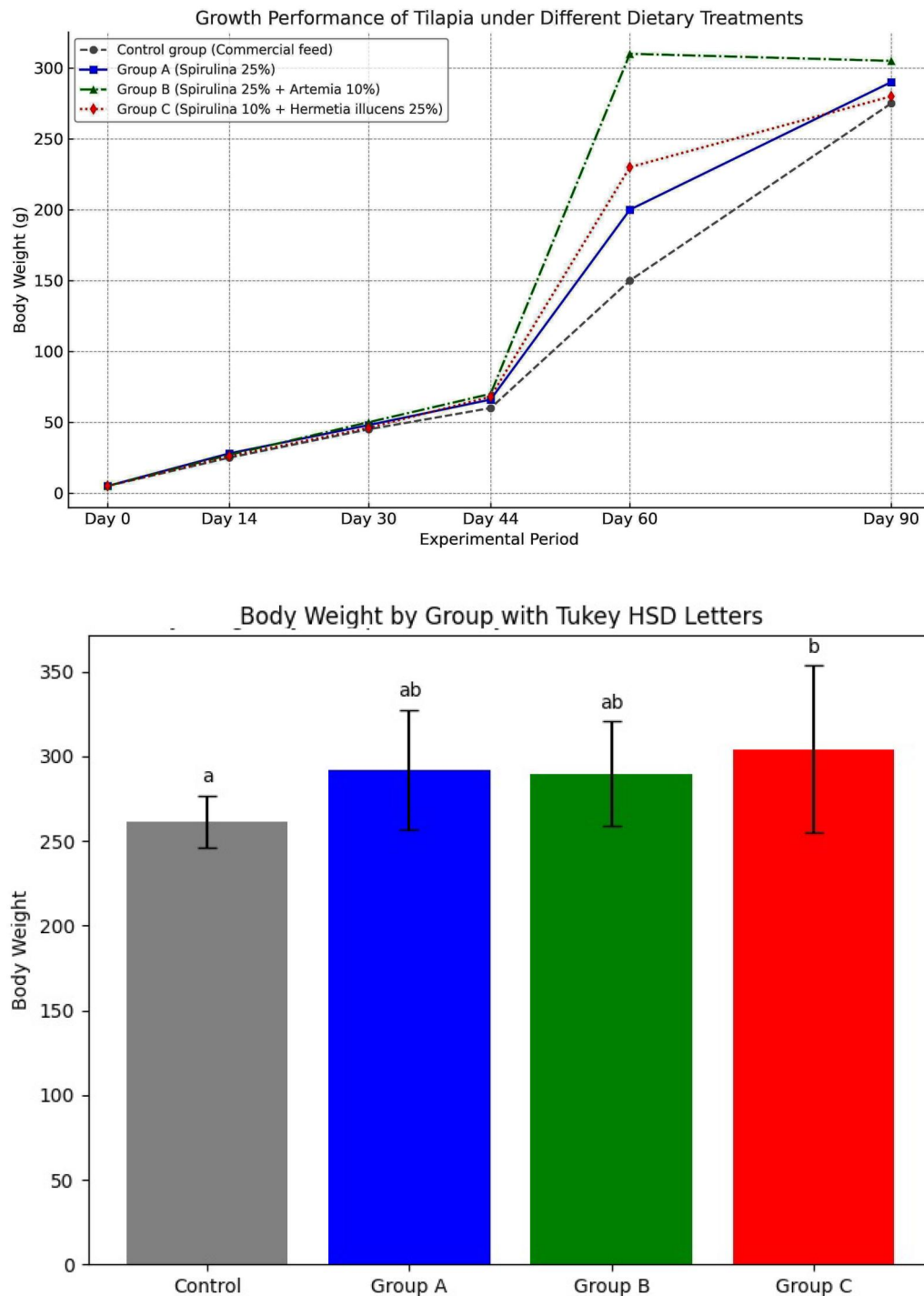


Figure 2. Study of the growth rate of tilapia in ontogenesis under the influence of technological factors, $x \pm SD$, $n = 25$ (I – start; II – after 14 days; III – 30 days; IV – 44 days; V – 60 days; VI – 90 days).

Spirulina biomass used for feeding was green and mildly saline. The *Spirulina* culture was cultivated under daily monitoring of illumination, bioreactor oxygen levels and biomass accumulation intended for subsequent filtration. Composition (%): moisture – 10; dry matter – 90; protein – 62; carbohydrates – 14.7; fat – 4; fibre – 3; ash – 6.3.

- *Artemia* nauplii: Cultivated following standard hydrochemical protocols (temperature: 25–28 °C; salinity: 30–80‰). Nauplii ranged from 0.3 to 0.6 mm in size and 0.01 to 0.06 mg in weight. Nauplii measured between 0.3 and 0.6 mm in length and weighed between 0.01 and 0.06 mg per individual. As a feeding substrate for *Artemia*, a

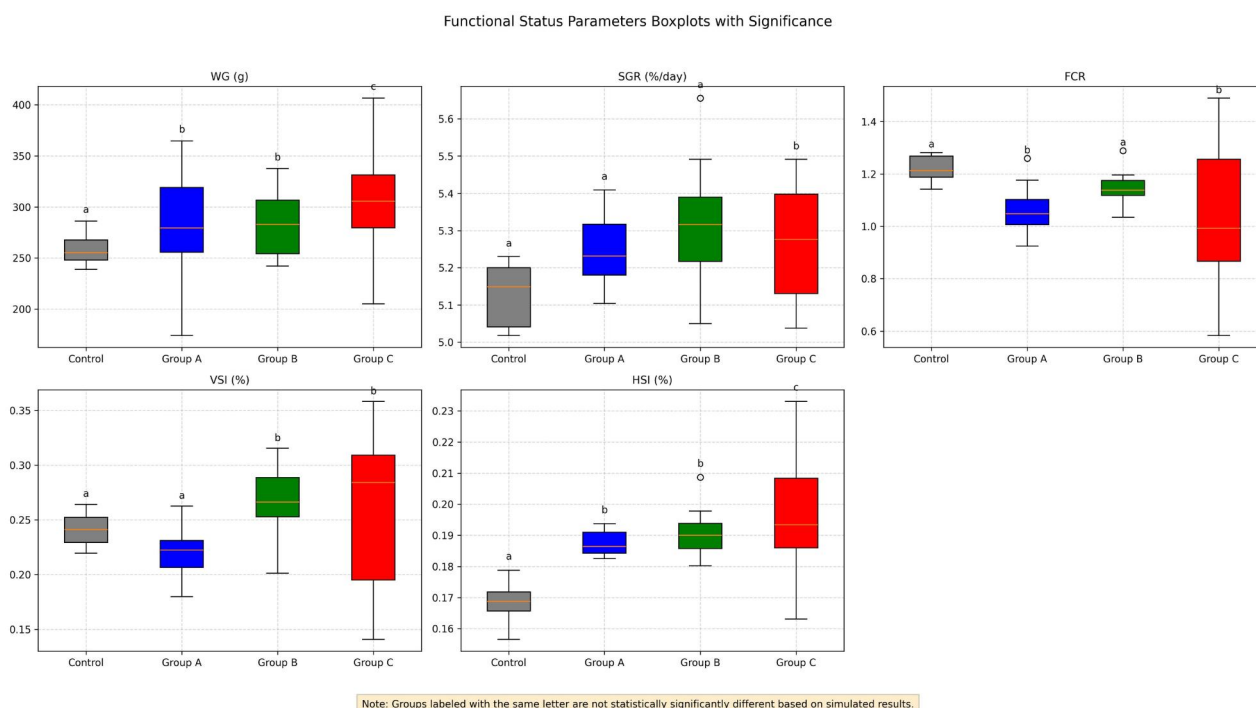


Figure 3. Analysis of tilapia organism development parameters under the influence of feeding when grown in a modular system with elements of multitrophic aquaculture ($\bar{x} \pm SD$, $n = 6$; WG: weight gain, g; SGR: specific growth rate, % day⁻¹; FCR: feed conversion ratio; survival, %; HSI: hepato-somatic index, %; VSI: viscero-somatic index, %).

culture of *Spirulina* and *Chlorella* obtained in a multitrophic aquaculture system was used. To obtain nauplii, it was assumed that approximately 3 g of cysts per litre were present. After hatching, the aeration was turned off and they were left for five minutes. The nauplii were then filtered with a general recommendation for stocking density of *Artemia* 10–12 pieces per mL tank. Composition (%): moisture – 12; dry matter – 88; protein – 53; carbohydrates – 12; fat – 3.2; fibre – 6.9; ash – 4.0. *Artemia* were used primarily for early feeding stages.

Water quality was monitored throughout the experimental period using a Palintest 7500 photometer (UK 2018; Gateshead, UK) and corresponding reagent kits, following aquaculture guidelines (Dehtiarov et al. 2001; Yevtushenko and Khyzhniak 2019). Dissolved oxygen, pH and temperature were measured daily, while ammonium (NH₄⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻) concentrations were analysed three times per week. The rearing environment was maintained within optimal ranges: temperature (26.2–28.2 °C), dissolved oxygen (6.5–7.2 mg/L), pH (7.4–7.6), NH₄⁺ (0.12–0.20 mg/L), NO₃⁻ (14.8–15.2 mg/L) and NO₂⁻ (0.035–0.040 mg/L) (Table 2). Measurements were conducted both by rapid on-site tests and laboratory analyses to ensure reliability.

For haematological and biochemical evaluation, blood samples (2 mL) were collected from the caudal vessels of six tilapia per tank using heparinised syringes. Four experimental groups were evaluated: a control group and three experimental diets (A, B and C), each in triplicate. Haematological parameters included red blood cell (RBC) count, haematocrit (Ht), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Biochemical indices in plasma – total protein, albumin, cholesterol, creatinine and glucose – were quantified photometrically using a Humalyzer 3000 biochemical analyser (Germany, 2010) with commercial kits (Human GmbH, Wiesbaden, Germany). Additionally, the chemical composition of muscle tissue was analysed in triplicate. Moisture content was determined by drying at 105 °C, protein by the Kjeldahl method, and ash content by standard incineration.

Growth performance was assessed through regular biometric monitoring of a representative subsample of 60 fish per group. For each specimen, total length and body weight were recorded, and the following indices were calculated: weight gain (WG), specific growth rate (SGR), Fulton's condition (Fc) factor, feed conversion ratio (FCR) and survival rate. Furthermore, the

liver and viscera of 12 randomly selected fish per group were excised and weighed to compute the hepato-somatic index (HSI) and viscero-somatic index (VSI). After dissection, fillets were skinned, homogenised and analysed to determine muscle composition.

All data are expressed as mean $\pm$ standard deviation (SD). Statistical comparisons were made using one-way analysis of variance (ANOVA). Differences between treatments were considered statistically significant at $p \leq 0.05$, with comparisons made relative to the control group.

Results

The method of growing tilapia with elements of a multitrophic modular system when adding natural components to the diet in all three experimental groups contributed to an increase in growth rate. In experimental groups B and C, the average body weight of tilapia increased in ontogenesis ($p < 0.001$) compared to the control group (Figure 2). The presented research results demonstrate both reliable differences in relation to the control group and only a trend of change. Therefore, all parameters were taken into consideration for a more comprehensive and integrated assessment. It was taken into account that the organism is a dynamic living system in which homeostasis has certain correlation and synergistic connections with the parameters of the functional status of fish.

Effect of alternative protein sources on the growth and physiological status of tilapia in a multitrophic aquaculture system

In this study (Figure 3), tilapia (*Oreochromis* sp.) were cultured in a modular multitrophic aquaculture system and fed four dietary regimens: control group (commercial feed only); group A (25% *Spirulina*); group B (25% *Spirulina* + 10% *Artemia*); and group C (25% *Hermetia illucens* + 10% *Spirulina*). The effect of these technological feeding strategies on growth performance and physiological parameters was assessed.

Weight gain analysis revealed that all experimental diets led to superior growth compared to the control. Group C achieved the highest WG (301.4 ± 49.3 g), which corresponds to an increase of $\sim 16.5\%$ over the control group (258.7 ± 15.6 g). Group A (289.3 ± 35.5 g) and group B (286.9 ± 31.1 g) also recorded marked gains, exceeding the control by 11.8% and 10.9%, respectively. There were no significant differences in these parameters for the control

group. But the difference in the experimental groups compared to the control from 10.9 to 11.8% allows us to note an increasing trend. The SGR was likewise improved: the control fish had an average SGR of $5.11 \pm 0.13\% \text{ day}^{-1}$, whereas group C reached $5.27 \pm 0.19\% \text{ day}^{-1}$, with groups A and B showing intermediate values ($5.22 \pm 0.15\%$ and $5.22 \pm 0.21\%$, respectively). Although the difference between groups was not statistically significant, a trend towards an increase in the growth rate of tilapia in the experimental groups could be noted, which was consistent with other parameters of fish development. Especially in the experimental group C. Feed conversion ratio, a critical indicator of feed efficiency, decreased significantly in the experimental groups. The control fish had an FCR of 1.25 ± 0.08 , while group C recorded the lowest value (1.03 ± 0.21 ; $p < 0.05$), followed by group A (1.06 ± 0.14 ; $p < 0.05$) and group B (1.12 ± 0.11 ; $p < 0.05$). These findings highlight the enhanced capacity of tilapia to convert feed into biomass when fed with insect and microalgae-based diets.

Fulton's condition factor (Figure 3) was slightly higher in experimental groups, suggesting improved somatic condition: group C had the highest Fc (1.80) compared to the control (1.73). In the experimental groups (A, B and C), the average body weight, SGR was higher than in the control, but the Fc did not have statistically significant differences. This is explained by the fact that the increase in weight was accompanied by a proportional increase in length, which maintained a stable weight/length ratio. Thus, the WG reflects mainly the intensity of growth, while the fatness index indicates the harmony of development without signs of disproportion or obesity.

Survival rates remained high and consistent across groups, ranging from 96.2% (control) to 98.6% (group C), indicating that dietary enrichment did not negatively impact survival.

Regarding somatic indices, the VSI remained statistically unchanged among groups: control (0.238 ± 0.014), group A (0.226 ± 0.027), group B (0.258 ± 0.037) and group C (0.249 ± 0.053). The determination of the VSI in tilapia did not reveal statistically significant differences between groups. Despite some fluctuations in the mean values, the variability within groups overlapped the differences between groups. In contrast, the HSI significantly increased in all experimental groups. The control fish had an HSI of 0.169 ± 0.006 , while group C reached 0.193 ± 0.013 ($p < 0.01$), and groups A and B recorded 0.189 ± 0.005 ($p < 0.001$) and 0.191 ± 0.09

Functional Status of Tilapia under Dietary Treatments (Boxplots with Significance)

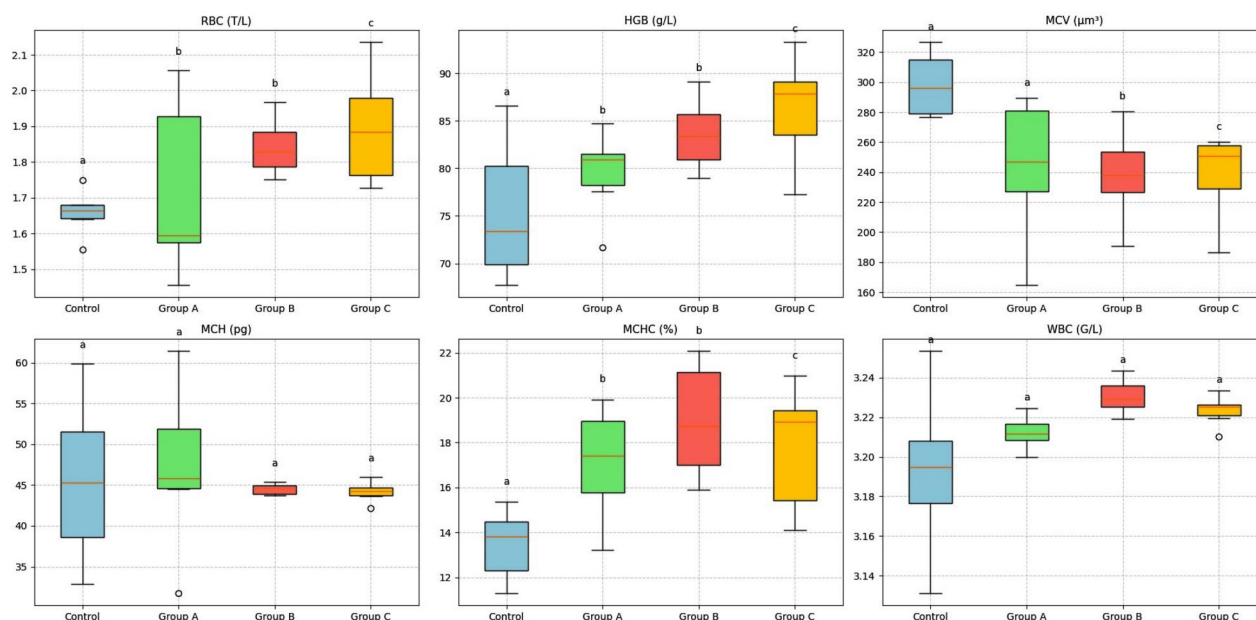


Figure 4. Functional status of tilapia organism under the influence of feed factor when grown in a modular multitrophic aquaculture system ($\bar{x} \pm \text{SD}$, $n = 6$; RBC: red blood cells; WBC: white blood cell count; HGB: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration).

($p < 0.001$), respectively, suggesting a possible increase in liver metabolic reserves or activity.

In summary, the integration of *Spirulina*, *Artemia* and *Hermetia illucens* into tilapia diets significantly improved growth performance, feed efficiency and condition factor, with group C demonstrating the most pronounced benefits. These results support the potential of alternative protein sources to enhance aquaculture productivity in a sustainable way.

Effect of alternative protein-enriched diets on haematological parameters of tilapia cultured in a multitrophic aquaculture system

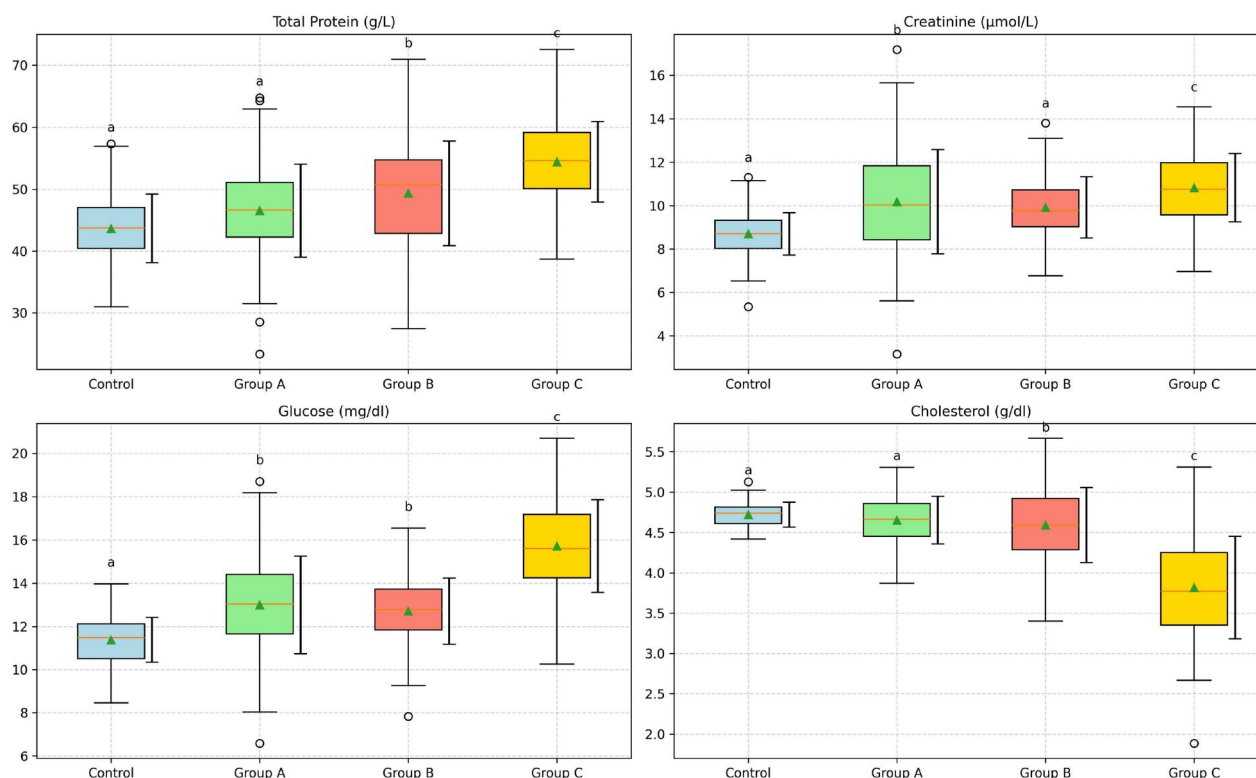
The total number of erythrocytes was higher in all experimental groups compared to the control (1.668 ± 0.087 T/L). Group C showed the highest RBC value (1.980 ± 0.152 T/L; $p < 0.05$), corresponding to an increase of approximately 18.7% over the control. Groups A and B also demonstrated elevated RBC levels (1.829 ± 0.347 T/L and 1.845 ± 0.067 T/L, respectively), indicating a stimulating effect of the alternative protein sources on haematopoiesis (Figure 4). Haemoglobin content followed a similar trend (Figure 4). The control fish had an HGB of 73.1 ± 5.8 g/L, while group C recorded the highest value (88.2 ± 4.9 g/L; $p < 0.001$). Groups A and B also showed significant increases (83.1 ± 5.6 g/L; $p < 0.05$

and 82.7 ± 3.6 g/L; $p < 0.01$, respectively). These results suggest enhanced oxygen-carrying capacity in fish fed enriched diets. A decrease in MCV was observed in experimental groups (Figure 4) compared to the control ($299.7 \pm 32.4 \mu\text{m}^3$). Group C showed the lowest MCV ($242.3 \pm 35.3 \mu\text{m}^3$; $p < 0.05$), and group B also recorded a significant reduction ($249.3 \pm 25.9 \mu\text{m}^3$; $p < 0.05$). This may reflect a physiological adaptation towards producing more numerous, smaller erythrocytes. Mean corpuscular haemoglobin (pg) values remained relatively stable across treatments: control (44.4 ± 6.5 pg), group A (46.7 ± 8.6 pg), group B (44.8 ± 0.6 pg) and group C (44.6 ± 1.4 pg), with no significant differences (Figure 4). The MCHC (%) increased significantly in all experimental groups. The control fish had an MCHC of $14.8 \pm 1.8\%$, whereas group A ($18.1 \pm 2.0\%$; $p < 0.05$), group B ($18.2 \pm 2.1\%$; $p < 0.05$) and group C ($18.7 \pm 2.5\%$; $p < 0.05$) exhibited higher values. This increase implies a greater concentration of Hb within erythrocytes (Figure 4).

The WBC (Figure 4) remained stable across groups, with values ranging narrowly around 3.2 G/L (control: 3.20 ± 0.06 G/L; groups A–C: 3.21–3.23 G/L), suggesting that the feeding interventions did not significantly influence innate immune cell levels ($p < 0.05$).

Figure 4 combined the haematological analysis demonstrates that diets supplemented with *Spirulina*,

Biochemical Parameters of Tilapia under Dietary Treatments (Boxplots with Standard Deviation Error Bars and Significance)



Note: Groups labeled with the same letter are not statistically significantly different based on simulated results.

Figure 5. Functional status of tilapia organism under the influence of feed factor when grown in a modular multitrophic aquaculture system ($\bar{x} \pm \text{SD}$, $n = 6$).

Artemia and *Hermetia illucens* positively affected haematopoietic function in tilapia. Group C consistently showed the most pronounced improvements, including the highest RBC and HGB levels, coupled with a significant increase in MCHC. These findings suggest that such diets can enhance oxygen transport capacity and potentially support better metabolic and physiological performance in fish reared under intensive aquaculture conditions.

Effect of optimised diets on blood biochemical parameters of tilapia cultured in a multitrophic aquaculture system

The use of diet optimisation contributed to notable shifts in blood biochemistry, most evident in experimental group C (Figure 5), in the control group, mean plasma total protein was 43.2 ± 5.7 g/L (approximate range: 37.5–48.9 g/L). Group C exhibited the highest total protein (53.9 ± 7.0 g/L; $p < 0.05$), corresponding to an increase of $\sim 24.7\%$ over the control. Intermediate values were observed in group B (49.6 ± 9.2 g/L) and group A (46.0 ± 7.6 g/L). This rise

suggests enhanced protein synthesis or retention, reflecting improved nutritional status.

Creatinine concentration, an indicator of muscle metabolism and kidney function, was 8.8 ± 1.1 $\mu\text{mol/L}$ in the control group. Group C recorded a higher creatinine level (11.0 ± 1.6 $\mu\text{mol/L}$; $p < 0.05$), showing an increase of $\sim 25.7\%$ compared to the control. Group A (10.4 ± 2.6 $\mu\text{mol/L}$) and group B (9.8 ± 1.5 $\mu\text{mol/L}$) also demonstrated elevated values, though without significant difference (Figure 5). In the experiment group C, tilapia, which received protein-enriched natural supplements, had the best growth indicators (Figures 2 and 3) and metabolic activity.

Blood glucose, reflecting energy metabolism, rose markedly in group C. The control group had a mean glucose of 11.6 ± 1.1 mg/dL, whereas group C reached 15.5 ± 2.1 mg/dL ($p < 0.01$), representing an increase of $\sim 33.5\%$. Groups A (13.1 ± 2.7 mg/dL) and group B (12.7 ± 1.5 mg/dL) showed moderate increases. Although elevated glucose can be a marker of stress or impaired insulin sensitivity, this was not supported here, since all experimental groups showed lower mortality, normal ethology and superior growth

Regression Curves of Blood Parameters vs Body Weight in Tilapia

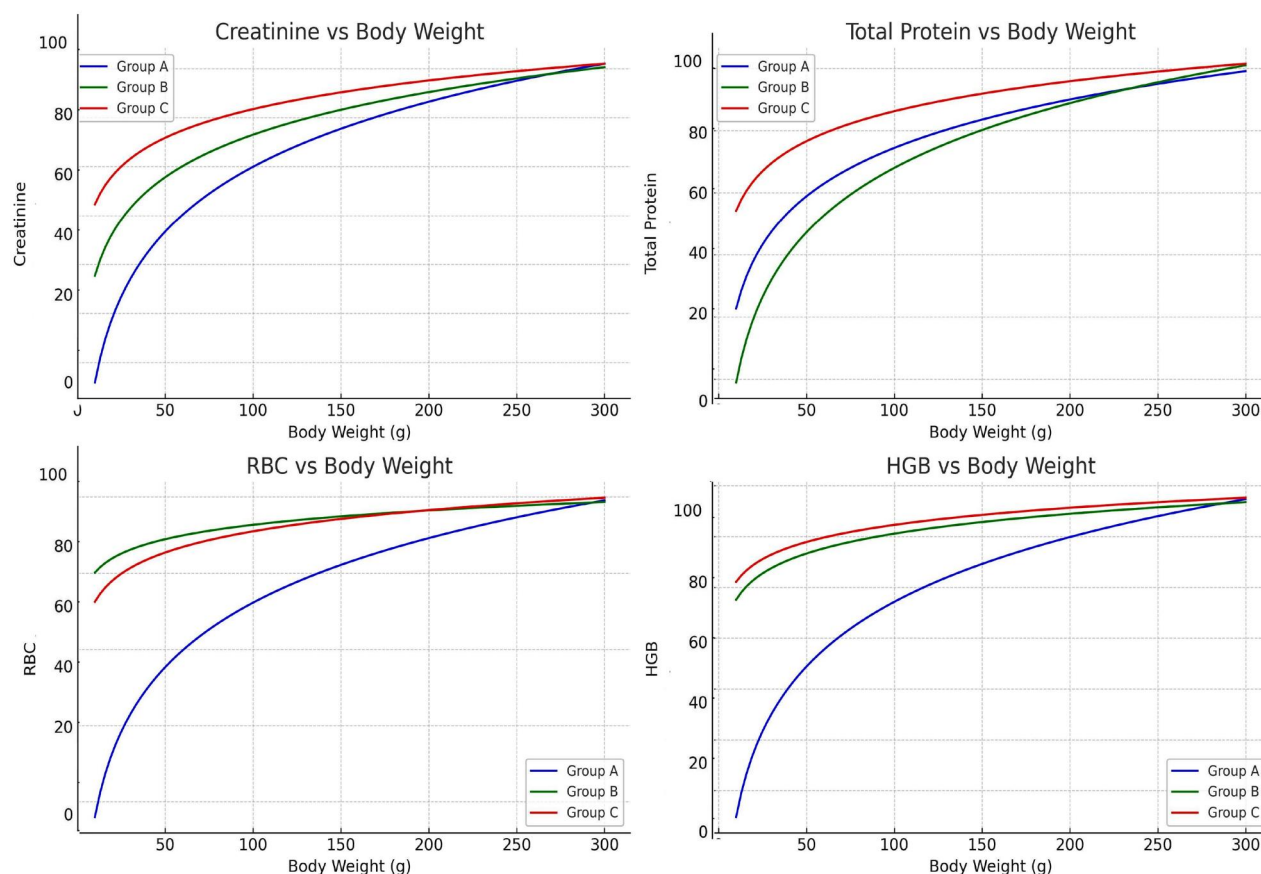


Figure 6. Coefficient of determination correspondences for dependence of blood parameters on body weight of tilapia. x , x^2 : body weight of tilapia; y : blood parameters.

performance compared to controls. Interestingly (Figure 5), cholesterol levels decreased in experimental groups. The control fish had a cholesterol concentration of 4.7 ± 0.15 g/dL. Group C showed the lowest value (3.8 ± 0.61 g/dL; $p < 0.01$), corresponding to a reduction of $\sim 19.8\%$ compared to the control. Groups A (4.7 ± 0.36 g/dL) and group B (4.5 ± 0.46 g/dL) displayed similar trends, though without statistical significance.

Overall, diet enrichment with *Spirulina*, *Artemia* and particularly *Hermetia illucens* led to higher total protein, creatinine and glucose concentrations in the blood of tilapia, alongside a significant reduction in cholesterol. The most pronounced changes were consistently observed in group C, indicating improved protein metabolism, energy status and potentially healthier lipid regulation. These biochemical adaptations complement the growth and haematological improvements previously observed, supporting the use of alternative protein sources to promote fish health and performance in sustainable aquaculture systems (Figure 5).

Relationship between biochemical composition of tilapia muscle tissue and physiological parameters under different dietary treatments

Curves in Figure 6 illustrate how to deepen the understanding of the impact of alternative protein-enriched diets on the physiological condition of tilapia (*Oreochromis* sp.), regression analysis was conducted to evaluate the dependence of muscle tissue biochemical composition on body weight and selected blood parameters. The analysis included three experimental groups: group A (25% *Spirulina*), group B (25% *Spirulina* + 10% *Artemia*) and group C (25% *Hermetia illucens* + 10% *Spirulina*).

In all groups, a positive logarithmic relationship was observed between body weight and muscle protein content. The coefficient of determination (R^2) reflects the strength of this relationship. The association was moderate in group A ($R^2 = 0.561$), whereas it was much stronger in group B ($R^2 = 0.8664$) and group C ($R^2 = 0.8532$). These high R^2 values in groups B and C indicate that body weight explains over 85% of the variation in muscle protein content, suggesting that

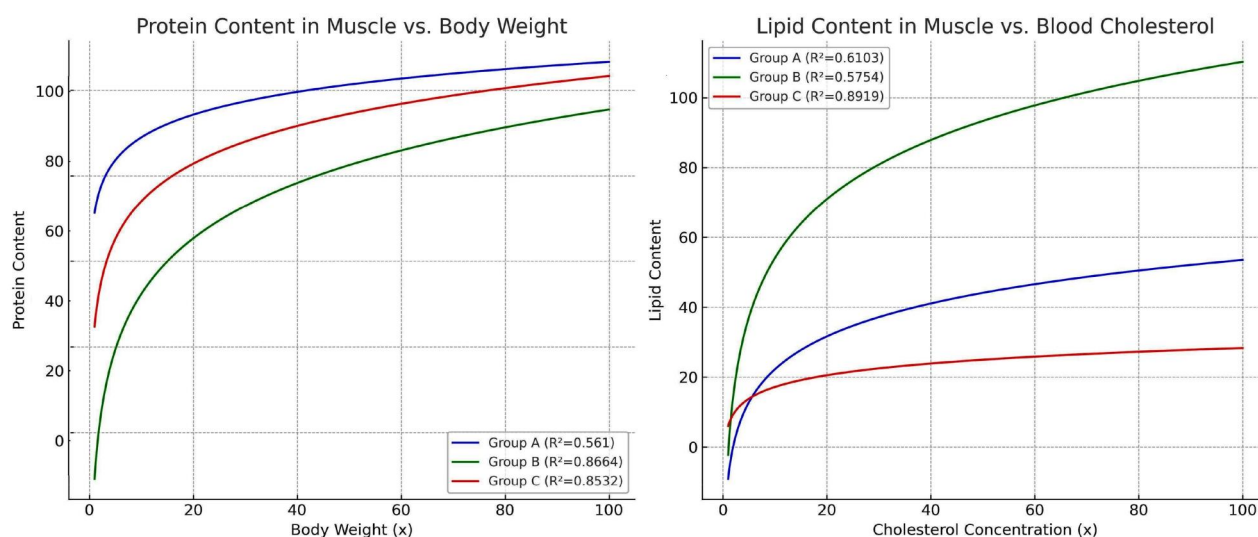


Figure 7. Coefficient of conformity of determination of the dependence of biochemical composition indicators of tilapia muscle tissue on body weight and blood indicators of tilapia (x , x^2 : body weight of tilapia and cholesterol content in muscle tissue; y : blood parameters).

diets enriched with *Artemia* or *Hermetia illucens* not only promote growth but also more consistently enhance protein accumulation in muscle tissue.

A similar analysis was performed to assess the relationship between muscle lipid content and blood cholesterol concentration. The association was moderate in group A ($R^2 = 0.6103$) and group B ($R^2 = 0.5754$), but very strong in group C ($R^2 = 0.8919$). This indicates that, in group C, nearly 90% of the variability in muscle lipid content can be explained by changes in blood cholesterol concentration. Such a high degree of dependence suggests a tightly regulated metabolic adaptation linked to the specific diet combining *Spirulina* and *Hermetia illucens*.

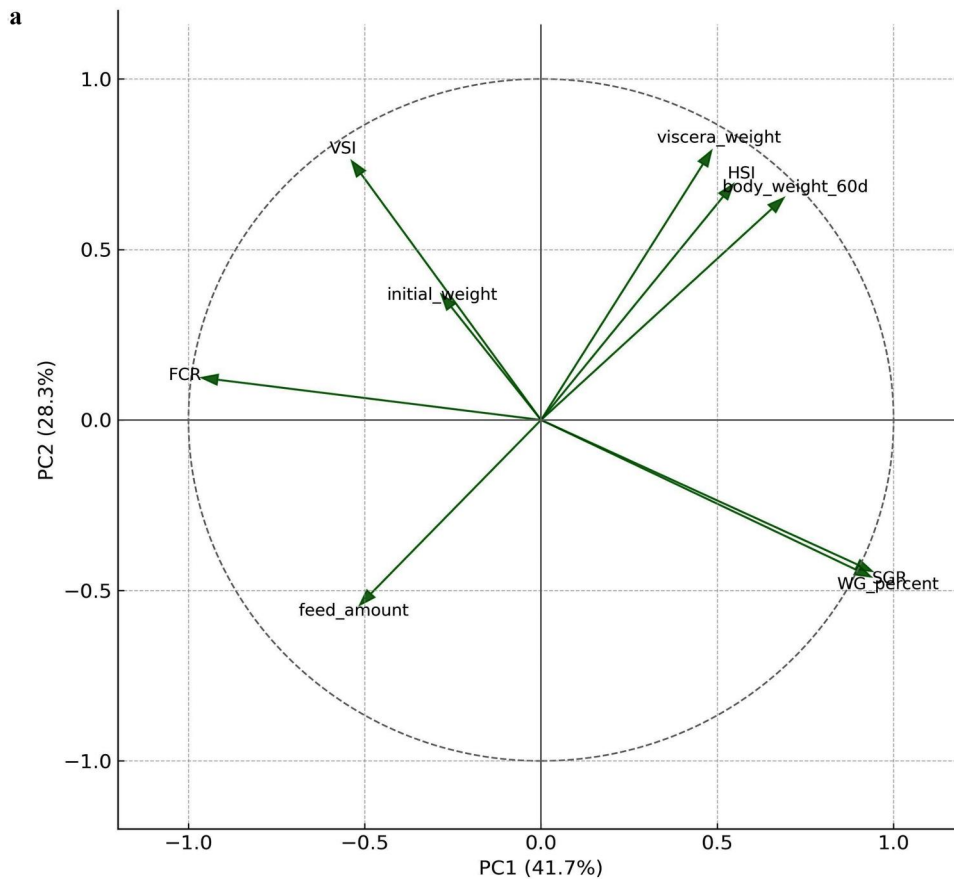
The regression analysis highlights that diets enriched with *Spirulina*, *Artemia* and especially *Hermetia illucens* not only improved growth and physiological parameters but also strengthened the predictive relationship between blood parameters and muscle biochemical composition. In particular, group C consistently showed the highest coefficients of determination (R^2), reflecting a more stable and coherent physiological adaptation in response to the diet. These results reinforce the value of diet optimisation strategies that incorporate alternative protein sources to enhance both the nutritional quality and metabolic efficiency of farmed tilapia.

The analysis of the last two curves highlights a clear logarithmic relationship between tilapia body weight and muscle protein content, as well as between blood cholesterol levels and muscle lipid content. In the first case, the protein content in muscle tissue shows a moderate correlation in group A ($R^2 = 0.561$),

suggesting that only around 56% of the variability can be explained by body weight, possibly due to other influencing factors such as diet composition or environmental conditions. In contrast, groups B ($R^2 = 0.866$) and C ($R^2 = 0.853$) demonstrate a much stronger relationship, indicating that the specialised feed tested in these groups supports a more predictable and consistent accumulation of muscle protein as fish grow.

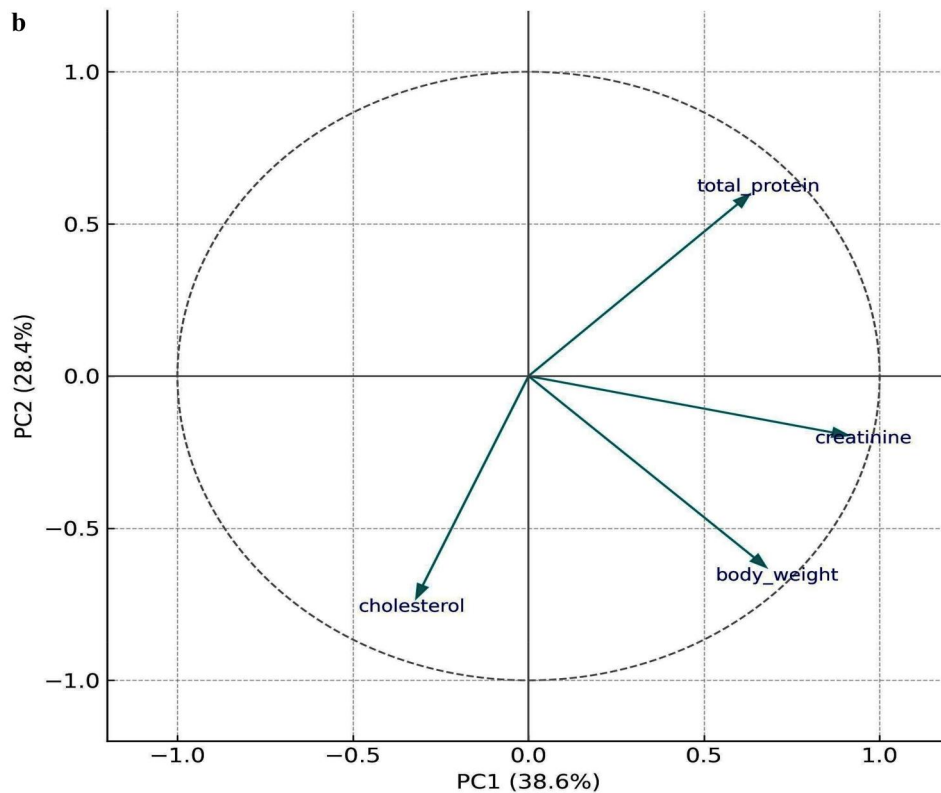
Similarly in Figure 6, the lipid content in muscle tissue plotted against blood cholesterol levels reveals a moderate association for groups A ($R^2 = 0.610$) and B ($R^2 = 0.575$). This suggests that in these groups, lipid accumulation in muscle is less directly linked to circulating cholesterol, possibly reflecting a less efficient lipid metabolism. On the other hand, group C shows a very strong correlation ($R^2 = 0.8919$), meaning that nearly 90% of the variability in muscle lipid content can be explained by cholesterol concentration in the blood. This points to a more stable metabolic response and a better adaptation of lipid deposition mechanisms under the influence of the tested feed.

Overall, the higher coefficients of determination observed especially in groups B and C confirm the beneficial effect of these experimental diets in promoting a more homogeneous and biologically coherent growth pattern. The logarithmic models also suggest that increases in protein and lipid levels become steeper at earlier growth stages before reaching a plateau, which reflects typical physiological growth dynamics. These findings collectively highlight the role of optimised feeding strategies in enhancing not only growth performance but also the biochemical



	PC1	PC2
RBC	0.344255	0.041499
Ht	-0.163119	-0.529278
WBC	0.186454	0.435914
Glucose	0.332574	-0.160665
BodyWeight_90d	0.330311	0.135062
Cholesterol	-0.304474	0.252077
TotalProtein	0.331649	0.007838
BodyWeight_30d	0.323659	-0.121262
Creatinine	0.326163	0.008764
BodyWeight_60d	0.333762	-0.120393
InitialBodyWeight	0.001805	0.619562
FeedConsumed90d	-0.274174	0.085619

Figure 8. (a) Principal component analysis (PCA) on growth and nutritional performance variables of dietary treatments on Nile tilapia. (b) Principal component analysis (PCA) on serum biochemical markers of dietary treatments on Nile tilapia. (c) Results of PCA haematological of dietary treatments on Nile tilapia.



Feature	PC1 Loading	PC2 Loading	
0	body_weight	0.643405	0.597314
1	cholesterol	-0.301675	0.688741
2	total_protein	0.596244	-0.565891
3	creatinine	0.866859	0.185578

Figure 8. Continued

composition and health status of tilapia in aquaculture systems.

Relationship between biochemical composition and physiological parameters

To assess the physiological determinants of muscle quality in *Oreochromis niloticus*, we analysed the relationship between muscle biochemical composition (protein and lipid content) and key physiological indicators (body weight and blood cholesterol levels) across the three experimental groups (A, B and C).

A logarithmic regression analysis revealed varying degrees of correlation between muscle protein content and fish body weight (Figure 7). The relationship was strongest in group B ($R^2 = 0.866$) and group C ($R^2 = 0.853$), suggesting a robust positive association

between somatic growth and protein accumulation in muscle tissue. In contrast, group A exhibited a weaker correlation ($R^2 = 0.561$), indicating greater interindividual variability or potentially lower metabolic efficiency in protein deposition.

These findings imply that in groups B and C, body weight is a reliable predictor of muscle protein content, likely reflecting optimised dietary assimilation or better physiological status. Similarly (Figure 7), a strong logarithmic relationship was observed between muscle lipid content and blood cholesterol concentration, particularly in group C ($R^2 = 0.892$). This suggests that lipid deposition in muscle may be closely linked to circulating lipid levels in this group. Groups A and B showed moderate correlations ($R^2 = 0.6103$ and $R^2 = 0.5754$, respectively), indicating a less consistent pattern. These results collectively demonstrate that

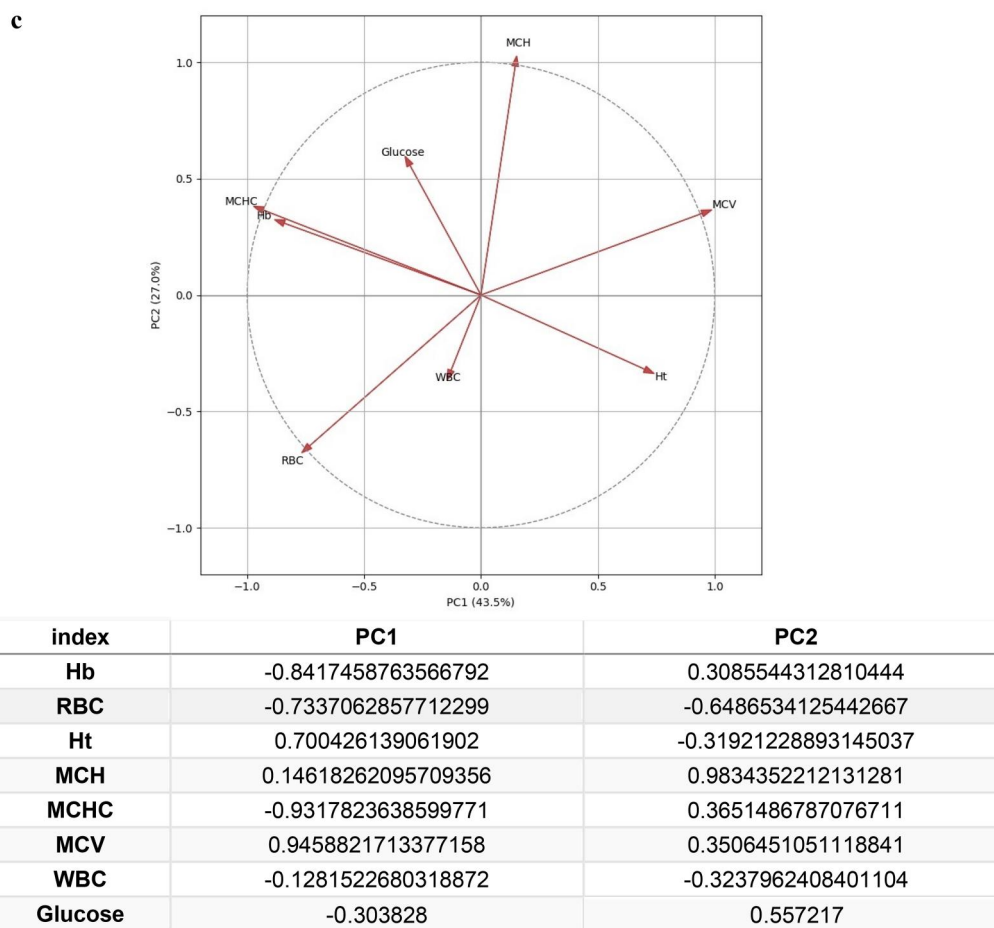


Figure 8. Continued

group C exhibits the most stable and predictive biochemical–physiological profile, potentially due to more effective nutrient utilisation or metabolic regulation (Figure 7).

To explore the impact of dietary treatments on Nile tilapia (Figure 8), a principal component analysis (PCA) was conducted on growth and nutritional performance variables (Figure 8(a)). The first two components explained 71.6% of the total variance, with PC1 accounting for 42.5% and PC2 for 29.1%. PC1 was positively associated with final body weight, SGR and viscera weight, while negatively correlated with FCR, defining a strong growth efficiency axis. Group C (*Spirulina* + *Hermetia illucens*) achieved the highest positive PC1 scores, indicating superior feed conversion, accelerated growth and better nutrient assimilation. The control and group A (*Spirulina*-only) had low PC1 scores, reflecting suboptimal growth and feed utilisation.

The PCA of serum biochemical markers captured 75.8% of the total variation, with PC1 explaining 49.3% and PC2 26.5% (Figure 8(b)). PC1 represented a metabolic performance axis, loading positively on final

body weight and creatinine, while PC2 highlighted protein metabolism and lipid balance, driven by total protein and cholesterol. Group C again scored highest on PC1, suggesting enhanced muscle growth and renal function, while the control group and group A were negatively aligned with both components, indicating weaker physiological responses. Group B (*Artemia* + *Spirulina*) showed intermediate values, suggesting moderate improvement but not as pronounced as group C.

Finally, the haematological PCA explained 70.52% of the variation, with PC1 contributing 43.49% and PC2 27.03%. PC1 was strongly associated with erythrocytic indices such as Hb, Ht and MCV, while PC2 reflected immune-metabolic variation linked to glucose and WBC. Group C was clearly separated from the others, showing elevated Hb and Ht, indicative of improved oxygen transport and physiological robustness. In contrast, the control group formed a distinct negative cluster, signalling reduced haematological performance (Figure 8(c)).

In conclusion, across all PCA analyses – growth/nutritional (71.6%), biochemical (75.8%) and

haematological (70.52%) – the diet combining *Spirulina* and *Hermetia illucens* (group C) consistently produced the most favourable physiological, metabolic and haematological responses. These findings clearly position group C as the most effective and scientifically validated formulation for enhancing health, growth and sustainability in Nile tilapia aquaculture.

Discussion

The primary focus of this study was to develop a comprehensive and ecologically sustainable method for cultivating juvenile tilapia, aiming to accelerate their development during ontogenesis. In the context of rapid growth in the aquaculture industry, modern approaches emphasise the synergy between multiple production systems and the efficient utilisation of aquatic resources. In the context of rapid expansion in global aquaculture, the results presented in this manuscript support that integration of biological, technological and ecological innovations is essential for sustainable productivity. However, unlike many previous studies that focused on single-factor improvements, for example, feed efficiency or water quality (Radhakrishnan et al. 2020; Abdel-Rahim et al. 2024; Mitra et al. 2025), this research examined the synergistic effects of combined natural feed components: *Spirulina* and *H. illucens* within an integrated system framework.

Numerous studies (Alexander et al. 2016; Freccia et al. 2020; Austin et al. 2022; Can and Seyhaneyildiz Can 2023) have demonstrated that integrated aquaculture management can enhance productivity while maintaining ecosystem balance. Our results align with these findings but extend them by incorporating modular production of feed components, providing a closed-loop model that reduces dependency on external protein sources. This systems-based approach contrasts with the more traditional optimisation studies that emphasise feed composition alone, suggesting that technological and ecological integration is equally crucial for resource-efficient aquaculture.

Previous studies have highlighted the benefits of incorporating feed additives like *Artemia*, *Spirulina* and black soldier fly larvae in aquaculture diets (El-Sheekh et al. 2014; Kals et al. 2024). The results of this study further confirm that the inclusion of *Spirulina* and black soldier fly meal had the most pronounced positive effect on the overall functional status of tilapia.

Our multidimensional PCA analysis further provide novel insight into the multifactorial drivers of growth and metabolic efficiency in tilapia. The strong loading

of growth and feed conversion traits on PC1 (42.5%) supports prior reports (Badr-Eldin et al. 2021; Youssef et al. 2023), yet the tighter clustering of *Spirulina* + *H. illucens* groups in our dataset suggests enhanced physiological coherence not seen in single-supplement studies. This implies that mixed natural feed components can produce integrative physiological responses that exceed the sum of individual effects. In contrast, Batista et al. (2023) and Omar (2023) reported only moderate improvements in metabolic markers when single insect meals were used: indicating that a combination strategy, as tested here, may be more efficient for sustainable production.

From a biochemical perspective, elevated serum protein and reduced lipid accumulation in experimental groups align partially, but not completely with prior research. For instance, Bittencourt et al. (2003) observed a general trend towards improved protein synthesis with natural feed additives, but without concurrent lipid regulation. Our findings thus extend current understanding by demonstrating that combined *Spirulina* and insect meal diets simultaneously enhance anabolic and lipid-regulatory processes, reflecting more efficient metabolic partitioning. The VSI may indicate that regardless of the feed composition, tilapia did not have significant changes in the relative weight of viscera, and the energy obtained from the diet was directed primarily to overall somatic growth, rather than to the deposition of visceral fat. This dual effect could have important implications for improving fillet quality and energy balance, which are rarely discussed in recent studies emphasising growth alone (Al-Deriny 2020; Al-Deriny et al. 2020).

Recent reviews (Aubin et al. 2019; Tikue et al. 2023) have emphasised the need to operationalise sustainability in aquaculture through circular production loops. In this context, the integration of multitrophic aquaculture elements and modular bioreactor-based feed production demonstrated in this study represents a step forward. Unlike conventional approaches that focus on either nutrient recycling or feed substitution, our framework links both processes, thereby minimising waste while maintaining high growth efficiency. This integrative perspective contrasts with most recent Egyptian studies, which, although highlighting the local potential of insect meal (Abd El-Gawad et al. 2025), did not evaluate ecosystem-level sustainability metrics.

In conclusion, this study not only corroborates but also extends recent advances in sustainable tilapia nutrition by demonstrating that combined natural feed components (*Spirulina* + *H. illucens*) yield

synergistic effects across growth, biochemical and haematological process (Tippayadara et al. 2021). The observed outcomes differ from prior single-factor studies by showing that nutrient synergy and ecological integration amplify physiological resilience and resource efficiency. Therefore, this research contributes a critical step towards the development of eco-efficient, circular aquaculture systems that align with the latest sustainability imperatives outlined in contemporary aquaculture science.

In our experiment, the most pronounced effects were observed in experimental group C, where the combined feeding approach resulted in a significant increase in growth rate and Fulton's condition factor (F_1), with values ranging between 1.755 and 1.80 across all experimental groups. These findings indicate a favourable general physiological state of the fish. Moreover, a strong correlation was found between blood biochemical parameters and muscle tissue composition, further validating the health status of the tilapia. These results confirm earlier studies that demonstrate the strong relationship between tilapia's functional status and haematological and biochemical indices, contributing to a balanced homeostasis (Bittencourt et al. 2003; Boucetta et al. 2025). In this study, the status of morpho-functional parameters of the blood of tilapia in experimental groups confirms the positive dynamics of the general functional status of the fish organism. Considering that RBC is a quantitative indicator and not a change in cellular properties, the lack of statistical difference in MCH can be explained as a positive haematological response to the supplement, where erythropoiesis in tilapia blood is enhanced without signs of micro- or macrocytic abnormalities. It is important to add to the question of blood composition, taken together, these results suggest that the higher glucose concentration in group C reflects adaptive energy metabolism, stimulated by the intake of glucogenic substrates from protein-enriched natural supplements. This interpretation is reinforced by the positive set of indicators (growth rate, FCR, survival), which consistently point to a favourable metabolic effect rather than a pathological state. Our results are consistent with research in this area in other scientific papers (Zhou et al. 2020; Xu et al. 2021).

In the biochemical and haematological PCA, PC1 explained 43.5% of variance and was associated with elevated Hb, Ht and creatinine, indicative of improved oxygen transport and renal function. Group C again showed the most positive scores, paralleling Egyptian studies reporting enhanced haematological resilience in tilapia fed insect-rich diets (Abd El-Gawad et al. 2025).

PC2 (27.0%) captured variation in total protein and immune markers (glucose, WBC), where again groups B and C performed better than *Spirulina* alone and the control, reflecting stronger immune-metabolic outcomes. Interestingly, creatinine levels were moderately elevated in group C ($11.0 \pm 1.6 \mu\text{mol/L}$; $p < 0.05$), representing a $\sim 25.7\%$ increase compared to the control. While creatinine can sometimes indicate renal stress, no clinical signs of stress (abnormal behaviour, reduced survival) were observed. Instead, the elevation is more likely linked to increased protein turnover associated with enhanced growth. Similarly, Ajeniyi and Solomon (2014) that the results indicated a significant increase in creatinine for all the groups as the weight increases in *Clarias gariepinus*. This shows that the criteria we look at in humans may differ in fish.

Conclusions

This study confirms that the implementation of a modular, IMTA system provides a viable pathway towards ecologically sustainable tilapia production. By incorporating trophically diverse, naturally derived feed components into a closed-loop system, we achieved enhanced control over environmental conditions and product quality. The natural diets – especially the combination of *Spirulina platensis* and black soldier fly (*Hermetia illucens*) larvae – proved highly beneficial for fish health.

The PCA highlighted experimental group C as the most effective diet, based on its superior scores across multiple growth, haematological and biochemical parameters. Fish in this group showed higher Fulton's condition factors, robust metabolic indicators and strong correlations between feed intake and tissue composition. These outcomes validate that natural, multitrophic feeding strategies accelerate tilapia ontogeny while supporting systemic sustainability.

In summary, the integration of multitrophic principles, natural feed additives and closed-loop management supports both ecological resilience and biological productivity. This model aligns with global sustainability trends in aquaculture and can serve as a blueprint for future fish-farming innovation.

Recommendations

- *Wider application of modular systems:* It is recommended that the integrated multitrophic modular aquaculture system be scaled up and tested in various aquaculture environments (e.g. RAS, pond systems, cages) to validate its adaptability and

efficiency under different ecological and economic conditions.

- *Further research on feed formulations:* Continued research should focus on optimising the proportion and combination of alternative protein sources (e.g. *Spirulina*, black soldier fly, *Artemia*) to further improve fish growth performance, immunity and product quality.
- *Economic and environmental assessment:* Future studies should evaluate the cost-effectiveness and environmental impact of this multitrophic approach in comparison with traditional feeding practices, to support decision-making for commercial adoption.
- *Policy support and certification:* It is advisable to develop certification protocols and guidelines to support the implementation of sustainable multitrophic aquaculture systems, ensuring traceability and consumer trust in eco-friendly aquaculture products.
- *Integration with circular agriculture:* Combining aquaculture systems with circular agriculture practices (e.g. hydroponics, vermicomposting) could create added value and minimise waste, supporting a fully closed-loop production model.

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Author contributions

Olena Honcharova: conceptualisation, writing – review, editing and statistics graphs; Sabine Boucetta: writing, statistics graphs, graphical abstract, review and editing; Mustafa Doğan: review; Erkan Can: supervision, writing, review, editing.

Ethical approval

The authors declare that this study complies with research and publication ethics. Research and procedures with tilapia were conducted in accordance with the recommendations of the Committee on the Ethics of Animal Experiments regarding the protection of animals used by scientists from KSAEU (Ukraine). The conditions for keeping aquatic organisms were completely favourable, maintaining the density of fish stocking and the conditions of the hydrochemical regime. All work was carried out in strict compliance with the provisions of the framework of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986); International Principles of the Declaration of Principles of Tolerance (28th session of UNESCO, 1995). All research involving animals must have been reviewed and approved

by an ethics committee prior to commencing the study and performed in accordance with relevant institutional and national guidelines and regulations (Excerpt from Protocol No. 4 of 2024 meeting of the commission on ethics and academic integrity of the Kherson State Agrarian and Economic University) (ASAB Ethical Committee/ABS Animal Care Committee 2023).

Consent form

Not available.

Disclosure statement

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ORCID

Olena Honcharova  <http://orcid.org/0000-0002-9702-7458>
 Sabine Boucetta  <http://orcid.org/0000-0003-2562-5499>
 Mustafa Doğan  <http://orcid.org/0000-0002-1882-6930>
 Erkan Can  <http://orcid.org/0000-0001-9440-7319>

Data availability statement

There were no data used in the present study.

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