Original Article

Hepatic Health and Humoral Immunological Parameters of Common Carp *(Cyprinus carpio)* Fed Lactic Acid-supplemented Diets

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ABSTRACT

Background: Organic acids and their salts are known as appropriate substitutes in feed for improving the health, growth, and performance of fish.

Objectives: The present study was conducted to determine the effects of dietary lactic acid supplementation on immunological factors, hepatic enzyme activity, and plasma proteins in common carp, *Cyprinus carpio*. The fish were fed the diets mentioned above for 56 days, then their growth performance, humoral immunity, and plasma hepatic enzymes were assessed.

Methods: A total of 180 fish (mean weight=25 g) were randomly distributed in twelve tanks (150 L water in each tank) as four treatments, fed diets containing 0, 2.5, 5, and 10 g/kg lactic acid (T0, T1, T2, and T3, respectively).

Results: At the end of the feeding trial, T2 showed significantly higher growth performance than T0. Plasma total protein, albumin, and globulin levels of T1-T3 were significantly higher than that of T0. However, plasma protein levels decreased significantly by elevation in dietary lactic acid concentration (10 g/kg). No significant differences were observed in plasma aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activity among the treatments, although alanine aminotransferase (ALT) activity decreased significantly in fish-fed dietary lactic acid supplementation (T1-T3) compared with the control group (T0). All humoral immunity parameters (lysozyme, complement, immunoglobulin, and bactericidal activity) increased significantly in T1-T3 treatments compared to the T0 group.

Conclusion: Overall, dietary lactic acid supplementation improves growth performance, humoral immunological parameters, and hepatic health. According to the results, dietary lactic acid (2.5-5 g/kg) is recommended for preparing common carp feed.

Keywords: Food additives, Common carp, Hepatic health, Immunity status, Lactic acid

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1. Introduction

Because of the world population growth and the need for more protein sources, fish farming is very important in the food industry. Common carp, *Cyprinus carpio*, is cultured in broad places worldwide due to rapid growth, high feed efficiency, and easiness of breeding (FAO, 2019). This species is an important farming species in Iran and is included in about 20% of fish in warm freshwater ponds (Iranian fisheries statistical report, 2020).

Improving performance and health is an important goal of aquaculture, and nutrition management, which directly and indirectly affects the economic and health administration of aquaculture production (Lee et al., 2015). Using economic and growth-promoting feedstuffs in fish diets may greatly reduce the production cost of fish farms. One of the most important effects of nutritional management in fish farms is boosting fish health, immunity, and disease resistance (Mohiseni, 2017). As a major aquaculture species, common carp have shown boosted immune and antioxidant power when fed diets supplemented with various feed additives (Alishahi et al., 2014; Abdulrahman et al., 2018).

Organic acids and their salts, known as "acidifiers", have been considered appropriate substitutes in food for improving the health, growth, and performance of livestock, including fish (Lim et al., 2015). It was reported that dietary acidifier supplementation positively influenced animals' performance by altering the feed quality, metabolism, and gastrointestinal tract health of animals (Huan et al., 2018). Acidifiers can increase the quality of feed in farms by inhibiting microbial growth via the reduction in the feed pH (Lim et al., 2015). Also, they positively affect nutrient digestion by improving digestive enzymes' activity in the stomach and intestine. This is especially noticeable when the animals are young, or feed is high in protein (Castillo et al., 2014). Organic acids have high gross energy and are easily absorbed by the intestinal epithelial cells due to their short-chain structure; therefore, they can contribute to different metabolic pathways for energy production (Luckstadt, 2008). Since the energy content of acidifiers is completely consumed in metabolism, they can increase the energy content of feeds. Collectively, all these properties of acidifiers improve the immune function of fish.

Lactic acid is one of the organic acids on which few studies have been conducted in aquatic animals (Hoseini et al., 2022). Improved growth performance and nutrient digestibility have been reported in beluga, Huso huso, fed with 10-20 g/kg lactic acid (Matani Bour et al., 2018). Hoseini et al. (2022) reported positive effects of lactic acid on immune and antioxidant responses in rainbow trout, Oncorhynchus mykiss. Safari et al. (2021) found an increase in growth performance and immune parameters of narrow-clawed crayfish Astacus leptodactylus, a fed diet supplemented by sodium lactate. As there is no study about the effects of lactic acid on the health and immune strength of common carp, the present study evaluated the impact of dietary lactic acid supplementation on hepatic health, plasma proteins, and innate immunity of common carp.

2. Materials and Methods

Diets preparation

To prepare the examined diets, the feed ingredients were mixed, as shown in Table 1. Then, 400 g of water was added per kg of mixed feedstuffs to form a paste. The paste was pelleted by a meat grinder with a 3 mm diameter and dried for 24 h. Lactic acid was added to the feedstuff mixture in exchange for the water. Finally, based on the amount of lactic acid added, four diets were prepared to contain 0 (T1), 2.5 (T2), 5 (T3), and 10 (T4) g/kg lactic acid (Hoseini et al., 2022). Diets were analyzed according to Hoseinifar et al. (2016).

Fish maintenance condition

A total of 220 juvenile common carp with an average weight of 25 g were obtained from a local farm and stocked in a fiberglass tank with a water volume of 1500 L for 10 days for acclimation. They were fed the control diet during the acclimation. Continuous aeration and water flow of 2 L/min were provided for the fish. After the acclimation, 12 tanks containing 150 L water were divided into 4 treatments considering the diets mentioned above (3 tanks per diet). A total of 180 fish with no external injuries were distributed in these tanks (15 homogeny-size fish per tank). The tanks were aerated and had a water flow rate of 0.3 L/min. The fish were fed 3% of biomass (three times a day) with experimental diets for 56 days. Then, growth performance was recorded in the treatments as follows (Alishahi & Jangeran Nejad, 2012) (Equation 1-4):

1. *Final biomass (g)=number of remaining fish per tank×individual weight of the fish,*

2. *Biomass gain (g)=final biomass–initial biomass,*

3. *Feed conversion ratio (FCR)=feed intake per tank during the experiment÷biomass gain,*

4. *Mean daily growth (g)=final biomass÷days of rearing*

Table 1. Ingredients and chemical composition of the diets

Diets	Treatments			
	T0	T1	T2	T3
Corn meal	100	100	100	100
Wheat meal	280	277.5	275	270
Soybean meal	200	200	200	200
Poultry byproduct[1]	380	380	380	380
Corn oil	10	10	10	10
Sunflower oil	10	10	10	10
Mineral premix[2]	10	10	10	10
Vitamin premix[3]	5	5	5	5
Methionine[4]	3	3	3	3
Lysine[5]	2	2	2	2
Lactic acid[6]	0	2.5	5	10

Diets	Proximate composition (g/kg)			
	T0	T1	T2	T3
Moisture	88.6	87.4	87.3	87.9
Crude protein	381	384	386	379
Crude fat	105	108	105	107
Crude ash	53.4	54.0	53.4	55.0
Crude fiber	42.1	42.0	41.0	42.3
Phosphorus	10.1	10.2	10.5	10.0
Calcium	16.3	16.5	16.2	16.5

[1]Crude protein 63%, Crude fat 16%, Crude ash 6%, Crude fiber 4%.

[2]Amineh Gostar Co. (Tehran, Iran). The premix provided the following amounts of the vitamin to the diets (per kg): A: 1600 IU, D3: 500 IU, E: 20 mg, K: 24 mg, B3: 12 mg, B5: 40 mg, B2: 10 mg, B6: 5 mg, B1: 4 mg, H: 0.2 mg, B9: 2 mg, B12: 0.01 mg, C: 60 mg, Inositol: 50 mg.

[3]Amineh Gostar Co. (Tehran, Iran). The premix provided the following amounts of minerals to the diets (per kg): Se: 0.15 mg, Fe: 2.5 mg, Co: 0.04 mg, Mn: 5 mg, Iodate: 0.05 mg, Cu: 0.5 mg, Zn: 6 mg, choline: 150 mg.

[4]CheilJedang Co., Seul, South Korea, [5]CheilJedang Co., Seul, South Korea, [6]Food grade, 85% (Mobtakeran Shimi Co., Tehran, Iran).

Sampling and plasma analysis

At the end of the experiment, two fish were taken from each tank and immediately anesthetized in a bath of 100 ppm eugenol (Hoseini et al., 2022). Then, the fish blood samples were taken from the caudal vein of the anesthetized fish using 2-mL heparinized syringes. The blood samples were centrifuged at 3000 rpm to separate the plasma. The plasma samples were kept at -20˚C until analysis.

Plasma total protein, albumin, globulin, and glucose levels and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities were determined by a spectrophotometer using Pars Azmun kits (Pars Azmun Co., Tehran, Iran).

Measurement of plasma total immunoglobulin (Ig) was done using the polyethylene glycol precipitation method (Siwicki & Anderson, 1993). The lysozyme activity of plasma was measured by a turbidimetric assay based on Ellis (1990) method. Alternative complement activity was determined according to Nayak et al. (2018) protocol and based on the sheep red blood cell hemolysis method.

Statistical analysis

Data normality and variance homogeneity were confirmed by the Shapiro-Wilk and Levene's tests, respectively. All data were analyzed by one-way ANOVA. Comparison among the treatments was performed by Duncan's test. All data were presented as Mean±SD, and $P<0.05$ was considered significant. SPSS software, version 22 was used for the statistical analysis

3. Results

Results showed that dietary supplementation with lactic acid had a beneficial effect on common carp growth performance (Table 2). Diets with T2 (5 g/kg lactic acid) significantly increased final biomass, biomass gain, mean daily growth, and decreased FCR, compared to T0. No mortality was observed during the experiment.

Total protein, albumin, globulin, and glucose in plasma are presented in Figure 1. Dietary lactic acid supplementation significantly affected plasma protein levels. Plasma protein levels of lactic acid treatments (T1, T2, and T3) were significantly higher than the control group (T0). However, plasma protein levels of lactic acid treatments had decreasing trend by increasing lactic acid concentration. T3 had the lowest plasma protein levels among lactic acid treatments. Dietary lactic acid supplementation did not affect plasma glucose levels.

Plasma hepatic enzyme activity is presented in Figure 2. Dietary lactic acid significantly affected ALT activity with no significant effects on ALP and AST activity. All concentrations of lactic acid supplement (T1, T2, and T3) significantly decreased the activity of the ALT enzyme compared with the control group (T0). The plasma ALT activity of T3 was significantly higher than that in the T1 and T2.

Humoral immunological parameters are represented in Figure 3. According to the results, all concentrations of dietary lactic acid significantly increased lysozyme, alternative complement, bactericidal activities, and total Ig level in the plasma. The alternative complement activity of T3 had no significant difference compared to T0.

Table 2. Growth performance of common carp fed with diets containing different levels of lactic acid

Variables	Mean±SD				P
	T0	T1	T2	T3	
Initial biomass (g)	387±2.30	387±3.06	385±1.15	387±2.31	0.760
Final biomass (g)	687±17.0^{a}	708±18.0^{ab}	746±25.0^{b}	690±21.5^{a}	0.029
Biomass gain (g)	300±15.9^{a}	321±21.0^{ab}	360±26.1^{b}	303±20.9^{a}	0.029
Feed conversion ratio	1.73±0.06^{b}	1.66±0.05^{b}	1.53±0.09^{a}	1.71±0.06^{b}	0.026
Mean daily growth (g)	5.37±0.28^{a}	5.74±0.37^{ab}	6.44±0.46^{b}	5.41±0.35^{a}	0.029

$^{a, b, ab}$significant differences (Duncan; n=3).

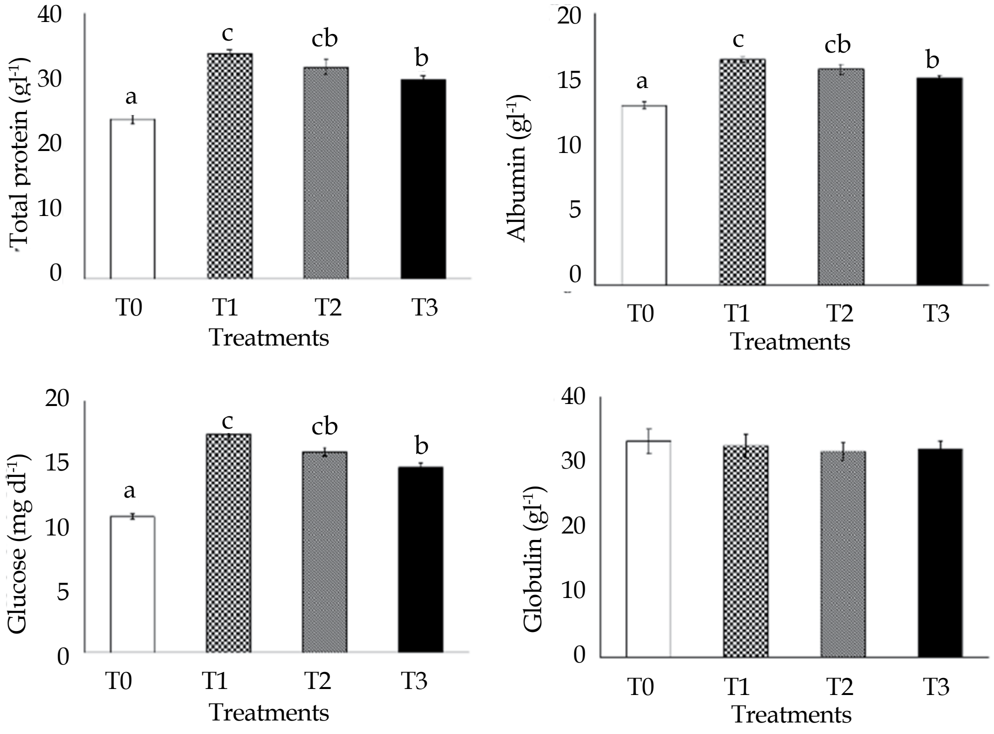

Figure 1. Plasma total protein, albumin, globulin, and glucose levels of common carp fed 0, 2.5, 5, and 10 g/kg lactic acid supplement in per kg diet (T0, T1, T2, and T3)

Different letters above the bars show significant differences (Duncan; n=6).

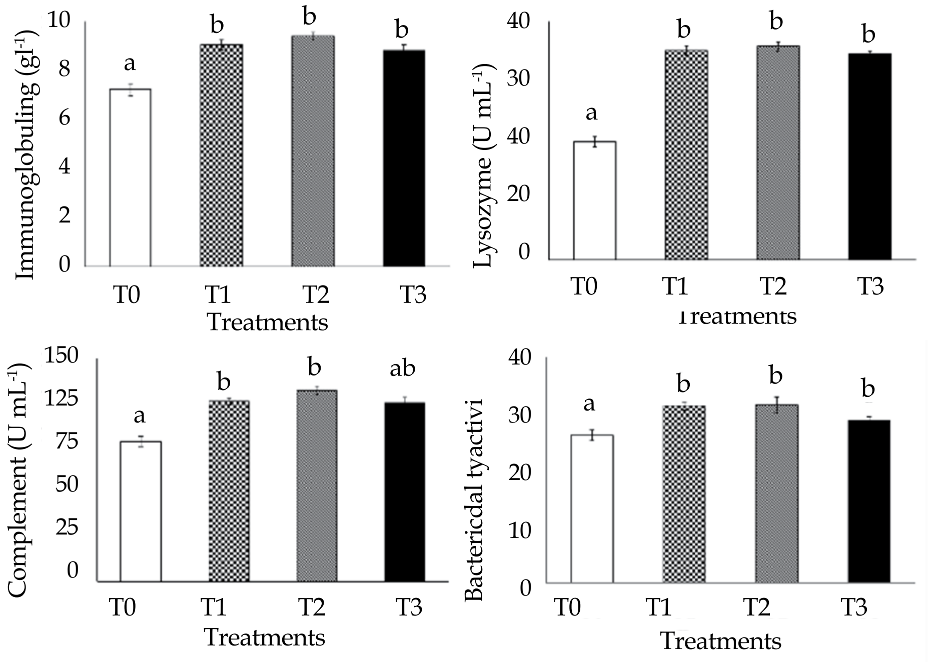

Figure 2. Plasma ALT, AST, and ALP levels of common carp fed 0, 2.5, 5, and 10 g/kg lactic acid supplement in per kg diet (T0, T1, T2, and T3)

Different letters above the bars show significant differences (Duncan; n=6).

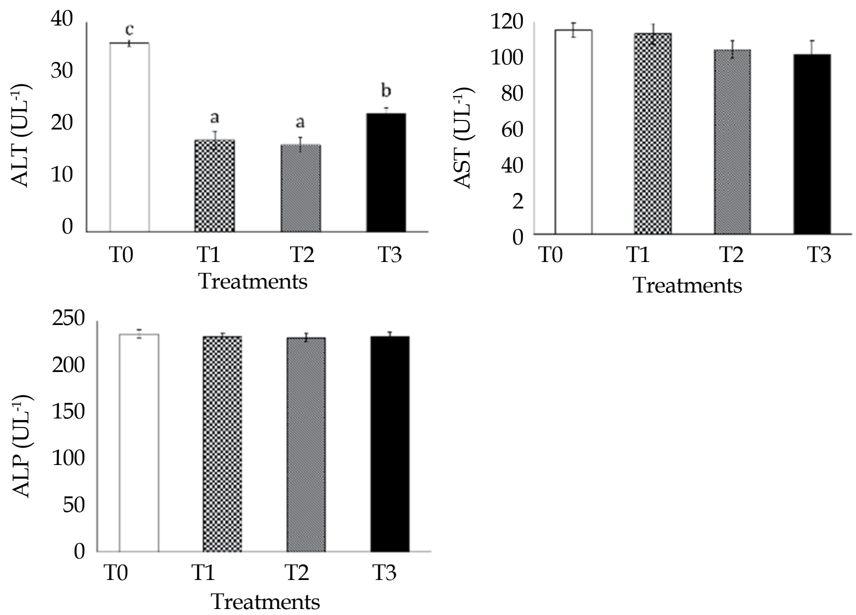

Figure 3. Plasma immunoglobulin, lysozyme, complement, and bactericidal activity of common carp fed 0, 2.5, 5, and 10 g/kg lactic acid supplement in per kg diet (T0, T1, T2, and T3)

Different letters above the bars show significant differences (Duncan; n=6).

4. Discussion

Lactic acid has not yet been investigated as a dietary additive for common carp. The present study showed that dietary lactic acid (5 g/kg) supplementation could improve the growth performance of common carp, which is partially similar to the results obtained in beluga (Matani Bour et al., 2018) and narrow clawed crayfish (Safari et al., 2021), except the effective lactic concentrations.

AST and ALT are important enzymes to control transferring an amino group of α-amino acids to α-ketoacids, and they are the main indicators of liver function and health. These enzymes give information regarding the damage to organs, particularly hepatocytes. Because during hepatocyte damage, large amounts of ALT and AST are released into the circulation (Walling et al., 2009). Lower values of plasma ALT were recorded in the present study for common carp fed 2.5 and 5 g lactic acid per kg diet, compared to the control group, suggesting healthy liver for common carp fed these diets.

Plasma proteins in fish are the main blood constituents that control the osmotic pressure, immune responses and carrying other compounds (Christiansen et al., 2015). In general, determining fish plasma protein is a useful way to recognize the physiological health of fish (Manera & Britti, 2008; Silva et al., 2010). Significant elevation in plasma total protein, albumin, and globulin of fish-fed lactic acid-supplemented diets may result from increased protein synthesis in the liver. Acidifiers improve amino acids' availability through the reduction of the stomach pH (increase in pepsin activity and protein digestion) and the breaking of phytate complexes (that release bonded amino acids) (Baruah et al., 2005). The plasma level of total protein, albumin, and globulin is used to monitor liver and kidney dysfunction (Ghelichpour et al., 2017). Therefore, the elevation in plasma proteins, along with the decrement in plasma ALT, confirms the promotion of liver health through lactic acid-supplemented diets. The present findings are in accordance with the results of previous research, which included dietary acidifiers such as sodium diformate (Wassef et al., 2017), malic acid (Hassaan et al., 2018), sodium acetate and sodium propionate (Sangari et al., 2021), and blend of formic acid and calcium propionate (Kumar et al., 2017) that increasing plasma total protein and albumin in European sea bass, *Dicentrarchus labrax*, Nile tilapia, *Oreochromis niluticus*, yellowfin seabream, *Acanthopagrus latus*, and mrigal carp, *Cirrhinus cirrhosus*, respectively.

Several investigations have reported the beneficial effects of dietary organic acids and their salts on the immune system of different fish species. For example, dietary butyric acid increased plasma lysozyme activity and total Ig concentration in Asian sea bass, Lates calcarifer, which indicates immunostimulation by butyric acid (Aalamifar et al., 2020). Hoseinifar et al. (2016) reported enhanced mucosal and humoral immune responses of Caspian white fish, *Rutilus kutum*, and fry-fed sodium propionate-supplemented diet (2.5 g/kg). Also, adding 1 and 2 g/kg of propionic acid to the Nile tilapia diet improved immunological parameters, including serum bactericidal index, lysozyme activity, and nitric oxide level (Reda et al., 2016). In the current study, increased plasma total protein and globulin of common carp fed with dietary lactic acid are in line with increased humoral immune parameters. The same finding was reported by Aalamifar et al. (2020), that fed Asian sea bass with butyric acid-supplemented diets. Also, other research studies showed increased plasma total Ig in different fish species fed with different kinds of organic acids, such as sodium propionate (Safari et al., 2021) and sodium butyrate (Abdel-Latif et al., 2021).

5. Conclusion

In conclusion, dietary lactic acid supplementation can increase growth performance, hepatic health, and immunological parameters in common carp. The best growth can be achieved at 5 g/kg dietary lactic acid, whereas improvement in hepatic health and immune responses is achievable at lower supplemental levels (2.5 g/kg). Hence, supplementing diets with 2.5-5 g/kg lactic acid is recommended for common carp feed.

Ethical Considerations

Compliance with ethical guidelines

All parts of the study were conducted under a protocol approved by the Committee of Ethics of the Faculty of Sciences of the University of Tehran (357; 8 November 2000).

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Authors' contributions

Conceptualization: Ali Taheri Mirghaed and Melika Ghelichpour; Methodology: Melika Ghelichpour and Abbasali Aghaei Moghaddam; Software: Seyed Hossein Hoseinifar; Formal Analysis: Melika Ghelichpour and Seyyed Morteza Hoseini; Data curation: Melika Ghelichpour; Writing-original draft preparation: Seyyed Morteza Hoseini and Seyed Hossein Hoseinifar Writing-review & editing and project administration: Ali Taheri Mirghaed.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

وضعیت شاخص‌های سلامت کبد و ایمنی خونی ماهی کپور معمولی (Cyprinus carpio) تغذیه‌شده با جیره‌های حاوی اسیدلاکتیک

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چکیده

زمینه مطالعه: اسیدهای آلی و نمک‌های آن‌ها به عنوان جایگزین‌های مناسب در خوراک برای بهبود سلامت، رشد و عملکرد ماهی شناخته‌می‌شوند.

هدف: مطالعه حاضر به منظور یافتن اثرات افزودن اسیدلاکتیک به جیره غذایی بر شاخص‌های ایمنی، فعالیت آنزیم‌های کبدی و پروتئین‌های پلاسما در ماهی کپور معمولی (*Cyprinus carpio*) انجام شد.

روش کار: ۱۸۰ ماهی (حدود ۲۵ گرم) به طور تصادفی در ۱۲ مخزن (۱۵۰ لیتر آب) به عنوان چهار تیمار با جیره‌های حاوی صفر (T0)، ۲/۵ (T1)، ۵ (T2) و ۱۰ (T3) گرم بر کیلوگرم اسیدلاکتیک توزیع شدند. ماهی‌ها به مدت ۵۶ روز با جیره‌های مذکور تغذیه شده و درنهایت شاخص‌های رشد، ایمنی، فعالیت آنزیم‌های کبدی و پروتئین‌های پلاسما بررسی شد.

نتایج: تیمار T2 به طور معنی‌داری شاخص‌های رشد بالاتری نسبت به تیمار T0 داشت. سطح پروتئین تام پلاسما، آلبومین و گلوبولین T1-T3 به طور معنی‌داری بیشتر از T0 بود. با این حال، سطح پروتئین پلاسما به طور قابل توجهی با افزایش غلظت اسیدلاکتیک در جیره (۱۰ گرم در کیلوگرم) کاهش یافت. تفاوت معنی داری در فعالیت آسپارتات آمینوترانسفراز پلاسما (AST) و آلکالین‌فسفاتاز (ALP) در بین تیمارها مشاهده نشد، اگرچه فعالیت آلانین آمینوترانسفراز (ALT) در ماهیان تغذیه‌شده با جیره‌های اسیدلاکتیک (T1-T3) در مقایسه با T0 به طور معنی‌داری کاهش یافت. تمام پارامترهای ایمنی خونی (لیزوزیم، کمپلمان فرعی، ایمونوگلوبولین و فعالیت باکتری‌کشی) در تیمارهای T1-T3 نسبت به T0 به طور معنی‌داری افزایش یافت.

نتیجه‌گیری نهایی: به‌طورکلی، افزودن اسیدلاکتیک به جیره برای بهبود رشد، پارامترهای ایمنی و سلامت کبد مفید است. با توجه به نتایج، مقدار ۲/۵ تا ۵ گرم بر کیلوگرم اسیدلاکتیک برای تهیه خوراک ماهی کپور معمولی توصیه می‌شود.

کلیدواژه‌ها: مواد افزودنی، کپور معمولی، سلامت کبد، ایمنی، اسیدلاکتیک

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