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THE ADDITION OF PUMPKIN SEED OIL TO COMMON CARP DIETS AFFECTS SEVERAL PHYSIOLOGICAL AND PRODUCTIVE CHARACTERISTICS

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ABSTRACT: This study aimed to evaluate the effect of adding different levels of pumpkin seed oil (PSO) (*Cucurbita pepo* L.) on specific growth and immune blood parameters in common carp (*Cyprinus carpio* L.). Six oil concentrations were tested, representing six treatments: PSO at 0% (control), 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%. All treatments were incorporated into a diet containing 29.14% protein. A total of 147 fish were divided into 21 cages, with three replicates per treatment, averaging 32±0.72 g in weight. The fish were fed at 3% of biomass per day, split into two meals; rations were adjusted biweekly according to measured biomass for 82 days. The 2% PSO treatment showed significantly better results (P<0.05) across all growth parameters studied, including Final Weight (FW), Weight Gain (WG), Daily Growth Rate (DGR), Relative Growth Rate (RGR), Specific Growth Rate (SGR), Food Intake (FI), Metabolic Growth Rate (MGR), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE), and Protein Efficiency Ratio (PER). Followed by the PSO 1.5% treatment. In blood parameters, PSO 2% also performed best in all measured aspects—Red Blood Cells (RBCs), Hemoglobin (Hp), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC)—with 1.5% PSO closely behind. In immunological parameters, 2% PSO increased the value of WBCs, TPS, and IgM, with 1.5% PSO just behind. Blood performance, which assesses overall blood health as a single measure, showed the highest performance in the 2% PSO treatment, significantly surpassing other treatments (P<0.05). Overall, the results indicated that adding different levels of PSO to carp diets has positive effects at a rate not exceeding 2%.

Keywords: Common carp, Cucurbita pepo, Fatty acids, Fish nutrition, Pumpkin.

INTRODUCTION

Dietary vegetable oils have various effects on the physiological characteristics of fish (Sutili et al., 2018). These oils are sources of metabolic energy (ATP) and are the most energy-dense nutrients (FAO, 2018). In addition, they provide essential organic acids (Ng and Koh, 2011). The addition of oils must be within proper proportions and tailored to the needs of the fish. Previous studies confirmed that using pumpkin seed oil (PSO) can act as a natural antibiotic, antioxidant, and indicator of normal fish growth, while also improved key feed properties such as taste, digestibility, and binding ability (Mahendra et al., 2019). Adequate supplementation with oils rich in polyunsaturated fatty acids has boosted the immune response of fish (Mesa-Rodriguez et al., 2017). A deficiency of these polyunsaturated fatty acids may lead to health issues in fish, such as altered intestinal morphology (Torrecillas et al., 2017), reduced bacterial resistance (Montero et al., 2010; Ferreira et al., 2015), and lowered immune parameters (Montero et al., 2003; Conde-Sieira, 2018). Increasing the ω3 PUFA content in the lipid profile of farmed fish is therefore crucial. These modifications also have significant effects on the quality of fish products, which may influence public perception of farmed fish (Sealey and Gatlin, 1999; Hixson, 2014, Mesa-Rodriguez et al., 2017).

Generally, pumpkin plants are propagated by seed and distributed worldwide. Their seeds are considered more durable and less prone to spoilage. These characteristics are reflected in the quality of the oil extracted from their seeds (Yadav et al., 2010). PSO contains the essential fatty acids linoleic acid and oleic acid. Additionally, the predominant fatty acids are stearic acid and palmitic acid, which make up about 98% of the total amount of fatty acids in pumpkin oil (Murkovic et al., 1996; Ardabili et al., 2011). The oil is extracted by cold pressing roasted or unroasted pumpkin seeds (Paris, 1989), with sensory differences in taste, color, and aroma, between the two oil types (Kim et al., 2012). Many previous studies analyzing the components of PSOs extracted from roasted seeds have shown that these oils are rich in monounsaturated and polyunsaturated fatty acids, minerals, vitamins, pigments, phytosterols, phenolic compounds, and pyrazine derivatives (Aktaş et al., 2018). Recently, much attention has been given to the essential fatty acid requirements of fish. It has also been proven that they need unsaturated fatty acids for normal growth. Fish fed diets lacking certain acids tend to have slower growth and show signs of essential fatty acid deficiency, indicating changes in cell membrane

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structure (Mesa-Rodriguez et al., 2017). Therefore, essential fatty acids must be included in their diet. Based on the background discussed above, the present study aims to examine the effect of adding different levels of PSO, which contains various types of fatty acids, on certain productive and immune traits. It also seeks to identify the optimal inclusion level in the feed and its effects on common carp *Cyprinus carpio* L.

MATERIALS AND METHODS

Experiment location and fish

The study was conducted at the First Agricultural Research and Experiments Station, affiliated with the College of Agriculture at Al-Muthanna University, located north of Samawah city, near the end of the Al-Atshan River, one of the branches of the Euphrates River, approximately 4 km before it joins the Al-Sabil branch. Water from this river was used as a water source for the experiment. Common carp fingerlings were obtained from a local hatchery; 147 fingerlings with an average weight of 32±0.72 g was transported in a specialized fish transport vehicle. The fish were placed in a 3% saline solution under observation and then placed in a specially prepared tank. After resting for 24 hours, they were distributed into the experimental cages at a rate of seven fish per cage as experimental units. The fish used in the experiment were acclimated for two weeks to the experimental conditions.

Experimental diets

During the experiment, the fish received a diet made up of several ingredients listed in (Table 1). The diet was given twice daily, in the morning and evening, at 3% of biomass per day, split into two meals; rations were adjusted biweekly according to the measured biomass. The ingredients were sourced locally, mixed thoroughly, and then pelletized using a heat press (Table 1). Pumpkin seed oil (PSO) was added at six levels: 0.5%, 1%, 1.5%, 2.0%, 2.5%, and 3.0%, along with a control group. Each treatment was replicated three times. The experiment used 21 cylindrical plastic cages (three cages for each treatment), each measuring 50 cm in diameter and 65 cm deep.

Table 1 - Ingredients of the experimental diet.

Ingredients	Percentage in the diet (%)
Soybean meal	40
AL-Wafi protein concentration*	20
Wheat bran	15
Corn	15
Barley	5
Wheat flour	3
Premix	1
Oil	1
Total	100
Chemical composition of diet	
Component	**(%) Result**
Moisture	5.03
Dry matter	94.97
Crude protein	29.14
Crude fat	1.74
Crude fiber	4.48
Nitrogen-free extract (NEF)	51.1
Ash	8.51
Gross energy (Kcal g^{-1}) **	396.726
Digestible energy (Kcal g^{-1}) ***	297.5445
Metabolizable energy (Kcal g^{-1}) ****	323.7654
Protein: Calorie ratio ****	979.34

*locally manufactured protein **Gross energy (Kcal g^{-1}) was calculated according to NRC (1993) by using factors 5.65, 9.45, and 4.22 Kcal g^{-1} of protein, lipid, and carbohydrate, respectively. *** Digestible energy (Kcal g^{-1}) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to NRC (1993). **** Metabolizable energy (Kcal g^{-1}) was calculated using a value of 4.5 Kcal g^{-1} proteins, 8.51 Kcal g^{-1} fat, and 3.48 Kcal g^{-1} carbohydrates according to Hepher et al. (1983). **** Protein: Calorie ratio it was calculated using by below equation: P: E = crude protein x 10000/digestible energy, according to Jauncey and Ross (1982).

Studied parameters

Weight gain (WG) = FW-IW (g); Daily growth rate (DGR)= "FW-IW"/("ΔT)" ; Relative Growth Rate (RGR) = $\frac{FW\text{-}IW}{IW}$; Specific growth Rate (SGR) = (Ln(Fw)-Ln(IW))/ΔT; Metabolic growth Rate (MGR) = ((WG/((⟦WI/1000)^0.8+(WF/1000)^0.8))/2)/ΔT (Dabrowski et al., 1986); Feed Conversion Ratio (FCR) = (Food intake (g))/WG; Feed conversion efficiency (FCE) = WG/(food intake (g))×1000; Protein Efficiency Ratio (PER) = WG/(protien intake); Blood Performance (BP) = Ln (Hb)+Ln (Ht)+Ln (RBC+Ln (WBC)+Ln (TP) (Esmaeili, 2021). Where FW is the final body weight, IW is the initial body weight, ΔT is the period time, Hb is the hemoglobin, RBC is the red blood cells, WBC is the white blood cells, and TP is the total protein.

Chemical analysis

The PSO sample was analyzed in the Iraqi Ministry of Science and Technology laboratories. The chemical analysis results showed that the oil contains the fatty acids shown in Table 2.

Table 2 - Fatty acid content of local pumpkin seed oil (PSO) (mean ± standard error)

Fatty acid	Content (%)
Gadoleic acid	1.04 ± 0.21
Linolenic acid	0.88 ± 0.24
Palmitoleic acid	1.38 ± 0.11
Linoleic acid	38.74 ± 0.52
Oleic acid	37.33 ± 0.41
Stearic acid	7.95 ± 0.57
Palmitic acid	12.68 ± 0.32

Statistical analysis

The data were presented as mean ± SD. The results were subjected to a one-way analysis of variance (ANOVA) to determine the impact of PSO inclusion on fish performance. IBM SPSS Statistics version 26 (IBM Corp.) was used to analyze the data. We looked at differences among means at the significance level ($P < 0.05$) using the least significant difference (LSD) multiple range test.

RESULTS

Environmental water measurements

The temperature ranged from 16.5°C at the start of the experiment to 30°C at the end. The pH was between 7 and 8.1. The water's salinity level varied from 4.981 to 6.730 g L^{-1}, due to water scarcity and the suspension of water releases into the river as a result of Iraq's ongoing water shortage. The amount of dissolved oxygen (DO) in the water ranged from 7.8 mg L^{-1} at the beginning to 7.2 mg L^{-1} at the end of the experiment.

Growth criteria

The current results in Table 3 show the most important growth parameters studied, which are Final Weight (FW), Weight Gain (WG), Daily Growth Rate (DGR), Relative Growth Rate (RGR), Specific Growth Rate (SGR), Food Intake (FI), Metabolic Growth Rate (MGR), Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE), and Protein Efficiency Ratio (PER). The results revealed significant differences among all treatments, with treatment T4 performing the best in all parameters, followed by treatment T3, which did not differ significantly ($P \geq 0.05$) from T4 in feed intake (FI). Then, treatments T2 and T5, respectively, showed no significant difference from each other in most of the parameters measured. Meanwhile, treatment T1 had the lowest values ($P \geq 0.05$) and did not differ significantly from the control group. All treatments significantly outperformed the control ($P < 0.05$), which showed the lowest values across all parameters.

Hematological parameters

The current results, as shown in Table 4, indicated that T4 was significantly different ($P < 0.05$) in all hematological parameters studied, namely: MCV, PCV, RBCs, Hb, MCH, and MCHC, followed in order by T3. T2 and T5 did not differ significantly from one another in any measured parameter. Likewise, treatments T1 and T6 did not differ significantly from each other ($P > 0.05$). Although all treatments showed significant variations, the control group exhibited the lowest values among all treatments.

Immunological parameters

Treatment 4 demonstrated a significant effect ($P < 0.05$) on all immune parameters, as shown in Table 5, where IgM, STP, and WBC were significantly affected by T3 across all studied parameters. Treatment 2 and T5 did not differ significantly from each other. Treatment 1 and T6 did not differ significantly ($P > 0.05$) in STP, but they showed significant differences in WBC and IgM.

Blood performance

As shown in Table 5, there were significant differences in blood performance among the treatments. The best blood performance was observed in T4 (13.19 ± 0.08), followed ($P < 0.05$) by T3, which showed no significant difference ($P > 0.05$) from T4. The remaining treatments varied considerably from each other with respect to this parameter.

Table 3 - Some of the studied growth parameters (mean ± standard error) for common carp fish fed on diets containing different levels of PSO during the experimental period.

Treatment	IW (g)	FW (g)	WG (g)	DGR (g day^{-1})	RGR (%)	SGR (% day^{-1})	FI (g Fish^{-1})	MGR (g Kg^{-1} day^{-1})	FCR	FCE	PER (%)
Control	32.87±0.08	138.46±0.33^{e}	105.59±0.30^{e}	1.27±0.00^{e}	334.44±1.08^{f}	1.75±0.00^{f}	248.21±0.18^{f}	9.43±0.01^{f}	2.33±0.01^{a}	42.94±0.09^{e}	1.48±0.00^{e}
T1	32.00±0.09	173.25±1.82^{d}	141.26±1.74^{d}	1.68±0.02^{d}	441.46±4.19^{d}	2.01±0.01^{d}	301.12±0.46^{d}	10.86±0.06^{d}	2.13±0.03^{b}	46.91±0.60^{d}	1.61±0.02^{d}
T2	32.00±0.06	185.61±0.31^{c}	153.61±0.30^{c}	1.83±0.00^{c}	480.03±1.22^{c}	2.09±0.00^{c}	321.53±0.55^{b}	11.30±0.01^{c}	2.09±0.01^{c}	47.78±0.14^{c}	1.64±0.01^{c}
T3	32.12±0.08	194.23±0.57^{b}	162.11±0.49^{b}	1.93±0.01^{b}	504.68±0.82^{b}	2.14±0.00^{b}	330.40±0.57^{a}	11.58±0.01^{b}	2.04±0.00^{d}	49.06±0.13^{b}	1.68±0.00^{b}
T4	32.28±0.16	214.81±0.54^{a}	182.53±0.59^{a}	2.17±0.01^{a}	565.60±3.95^{a}	2.26±0.01^{a}	331.53±0.42^{a}	12.20±0.03^{a}	1.81±0.01^{e}	55.06±0.23^{a}	1.89±0.01^{a}
T5	32.11±0.08	185.08±0.81^{c}	152.98±0.75^{c}	1.82±0.01^{c}	476.48±1.72^{c}	2.09±0.00^{c}	315.23±0.90^{c}	11.27±0.02^{c}	2.06±0.01^{cd}	48.53±0.14^{bc}	1.67±0.00^{bc}
T6	32.36±0.11	170.89±1.50^{d}	138.53±1.54^{d}	1.65±0.02^{d}	428.09±5.50^{e}	1.98±0.02^{e}	287.90±2.96^{e}	10.72±0.06^{e}	2.08±0.00^{c}	48.12±0.08^{c}	1.65±0.00^{c}

Data with different superscripts are significantly different ($P < 0.05$). IW: Initial weight; FW: Final weight (g); WG: Weight gain (g); DGR: Daily Growth Rate (g day-1); RGR: Relative Growth Rate (%); SGR: Specific Growth Rate (% day-1); FI: Food Intake (g Fish-1); MGR: Metabolic Growth Rate (g Kg-1 day-1); FCR: Feed Conversion Ratio; FCE: Feed Conversion Efficiency; PER: Protein Efficiency Ratio (%).

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Table 4 - Some heamatological parameters (mean ± standard error) for common carp fish fed on diets containing different levels of PSO during the experimental period.

Treatment	Concentration	RBC (106 mm$^{3-1}$)	Hb (%)	PCV (%)	MCV (μm^{3})	MCH (pg)^{3}	MCHC (g dl^{-1})^{3}
Control	PO 0.0 %	1.05±0.04^{e}	7.09±0.06^{f}	20.56±0.34^{e}	164.34±1.94^{e}	81.70±0.40^{e}	33.72±0.40^{e}
T1	PO 0.5 %	1.23±0.01^{d}	9.43±0.06^{e}	23.00±0.70^{d}	176.17±3.68^{d}	87.05±1.35^{d}	35.73±0.41^{cd}
T2	PO 1.0 %	1.35±0.01^{c}	10.20±0.07^{cd}	26.46±1.15^{bc}	193.92±1.80^{c}	91.200.10^{c}	37.21±0.04^{c}
T3	PO 1.5 %	1.580.01^{b}	11.85±0.09^{b}	28.18±0.04^{b}	215.55±1.65^{b}	94.80±0.60^{b}	40.48±0.27^{b}
T4	PO 2.0 %	1.77±0.05^{a}	12.97±0.06^{a}	30.82±0.37^{a}	250.81±2.71^{a}	99.30±0.60^{a}	44.34±0.43^{a}
T5	PO 2.5 %	1.44±0.06^{c}	10.63±0.32^{c}	24.96±0.75^{cd}	199.20±0.95^{c}	90.25±0.35^{c}	37.04±0.18^{c}
T6	PO 3.0 %	1.22±0.01^{d}	9.72±0.18d^{e}	22.82±0.70^{d}	182.60±1.00^{d}	85.10±0.20^{d}	36.49±2.04^{cd}

Data with different superscripts are significantly different ($P < 0.05$). RBC: Red blood cell; Hb: Hemoglobin (%); PCV: Packed Cell Volume (%); MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration.

Table 5 - Some immunological parameters (mean ± standard error) for common carp fish fed on diets containing different levels of PSO during the experimental period.

Treatment	Concentration	WBC (10^{3} mm$^{3-1}$)	STP	BP	Ig M (μg ml^{-1})^{5}
Control	PO 0.0 %	189.71±3.32^{a}	2.00±0.07^{d}	10.97±0.03^{e}	0.0023±0.00005^{f}
T1	PO 0.5 %	200.75±0.53^{d}	2.37±0.05^{c}	11.75±0.03^{d}	0.0032±0.00004^{e}
T2	PO 1.0 %	206.72±0.44^{cd}	2.60±0.01^{bc}	12.44±0.09^{b}	0.0047±0.00004^{c}
T3	PO 1.5 %	215.39±1.77^{b}	2.71±0.04^{b}	12.63±0.02^{b}	0.0051±0.00007^{b}
T4	PO 2.0 %	225.89±3.18^{a}	3.36±0.15^{a}	13.19±0.08^{a}	0.0064±0.00007^{a}
T5	PO 2.5 %	209.85±1.41^{bc}	2.48±0.07^{bc}	12.19±0.04^{c}	0.0051±0.00010^{b}
T6	PO 3.0 %	209.00±1.87^{bc}	2.40±0.06^{c}	11.81±0.08^{d}	0.0040±0.00016^{d}

Data with different superscripts are significantly different ($P < 0.05$). WBC: White Blood Cell; STP: Scientist Training Programme; BP: Blood Pressure; Ig M: Immunoglobulin M

DISCUSSION

Growth standards

Previous studies that examined various types of fatty acids have shown that it is better to combine these acids rather than use a single type. This is because they work together to reduce or inhibit the growth of pathogenic bacteria and lower the pH, especially in the front part of the intestine (Ng and Koh, 2017; Huang et al., 2022). Additionally, the synergistic effect of different fatty acids among each other may enhance their effectiveness and benefits for animals (Abdel-Tawwab et al., 2019; Pearlin et al., 2020). The present study was based on this concept. The results indicated that the group of fatty acids in PSO affected all the growth characteristics studied. In WG and FW, all treatments showed significant differences from the control, and treatment T4 achieved the greatest differences compared to the control and the other treatments. Treatment T3 also produced results similar to T4. This suggests that fatty acids like linoleic, oleic, stearic, and palmitic significantly influenced all the growth traits studied, particularly FW, WG, DGR, RGR, and SGR. Overall, dietary lipid serves as a more important energy source than carbohydrate for feeding carnivorous fish and has a sparing function for dietary protein (Lee et al., 2002). On the other hand, growth performance may have improved because PSO, containing these acids, reduced Gram-negative bacteria populations or because of a suitable concentration that favored the growth of Gram-positive bacteria, in addition to maintaining the optimal pH for digestive enzyme activity. Combining organic acids creates a diverse activity within the intestine, counteracting many types of bacteria, especially pathogenic ones. This allows beneficial bacteria to proliferate more freely by reducing competition within the lower intestine (Banerjee and Ray, 2017). In this study, it was observed that higher oil concentrations led to a decrease in growth performance. This aligns with our previous research on the synergistic effects of butyric and propionic acids in common carp diets, where combined supplementation yielded greater benefits than individual additions (Alhamadany et al., 2023). Additionally, there was a notable increase in the metabolic growth factor across all parameters. This could be because fatty acids lower the pH in the front part of the intestine, providing a larger area for enzyme activity (Lückstädt, 2008), or because these oils are absorbed directly without requiring digestion, as demonstrated in a study on Atlantic salmon fed graded triglyceride levels, which showed decreased carbohydrate digestion and increased fat absorption (Nordrum et al., 2003). The reduced growth performance observed in treatments T5 and T6 might be due to excessively high concentrations that reduced palatability due to the oil's strong taste. Excessive fatty acids can decrease palatability, reducing food intake due to the unpleasant smell or taste (Xie et al., 2003).

From Table 3, it is observed that a high concentration of PSO slightly increased the protein efficiency ratio. This confirms the findings of Zhang et al. (2021), who used different levels of complex acidifying substances and an extensive range of acidifiers to study the growth performance and intestinal health of young fish. In the American eel, Anguilla rostrata, these compounds improved the PER values when low levels of acidifying materials were used. However, at

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higher concentrations, the measured parameters, including the protein efficiency ratio, began to decline. This indicates that using high levels of acidifying agents may negatively affect some performance indices.

Blood standards

Many studies have documented an increase in the number of RBCs when feeding fish with some functional additives, including studies by Zarei et al. (2021), Raissy et al. (2022), and Mohamed et al. (2022). These studies confirmed that acids and functional additives boost the number of RBCs, hemoglobin, and other blood parameters. The present results align with this, as MCV, PCV, RBCs, Hb, MCH, and MCHC increased across all treatments, showing a significant difference ($P<0.05$) compared to the control. This can be explained by hematopoietic activity that increases blood volume in fish, which may result from the synergy of various nutritional factors. These include vitamins such as B12, B6, K, and D; fatty acids and other nutrients; and minerals like iron, zinc, and copper (Choi and Kim, 2005). The role of acids in enhancing blood activity is clear, as fatty acids can improve nutrient digestion and stimulate digestive enzyme activity, leading to increased growth as a natural consequence of heightened metabolism. Consequently, fish require larger blood volumes to support greater metabolic demand. Increased metabolic activity and blood volume can thus contribute to increases in body mass. A study by Hongyan et al. (2023), which investigated the relationship between body mass and blood indicators in young yellowfin tuna *Thunnus albacares* concluded that larger body mass is generally linked to higher metabolism and correlates with increased blood volume. This study confirms that the rise in metabolic rate was accompanied by higher levels of PSO, which in turn increased the fatty acids present in the diet.

Immunological standards

The synergistic mixture of acids found in PSO, resulting from various types of fatty acids, appears to have a definite effect on the values of immune parameters IgM, STP, and WBCs. Heshmatfar et al. (2023) demonstrated that the synergistic effect of fatty acids can lead to improved results, as they observed an increase in total protein and immunoglobulin during their experiment investigating the effects of combined or single administration of formic acid and *Pediococcus acidilactici* on stress resistance, growth performance, immune responses, and gene expression in fish. This was also supported by Hamed et al. (2023), who studied the effect of certain fatty acids on enzymatic parameters and immune responses in common carp, finding noticeable improvements in immune parameters. Immunoglobulin (IgM) is also considered the first line of defense for fish against diseases, helping to identify and weaken pathogenic bacteria and viruses (Giri et al., 2012). The results in Table 5 demonstrated the effectiveness and superiority of the T4 treatment on blood globulin levels (Zarei et al., 2021). Their study also confirmed that adding certain fatty acids improved the performance of fingerlings in yellowfin sea bream *(Acanthopagrus latus*). The total immunoglobulin content and lysozyme activity in skin mucus increased as the amount of fatty acid supplemented increased. It was also observed in our study that fish had a high mucus content on their skin, which serves as the body's first defense mechanism against many pathogens, such as fungi and bacteria that infect fish (Hussain and Ghosh, 2023).

Blood performance

The mathematical formula for the blood performance standard is based on the idea that any component of the blood - Hb, RBCs, WBCs, or total serum protein - cannot always serve as a reliable biomarker for fish growth or health. For this reason, blood performance may be a better choice because it considers all these variables within one composite formula. The blood performance parameter is reliable and suitable for comparing parameters within each experiment, but it is not ideal for comparing results across separate experiments. A high value of this parameter indicates better growth or overall fish health. The values of this criterion ranged between 10.68 and 18.24, based on analyzing 441 samples, with an average of 14.43 as reported in the study by Esmaeili (2021). It was also confirmed that blood serum proteins and WBCs have the greatest impact on blood performance, while hemoglobin has a lesser effect. Additionally, these five parameters account for 95% of the influence on blood performance. Generally, when we observe differences in these five parameters across treatments, this formula becomes more meaningful and reliable, as it integrates all the components that collectively explain the fish's physiological response to dietary treatments. The natural logarithm (Ln) is added to the formula to reduce the variance among variables. The present results were within the range reported by Esmaeili (2021), corresponding to the normal blood performance standard. This can be considered an indicator of fish health and is presented in Table 5.

The blood performance standard can be viewed in two ways

First, consider the relationship between blood performance and growth. After absorbing energy from feed, this energy contributes to three main physiological components: growth, respiration, and energy loss through feces and nitrogenous excretory products (Jobling, 1994). In other words, increases in growth parameters are associated with higher blood performance values. Although glucose serves as an essential energy source, it appears to be less important for fish metabolism. The physiological underpinnings of fish's apparent glucose intolerance have not yet been thoroughly investigated. The relative inability of fish to effectively utilize dietary glucose may be explained by their distinct regulation of hepatic glucose production (gluconeogenesis)and glucose utilization (glycolysis) (Enes et al., 2009). It is also known that fatty acids contain a large amount of energy. For example, wheat provides five times less energy than propionic acid (Freitag, 2007; You et al., 2023). The high energy content in PSO contributed to the increase in RBC count, based on the findings. Accordingly, the observed rise in RBC count corresponded with an overall increase in blood performance.

Second, the relationship between blood performance and the immune system: The results indicate an increase in blood performance with higher levels of WBC, IgM, and STP. This was confirmed by Esmaeili (2021), who showed a close connection between the blood performance standard and the studied immunological markers, particularly WBCs, total blood proteins, and globulin. These findings align with those of Zarei et al. (2021) and a study by Hassaan et al. (2018). All these studies confirm that fatty acids and functional additives boost the number of RBCs, hemoglobin, and other blood characteristics, as well as enhance immune parameters.

CONCLUSION

Adding oils to diets significantly boosts the growth and overall health of common carp, especially oils rich in diverse fatty acids. Combining these acids provides the most effective approach for maximizing physiological and immune performance. However, aquaculture professionals are advised to use these PSOs at a rate not exceeding 2% to achieve optimal growth.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

A.S.A-A. Alhamadany and H.A. Fazaa contributed in formal analysis, methodology; investigation, writing, preparation of original draft, writing, review & editing. K.Z. Negaud performed conceptualization, formal analysis, acquisition of funding, research, and methodology; and R. M. Sayed-Lafi performed writing, review & editing.

Ethical regulations

All parts of this research were conducted in accordance with the regulations and instructions of the Research Ethics Committee at the College of Agriculture, Al-Muthanna University, and in accordance with the Iraqi Animal Health Law No. 23 of 3013. All necessary measures were taken to minimize pain and stress to the experimental fish and to minimize the measurements made for this research.

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Competing interests

The authors declare no competing interests in this research and publication.

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