

Modulatory role of dietary curcumin and resveratrol on growth performance

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² Modulatory role of dietary curcumin and resveratrol on growth performance, serum immunity responses, mucus enzymes activity, antioxidant capacity and serum and mucus biochemicals in the Common carp, *Cyprinus carpio* exposed to Abamectin

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Abstract

The potential of dietary curcumin and resveratrol on blood biochemistry, immune response and resistance to the toxicity of pesticides, abamectin is investigated in this study. The distribution of carp with a total (of 30.78 ± 0.17 g) was carried out randomly into 18 tanks (with a total of 30 fish in each tank), which were used as six experimental groups (T1: fish that did not receive supplements and fish that were exposed, T2: 300 mg/kg curcumin, T3: 300 mg/kg resveratrol, T4: 12.5% LC50 abamectin, T5: 300 mg/kg curcumin + 12.5% LC50 abamectin, T6: 300 mg/kg resveratrol + 12.5% LC50 abamectin). The use of 300 mg/kg resveratrol in fish feed exposed to non-abamectin increased growth performance ($P < 0.05$) improved by fish feed exposed to non-abamectin containing 300 mg/kg resveratrol, while no effect was observed on curcumin. ($P > 0.05$). Final weight (FW), feed conversion ratio (FCR) and body weight gain (BB) between control and fish treated with resveratrol + abamectin and curcumin + abamectin ($P < 0.05$) were not different. Various patterns of changes are seen in immune components in blood [lysozyme, complement activity, total immunoglobulin (total Ig), protease, myeloperoxidase (MPO), nitro-blue-tetrazolium (NBT), peroxidase, albumin] and mucus [acid phosphatase (ACP), alkaline phosphatase (ALP), esterase, antiprotease] and antioxidant enzymes [(superoxide dismutase (SOD), glutathione peroxidase (GPX)) when juxtaposed with the control group, however, the fish that were supplemented with curcumin and resveratrol on abamectin-free media were the majority of the components this was higher than the control and other groups ($P < 0.05$). There was no significant difference between the resveratrol + abamectin and curcumin + abamectin treatments ($P > 0.05$), as indicated by the levels of immune components and antioxidants in the controls in the majority of cases. Abamectin induced oxidative stress in fish because there was a significant increase in malondialdehyde (MDA) levels in exposed fish when compared to the unexposed group ($P < 0.05$). It seems that oxidative stress was prevented effectively by curcumin and resveratrol because the exposed fish had higher levels of MDA (abamectin, curcumin + abamectin, resveratrol + abamectin) when compared to controls and individuals who were not exposed ($P < 0.05$).

Curcumin and resveratrol also showed a protective effect on the liver because fish that were supplemented with abamectin-free

media had lower levels of liver metabolic enzymes [aspartate transaminase (AST), ALP, lactate dehydrogenase (LDH)] compared to controls ($P < 0.05$). The stress response in the exposed fish was reduced with curcumin and resveratrol. A significant reduction in the supplemented fish was seen in their cortisol and glucose levels ($P < 0.05$). In conclusion, this study found that growth and immunity in carp can be suppressed using abamectin. Although the toxic effect of abamectin is reduced in the presence of resveratrol and curcumin, it seems that the effectiveness of resveratrol is higher when compared to curcumin.

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1. Introduction

The use of pesticides increased significantly to control pests in line with growing agricultural activities [1,2]. However, the environment and humans have always had problems with insecticides. Includes ecosystems, the life of aquatic organisms are threatened by insecticides and their derivatives which contaminate water through runoff and drainage [3]. The transmission and expansion of insecticides from a biological point of view in aquatic ecosystems can be carried out in the food chain without exception, where the toxicity is intensified, especially to the end consumer [4-7]. In aquatic ecosystems, one of the organisms that are adversely affected by insecticides and pesticides is fish [8]. The toxic effects of pesticides on fish can be reduced by using immune-stimulating food supplements, some studies have shown [9-15]. Antioxidant and immunogenic properties of medicinal plants and their derivatives have recently attracted much human attention [16-25]. Some studies related to this have shown that fish get a protective effect against pesticide toxicity in the presence of medicinal plants and their derivatives [11-14, 26]. In China and India, *Curcuma longa* is a well-known plant whose rhizomes can be used as a medicine that cures many diseases [27-29]. Curcumin, or *C. longa*, is a yellow pigment that is the origin of curcumin or diferuloylmethane; its functions are antibacterial, anti-inflammatory, immunomodulatory, and antioxidant [30-32]. The immune-stimulating properties of curcumin in fish are obtained from many studies that have been conducted [33-38].

Another plant-derived polyphenolic compound that functions as an antioxidant and anti-inflammatory is

resveratrol [39–42]. Research has also found fish to exhibit the antioxidant and immune-stimulating effects of resveratrol [43–49]. The effects of pesticide-induced immunotoxic and oxidative stress are rarely known, although there have been reports on the immunogenic and antioxidant functions of curcumin and resveratrol in fish.

needs use the most abundant avermectin compound (a macrocyclic

lactone compound extracted from the fungus *Streptomyces avermitilis*)

called abamectin. Abamectin is a neurotoxic insecticide whose action uses glutamate and γ -amino butyric acid-gated chloride channels in the brain [50,51]. Toxic effects on fish have been widely reported, although abamectin is relatively safe for humans [52–55]. The advice given in this study is to increase the natural carp immune system against the toxicity stimulated by abamectin.

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2. Materials and methods

2.1. Fish and experiment design

A local farm in Isfahan province, Iran, prepared 600 juvenile goldfish with an average weight of 27.18 ± 0.2 g and then acclimated to laboratory conditions within two weeks at a water temperature of 24–25 °C. Fish get food in the form of staple food ad libitum daily in this period (Table 1). When the adaptation period was complete, the fish (n 540, average weight: 30.78 ± 0.17 g) were separated into 18 tanks. One tank contained 30 fish randomly; the purpose of this separation was to carry out six experiments which were repeated three times. The experimental was grouped into control (T1): cultured fish on abamectin-free media that did not get supplements, T2: cultured fish on abamectin-free media supplemented with 300 mg/kg curcumin, T3: cultured fish on abamectin-free media supplemented with resveratrol 300 mg/kg, T4: cultured fish in media containing 12.5% abamectin LC50 without supplementation, T5: cultured fish in media containing 12.5% abamectin supplemented with 300 mg/kg curcumin, T6: cultured fish in media containing 12.5% LC50 abamectin supplemented with 300 mg/kg resveratrol. Abamectin concentration adjustments and 75% water replacement was done daily during the experimental period. Aeration is continuously carried out in the tanks, suction of suspended particles is carried out every day, and every two weeks, the biomass of each tank is weighed so that the feeding rate can be adjusted. Water quality parameters are checked every day, which include: temperature: 24.05 °C, dissolved oxygen $6.50.78$ mg/l (Portable Dissolved OXYgen Meter, Hanna, HI9146, Hanna instruments CO., UK), pH: 7.3 ± 0.2 (pH meter, Hanna-HI 98128, Hanna instruments CO., UK) and un-ionized ammonia 0.03 ± 0.025 mg/l (Hi-700 Ammonia Low Range Colorimeter – Examiner, Hanna Instruments CO., UK). Feed is given a maximum of twice a day [56].

2.2. Preparation of experimental diets

Merck CO. and Sigma Aldrich CO provided curcumin (820354, purity:75%) and resveratrol (R5010, purity: 99%). To be able to prepare a trial ration, the first step is to prepare and weigh the food ingredients and then mix the ingredients evenly. The next step is to form the dough by mixing water with the supplement at an adjusted level. Next is the production of pellets using a meat grinder and drying the pellets at 37 °C and the storage process at 4 °C. [56,57].

2.3. Determination of acute toxicity of abamectin and exposure trial

For the dose to be given correctly, the lethal range and acute concentration of carp abamectin must be known before the main

exposure test. That is why exposure of carp to different concentrations of abamectin is carried out after the adaptation period to obtain a lethal range. To achieve this goal, the fish (n 30, 10 fish per replicate) were exposed to concentrations of 0 (control), 0.25, 0.5, 1, 1.5, 2 and 4 mg/l abamectin agar LC50 according to the standard method, which can be specified [58] Recording of fish mortality was carried out for 96 hours at 24, 48, 72 and 96 hours after the fish were exposed. The final step, referring to the probit statistical analysis method, calculates the LC10, LC30, LC50, LC70, and LC90 values at 24, 48, 72 and 96 hours (Table 2).

2.4. Sampling

Feeding the fish for 24 hours, anaesthesia using eugenol solution (100 mg/l) and calculating the growth index and nutrition using fish biometry were carried out at the end of the 30th day, in addition to using the following formula to determine the feed consumed [59]:

Weight gain (WG; g) = $\frac{\text{Final weight (FW)} - \text{Initial weigh (IW)}}{\text{FW} - \text{in IW}}/d$. Specific growth rate (SGR; %) 100 [(in

Feed conversion ratio (FCR) = $\frac{\text{Feed intake}}{\text{FW-IW}}$.

Survival rate (SR;%) = $\frac{\text{Number of alive fish}}{\text{total number of fish}} \times 100$.

Eugenol solution (100 mg/l) was used to anaesthetize three fish from each tank to allow the evaluation of serum immune parameters to be carried out [59]. A 2 ml syringe takes blood samples from the caudal vein. The blood sample was poured into a tube and stored at room temperature for 2 hours. Subsequently, centrifugation (3000 g for 10 min at 4 °C) was used to separate the serum samples. The serum obtained was stored at 70 °C until the biochemical tests were carried out. Randomly capturing three fish from each tank and transferring them to a polyethene bag containing 10 ml of 50 mM saline solution was carried out so the slime samples could be collected. Slime centrifugation was carried out after 3 minutes (2500×g for 10 minutes at 4 °C) and supernatant storage at -80 °C [60].

2.5. Serum and mucus immune assays

Ellis described the turbidity method, which is the basis for measuring the activity of serum and mucus lysozyme [61] *Micrococcus lysodeikticus* in phosphate buffer (0.2 mg/ml in 0.05 M sodium phosphate buffer (pH 6.2) was used as a target. The amount of protein before and after polyethene glycol was added as a basis for the assay of total serum immunoglobulin (total Ig) (mg/dL). A commercial assay kit (Pars Azmun Co., Tehran, Iran) with ELISA [(ELX800), Bio-Tek, Vermont, USA)] was used to determine serum complement component concentrations (C3 and C4) (mg/dL). Yano describes the method on which the evaluation of alternative complementary activities (ACH50) is based [62]. Sheep red blood cells in the veronal buffer containing EGTA and Mn are the targets in this method. Preparation of different serum sample concentrations (0.312, 0.625, 1.25, 2.5, 5 and 10%) and mixing of 25 μ l of serum with 125 ml of buffer containing 50 μ l of blood cells was carried out. Centrifugation (13000 \times g for 5 minutes) of the mixture was carried out after 2 hours of incubation and storage was carried out at room temperature; then, the absorption rate was recorded at 412 nm.

In this method, mixing 100 μ l of slime with 100 μ l of ammonium bicarbonate buffer (100 mM) was performed. The mixture contained 0.7% azocasein solution and was then incubated at 30 °C for approximately 20 hours. Trichloroacetic acid was used to stop the reaction, and the supernatant was not removed by centrifugation (15 000 g, approximately 5 minutes). Then, the supernatant was mixed with 0.5 N hydroxide, and the absorption rate was recorded at 450 nm. Hank's Buffer (HBSS) at 450 nm wavelength refers to the method directed by Quade and Roth, was used to measure serum peroxidase activity [64]. Pars Azmun (Pars Azmun Co., Tehran, Iran), which did not contradict the manufacturer's protocol, was used to test the mucilage alkaline phosphatase activity.

The Anderson and Siwicki method was used to test nitro-blue-tetrazolium (NBT) serum [65]. It can be said, add 100 μ l blood to 100 μ l NBT 0.2% and incubate the mixture for approximately 25 minutes at 25 °C. About 50 μ l of the resulting suspension was poured into 1 ml of N, N-dimethyl formamide, then centrifuged at 3000 g for approximately 5 minutes, and the adsorption of the supernatant was recorded at 540 nm.

The Quade and Roth method measured serum myeloperoxidase (MPO) activity [64]. The 96-well microplate reader well at this stage obtained an additional ten μl of serum and 90 μl of Na^+ and Mn^{2+} mixed without Hank's balanced salt solution (HBSS) (Thermo Fisher Scientific, Inc., USA). Furthermore, the wells received an additional 35 μl of the tetramethylbenzidine hydrochloride (TMB) system. 35 μl sulfuric acid (0.5 M) was added to stop the color change, and the absorption rate was recorded at 450 nm.

Garen and Levinthal reported p-nitrophenyl phosphate as the substrate used to test the activity of mucus acid phosphatase (ACP) at 37 °C in 0.1 M acetate buffer (pH 5.0) [66]. Trypsin activity is slowed by using the ability of mucus to be the basis for measuring total antiprotease activity [67]. Ten μl of mucus was incubated with 5 mg trypsin at 22 °C for 10 minutes. Next, the mixture was subjected to adding 100 μl of ammonium bicarbonate (100 mM) and 125 μl of 0.7% azocasein. Samples were incubated for 2 hours at 30 °C, and then 250 μl of trichloroacetic acid was added. The mixture was centrifuged at 6000 \times g for 10 minutes. The supernatant was placed into a 96-well microplate reader well containing 100 μl sodium peroxide, and absorption rate readings were taken at 450 nm. Guardiola is the basis for measuring esterase activity [67]. Incubation was performed with equal volumes of mucilage and 0.4 mM nitrophenyl meristic in ammonium bicarbonate buffer containing 0.5% Triton X100 at 30 °C, and absorption rate readings were carried out at 405 nm in this method.

2.6. Serum and mucus biochemical parameters

A commercial assay (IBL Co., Gesellschaft für Immunchemieund Immunbiologie, Germany) with ELISA was used to test serum and mucus cortisol levels (ng/ml). A commercial assay (Pars Azmun Co., Tehran, Iran) was used to determine serum and mucus glucose concentrations (mg/dL). Measurement of each oxidation rate of glutathione oxide and reduction rate of cytochrome C (ZellBio GmbH, Veltinerweg, Germany) is a medium for testing the activity of serum glutathione peroxidase (GPX) (U/ml) and superoxide dismutase (SOD) (U/ml). The decomposition rate of H_2O_2 was estimated to calculate the serum catalase (CAT) activity (U/ml), as Goth described [68]. The thiobarbituric acid (TBARS) method with a commercial assay kit (ZellBio GmbH, Veltinerweg, Germany) was used to evaluate the lipid peroxidation indicator, malondialdehyde (MDA). A commercial assay kit

(Pars Azmun CO., Tehran, Iran) with biochemical autoanalysis (Beckman Coulter, Avanti J-26 XPI, CA, USA) was used to measure the activities of the enzymes ALP, AST and alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) (U/l) in serum.

2.7. Data analysis

SPSS software is used to analyze the data in this study. The Kolmogorov-Smirnov test was used to evaluate the normality of the data. One-way analysis of variance (ANOVA) was used to determine the differences among means, and then Tukey's test ($P < 0.05$) used to compare means. Mean \pm SE is the data in the figures and tables presented.

3. Results

3.1. Growth performance

In the T3 treatment (300 mg/kg resveratrol), the FW value was significantly higher compared to the control after receiving 30 days of feed (Table 3, $P < 0.05$). In addition, the FW value at T5 (Table 3, $P < 0.05$) was lower than T2. It can be seen from FW that there was no significant difference between the other groups (Table 3, $P > 0.05$). Compared with the control, the WG value increased significantly at T3; the lowest WG value was seen at T4 (Table 3, $P < 0.05$). There was no significant difference in the WG between the control and other groups (Table 3, $P > 0.05$). The lowest and highest FCR values were observed at T3 and T4 (Table 3, $P < 0.05$). Compared to the control, the FCR value at T3 shows a significant decrease, while at T4 (Table 3, $P < 0.05$), it has increased. Between T3, T5, and T6 (Table 3, $P > 0.05$), there was no significant difference in FCR. Compared with the other experimental groups (Table 3, $P < 0.05$), the SGR value at T4 was significantly lower. There was no significant difference between groups in SGR (Table 3, $P > 0.05$). Between all groups (Table 3, $P > 0.05$), there was no significant difference in SR.

3.2. Serum immune components

The T2 and T3 treatments showed a significant increase in lysozyme activity compared to the control and other groups (Table 4, $P < 0.05$). There was no significant difference in the training of lysozyme between the management and other groups (Table 4, $P > 0.05$). At each T2 and T4 ACH50 activity significantly compared to the control (Table 4, $P > 0.05$) showed higher and lower action. In other groups, ACH50 activity, compared to controls (Table 4, $P > 0.05$), did not show a significant difference. In the T3 treatment, there was an increase in the total concentration of Ig compared to control (Table 4, $P < 0.05$). Significant differences with the other groups (Table 4, $P > 0.05$) were not seen in total Ig concentrations in the controls. There was no significant difference in total Ig concentrations between T2, T3, and T6 and between T5 and T6 (Table 4, $P > 0.05$). In the T2 treatment compared to the control (Table 4, $P < 0.05$), there was a significant increase in protease activity. There was no significant difference with the other groups (Table 4, $P > 0.05$) in the protease activity of the control group. There was no significant difference in protease activity between T3 and T5 and between T4, T5, and T6 T2 than in T4 (Table 4, $P < 0.05$). The NBT activity

Significant differences with the other groups (Table 4, $P > 0.05$) were not seen in total Ig concentrations in the controls. There was no significant difference in total Ig concentrations between T2, T3, and T6 and between T5 and T6 (Table 4, $P > 0.05$). In the T2 treatment compared to the control (Table 4, $P < 0.05$), there was a significant increase in protease activity. There was no significant difference with the other groups (Table 4, $P > 0.05$) in the protease activity of the control group. There was no significant difference in protease activity between T3 and T5 and between T4, T5, and T6 (Table 4, $P > 0.05$). There was no significant difference with the other MPO activity groups in control (Table 4, $P > 0.05$). However, compared to T4 (Table 4, $P < 0.05$), MPO activity was significantly lower than T2. In the T3 and T6 treatments, when compared to the controls (Table 4, $P < 0.05$), there was a significant increase in NBT activity. There was no significant difference in NBT activity between T3 and T6 and between controls, T2, T4, and T5 (Table 4, $P > 0.05$). There was a significant increase in peroxidase activity in the T3 treatment compared to the control (Table 4, $P < 0.05$). Furthermore, compared to T4 and T5 (Table 4, $P < 0.05$), the peroxidase activity was significantly lower than that of T2. In the T2 and T3 treatments,

5 compared to the controls (Table 4, $P < 0.05$), there was a significant increase in C3 activity. In addition, T4 and T5 activity (Table 4, $P > 0.05$), when compared to C3 at T2, showed lower levels. Between the control and T4, T5, and T6 (Table 4, $P > 0.05$), there was no significant difference in C3 activity. Although C4 training in the T3 treatment was not lower than in T4, there was no significant difference in this component between the control and other groups (Table 4, $P > 0.05$).

3.3. Mucus immune components

Protease activity showed a significant decrease. In the T4 treatment, there was a decrease in protease activity compared to T3 (Figure 1A, $P < 0.05$). 9 There was no significant difference in protease activity with the control in the other groups (Fig. 1A, $P > 0.05$). Between all groups, there was no significant difference in lysozyme activity (Fig. 1B, $P > 0.05$). It can be seen from the ACP activity that the T2 and T3 treatments increased, significantly when 4 compared to the control (Fig. 1C, $P > 0.05$). difference In ACP activity, there was no significant difference between control and treatment, T4, T5, and T6 (Fig. 1C, at T3, ALP activity increased significantly compared to controls (Fig. 47 1D, $P > 0.05$). The ALP activity with the control difference is shown in Figure 1D (Fig. 1D, $P > 0.05$). 46

In the T2 treatment, there was a significant increase in esterase activity compared to the control (Fig. 1E, $P > 0.05$). 4 There was no significant difference in the esterase activity between the control and the other groups (Fig. 1E, $P > 0.05$). 25 In addition, in the T4 treatment, the esterase activity was significantly lower when compared to T3 (Figure 1E, $P > 0.05$). 41 A significant increase in T3 compared to the control can be seen in the antiprotease activity (Fig. 1F, $P > 0.05$). In addition, in the T2 treatment, the antiprotease activity was higher when compared to T4, T5, and T6 (Fig. 1F, $P > 0.05$).

There was no significant difference in the antiprotease activity between the control and treatment, T4, T5, and T6 (Fig. 1F, $P > 0.05$). 35 In addition, there was no significant difference in the antiprotease activity between T4, T5, and T6 (Figure 1F, $P > 0.05$).

3.4. Serum biochemicals

There was no significant difference between the experimental groups after the feeding period, as indicated by CAT activity (Table 5, $P > 0.05$). Compared to T4, SOD activity was significantly higher in T3 (Table 5, $P < 0.05$). There was no significant difference between the control and other groups in SOD activity (Table 5, $P < 0.05$). There was no significant difference in GPx activity between the control and other groups (Table 5, $P > 0.05$). In the T4, T5, and T6 treatments, compared to the control and other groups, there was a significant increase in the MDA level (Table 5, $P < 0.05$).

There were no significant differences in the MDA between controls, T2 and T3, T4, T5, and T6 (Table 5, $P > 0.05$). The experimental diet affected liver metabolizing enzymes in serum (Table 6, $P < 0.05$).

In the T3 treatment, when compared to the control, there was a significant reduction in the ALT level (Table 6, $P < 0.05$). There was no significant difference in the ALT level between the control and the other groups (Table 6, $P > 0.05$). There was no significant difference between the different groups' ALP and AST levels in the controls (Table 6, $P > 0.05$).

However, compared to T4, there was a significant decrease in the ALP level at T3 (Table 6, $P < 0.05$). In the T2 and T3 treatments, when compared to the controls, there was a significant reduction in LDH levels (Table 6, $P > 0.05$). There was no significant difference in LDH activity between the control and other groups (Table 6, $P > 0.05$).

There was no significant difference between the experimental groups after the feeding period in total protein and globulin concentrations (Table 7, $P > 0.05$). At T3, when compared to the control, there was a significantly increased albumin level (Table 7, $P < 0.05$). There was no significant difference in albumin concentrations between the control and other groups (Table 7, $P > 0.05$).

At T3, compared to the control, glucose concentration was significantly decreased (Table 7, $P < 0.05$). There was no significant difference in glucose concentration with the control group (Table 7, $P > 0.05$). At T3, when compared to controls, cortisol levels decreased significantly, while at T4, there was an increase (Table 7, $P < 0.05$). There was no significant difference between the controls, T5 and T6, in cortisol levels (Table 7, $P > 0.05$).

3.5. Mucus biochemicals

In treatment T3, compared to treatments T4, T5, and T6, there was a significant decrease in cortisol levels (Figure 2A, P <0.05).²⁰ Between the control and other groups, there was no significant difference in cortisol levels (Fig. 2A, P < 0.05). In the T4 treatment, when compared to T4, the glucose concentration increased significantly (Fig. 2B, P <0.05).¹ There was no significant difference in glucose concentrations between the control and other groups (Fig. 2B, P<0.05).³ There was no significant difference between the experimental groups after the feeding period in lactate concentration (Fig. 2C, P > 0.05)

4. Discussion

Growth performance in this study increased with 300 mg/kg of resveratrol in fish feed exposed to non-abamectin because the FW and WG values increased and the FCR in this treatment decreased compared to other groups. Curcumin was not observed for such an effect because the growth performance in the 300 mg/kg curcumin treatment was not different from the control. There are many reports on the growth-promoting effects of resveratrol and curcumin in fish [31,36,40,47,49,57,69–75]. However, fish species, level of supplementary feed, feeding duration,

and experimental conditions affect growth results and may vary. For example, the combination of resveratrol (400-800 mg/kg) and probiotics, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in the rainbow trout diet resulted in improvements in fish growth and immunity [49].

In the crucian carp *Carassius auratus*, which received a curcumin supplement of 5 g/kg of feed, its growth index increased, and the activity of digestive enzymes in the fish which received the supplement was a related factor [69]. Growth in the common carp, *Ctenopharyngodon Idella*, was boosted by curcumin at a dietary level of 438.20 mg/kg, which was associated with increased immune and antioxidant systems in fish receiving curcumin supplements [36]. Research by Ashry et al. [73] also obtained similar results, where adding 2-3% curcumin increased the growth performance of gilthead seabream and *Sparus aurata*. Li et al. [72] reported that the growth of fish could be encouraged by making growth and development of the intestine and the ability of the

intestine to absorb and transport amino acids increase. Curcumin can also increase feed intake in fish. It can also be increased with amino acids because of its unique taste [31,76]. Beneficial effects on turbot, *Scophthalmus Maximus* [47] and southern flounder, *Paralichthys lethostigma* [40], pacu, *Piaractus mesopotamicus* [71] and silverfish, *Channa argus* is also produced by curcumin and resveratrol [74]. Repair of intestinal damage [47], reduction of protein degradation [40], improvement of antioxidant defense [71] and lipid and glucose metabolism all have ⁶ the effects of resveratrol boosting on fish growth [77]. In this study, fish exposed to abamectin-free supplementation were the same as those in previous studies, namely reduced growth [78,79]. Reduced food intake, suppressed digestive enzymes and growth hormone, induction of intestinal tissue damage accompanied by impaired digestion and absorption of nutrients, and impaired liver function makes fish growth reduced in the presence of pesticides, including abamectin [55,80-87]. FW showed a higher FW value in the fish in this study resulting in the 300 mg/kg curcumin treatment compared to the 300 mg/kg curcumin 12.5% LC50 abamectin treatment. Not only that, no differences in FW, FCR and WG between control and treated fish, resveratrol abamectin and curcumin abamectin were observed, which allows suggestions of a remedial function for supplementation on the growth inhibitory effect of abamectin..

Immune components in the blood (lysozyme, C3, ACH50, total Ig, protease, MPO, NBT, peroXidase, albumin) and mucus (ACP, ALP, esterase, antiprotease) and antioxidant enzymes (SOD, GPX) in this study several changes can be seen when ⁴⁹ compared to the control group, however, in fish that received curcumin and resveratrol in abamectin-free media, almost all of these ²⁴ components were higher than the control and other groups. Several studies also concur with our study in that they have found immune-stimulating effects of curcumin and resveratrol in fish [33,35, 38,44,74,88-91]. Studies in other vertebrates show that their involvement in the system may occur by influencing cytokine production and modulating the inflammatory response. However, the mechanism of action of curcumin and resveratrol in fish is unknown. [92–94].

Oxidative stress can cause free radicals in fish which the presence of an antioxidant system can fight as the first step of resistance. In this study, oxidative stress in the fish was stimulated by abamectin because indicators of oxidative stress in MDA levels showed a significant increase in exposed fish. Scavenging effects on free radicals acquired in oxidative stress are known to exist in curcumin and resveratrol [95-97]. It has also been reported that fish have this scavenging function [45,98,99]. Thus, the immune system can be strengthened with these two supplements. However, it seems that oxidative stress can be prevented effectively by both curcumin and resveratrol because the treatment got lower MDA levels; curcumin and resveratrol complemented the control and unexposed fish when compared to only abamectin and abamectin supplements.

Significant differences between groups were not shown by the levels of immune components and antioxidants in the controls in this study; an indication of the moderate action of these supplements on the immunotoxic effects of abamectin may well be seen from abamectin's resveratrol and abamectin's curcumin.

Liver disorders and damage are generally indicated by several causes, such as elevated blood levels of hepatic metabolic enzymes (LME), although this is not necessarily a specific symptom [100,101]. In fish, elevated blood LME levels have been found after exposure to contaminants, especially pesticides, which are associated with liver damage due to toxins [102-104]. There was no significant difference in LME levels in the blood ⁴⁸ of the control group and the abamectin-exposed group in our study, which could indicate a non-significant effect of the pesticide at a dose of 12.5% LC50 on the liver. A significant reduction was shown in fish exposed to non-abamectin, levels of ALT, LDH and ALP in fish that received resveratrol supplements and LDH in fish that received curcumin supplements compared to controls, which allows the protective role of the supplement to be seen with the liver. Cortisol is the most important stress hormone; secretion is carried out into the bloodstream in response to stressors and separates glycogen in the liver so that glucose can be produced and energy needs in stressful situations can be provided [105]. In the present study, there was an increase in cortisol levels ¹⁷ in non-supplemented fish after exposure to abamectin; ¹⁶ the stress-inducing effect of the pesticide was apparent in this, as previously reported concerning other pesticides in fish [106-110]. Our results showed a significant decrease in non-exposed fish supplemented with cortisol and glucose levels, suggesting a stress-mitigating effect for resveratrol. Not only that, the glucose and cortisol levels in control were the same as those in resveratrol abamectin and curcumin abamectin, which strengthened this mitigating effect.

5. Conclusion

It can be concluded that the growth and immunity of carp can be decreased in the presence of abamectin. Resveratrol is more effective than curcumin, although both can reduce the disturbances brought on by abamectin. Abamectin ¹⁶ did not significantly affect the survival rate of fish even though ¹⁶ the growth and immunity in the treatment without supplements were reduced.

5. References

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- 791

792 Table 1. Feedstuffs and compositions of the basal diet.

793

| Ingredients | g/kg | Proximate composition | % in dry basis |
|--------------------------|------|-----------------------|----------------|
| Fishmeala | 160 | Crude protein | 393 |
| Soybean meal b | 170 | Crude lipid | 88.7 |
| Wheat flour (Res or cur) | 381 | ash | 62.1 |
| Poultry mealc | 150 | Dry matter | 908 |
| Wheat glutend | 100 | | |
| Phytasee | 5 | | |
| Fish oil | 10 | | |
| Lysine f | 6 | | |
| Soybean oil | 10 | | |
| Methioninef | 3 | | |
| Mineral mixg | 2.5 | | |
| Vitamin mixh | 2.5 | | |
| Total | 1000 | | |

794 a Peygir Co (crude protein 55.8 %). 795 b Soyabean Co (crude protein 45.5 %). 796 c Peygir Co (crude protein 50.0 %).

d Shahdineh Aran Co (crude protein 78.3 %).

e CheilJedang Co.

f Golbid Co (10,000 IU).

g The premix provided following amounts per kg of diet: Mg: 350 mg; Fe:

13 mg; Co: 2.5 mg; Cu: 3 mg; Zn: 60 mg; NaCl: 3 g; dicalcium phosphate:

802 10 g.

803 h The premix provided following amounts per kg of feed: A: 1,000 IU;

804 D3: 5,000 IU; E: 20 mg; B5: 100 mg; B2: 20 mg; B6: 20 mg; B1: 20 mg;

805 H: 1 mg; B9: 6 mg; B12: 1 mg; B4: 600 mg; C: 50 mg

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808 Table 2. Lethal Concentrations (LC10-90) of Abamectin depending on time (24-96h) for Cyprinus

809 carpio (mean \pm SE) Point Concentration (ppm) (95 % of confidence limits)

| | 24h | 48h | 72h | 96h |
|------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| LC ₁₀ | 0.95 \pm 0.38 | 0.84 \pm 0.33 | 0.70 \pm 0.33 | 0.62 \pm 0.41 |
| LC ₃₀ | 1.24 \pm 0.38 | 1.15 \pm 0.33 | 1.01 \pm 0.33 | 0.87 \pm 0.41 |
| LC₅₀ | 1.45 \pm 0.38 | 1.37 \pm 0.33 | 1.22 \pm 0.33 | 1.04 \pm 0.41 |
| LC ₇₀ | 1.65 \pm 0.38 | 1.59 \pm 0.33 | 1.43 \pm 0.33 | 1.22 \pm 0.41 |
| LC ₉₀ | 1.95 \pm 0.38 | 1.91 \pm 0.33 | 1.74 \pm 0.33 | 1.47 \pm 0.41 |

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812 Table 3. The growth and survival of the common carp, *Cyprinus carpio* over 30 days feeding with 813 experimental diets: T1: non-supplementd and non- abamectin exposed fish, T2: non- abamectin 814 exposed fish supplemented with 300 mg/kg curcumin, T3: non-abamectin exposed fish 815 supplemented with 300 mg/kg resveratrol, T4: non-supplementd fish exposed to 12.5 % LC50 816 of abamectin, T5: 300 mg/kg curcumin supplemented fish exposed to 12.5 % LC50 of abamectin, 817 T6: 300 mg/kg resveratrol supplemented fish exposed to 12.5 % LC50 of abamectin. Data are 818 presented as mean \pm SE. Different letters in the same row show significant differences ($P < 0.05$).

| Parameters | T1 (control) | T2 | T3 | T4 | T5 | T6 |
|------------|---------------------|--------------------|-------------------|-------------------|--------------------|--------------------|
| IW (g) | 30.43 \pm 0.34a | 31.43 \pm 0.47a | 30.93 \pm 0.43a | 30.46 \pm 0.43a | 30.83 \pm 0.72a | 30.60 \pm 0.20a |
| FW (g) | 46.66 \pm 0.72bcd | 49.83 \pm 0.92ab | 50.16 \pm 0.72a | 43.36 \pm 0.44d | 46.46 \pm 0.77cd | 46.73 \pm 0.53bc |
| WG (g) | 16.23 \pm 0.39b | 18.40 \pm 0.49ab | 19.23 \pm 0.88a | 12.90 \pm 0.66c | 15.63 \pm 0.36bc | 16.13 \pm 0.72b |
| FCR | 1.54 \pm 0.04b | 1.38 \pm 0.03bc | 1.29 \pm 0.04c | 1.86 \pm 0.05a | 1.46 \pm 0.06bc | 1.51 \pm 0.06bc |
| SGR (%/d) | 1.42 \pm 0.01ab | 1.53 \pm 0.01ab | 1.61 \pm 0.07a | 1.17 \pm 0.06c | 1.36 \pm 0.04bc | 1.41 \pm 0.05abc |
| SR (%) | 96.33 \pm 2.02a | 97.33 \pm 1.33a | 98.66 \pm 1.33a | 93.00 \pm 1.73a | 95.00 \pm 1.00a | 96.33 \pm 2.02a |

819 *IW: initial weigh; FW: final weight; WG: weight gain; FCR: feed conversion ratio; SGR: specific
820 growth rate; SR: survival rate.

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823 Table 4. The serum immune components of the common carp, *Cyprinus carpio* over 30 days 824 feeding with experimental diets: T1: non-supplementd and non- abamectin exposed fish, T2: non- 825 abamectin exposed fish supplemented with 300 mg/kg curcumin, T3: non-abamectin exposed fish 826 supplemented with 300 mg/kg resveratrol, T4: non-supplementd fish exposed to 12.5 % LC50 827 of abamectin, T5: 300 mg/kg curcumin supplemented fish exposed to 12.5 % LC50 of abamectin, 828 T6: 300 mg/kg resveratrol supplemented fish exposed to 12.5 % LC50 of abamectin. Data are 829 presented as mean \pm SE. Different letters in the same row show significant differences ($P < 0.05$).

| Parameters | T1 (control) | T2 | T3 | T4 | T5 | T6 |
|--------------------------------|--------------------------------|--------------------------------|---------------------------------|-------------------------------|--------------------------------|---------------------------------|
| (U/ml) Lysozyme | 22.60 \pm 1.51 ^{cd} | 28.93 \pm 0.86 ^{ab} | 31.36 \pm 1.19 ^a | 18.76 \pm 0.72 ^d | 22.10 \pm 1.15 ^{cd} | 25.23 \pm 0.67 ^{bc} |
| (U/ml) ACH₅₀ | 106.50 \pm 3.29 ^b | 123.50 \pm 2.46 ^a | 118.03 \pm 1.88 ^{ab} | 92.33 \pm 3.48 ^c | 106.73 \pm 2.74 ^b | 104.63 \pm 3.44 ^{bc} |
| (%) Protease | 5.73 \pm 0.66 ^{bc} | 8.33 \pm 0.48 ^a | 7.93 \pm 0.52 ^{ab} | 3.76 \pm 0.43 ^c | 6.13 \pm 0.49 ^{abc} | 5.23 \pm 0.43 ^c |
| (OD 450) Total Ig | 17.50 \pm 0.81 ^{bc} | 21.43 \pm 0.80 ^{ab} | 22.23 \pm 1.01 ^a | 13.36 \pm 0.78 ^c | 7.33 \pm 1.20 ^{bc1} | 18.50 \pm 0.73 ^{ab} |
| MPO | 1.23 \pm 0.20 ^{ab} | 2.21 \pm 0.34 ^a | 1.63 \pm 0.24 ^{ab} | 1.00 \pm 0.17 ^b | 1.50 \pm 0.15 ^{ab} | 1.30 \pm 0.17 ^{ab} |

NBT (OD 540)

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|--------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|------------------------|
| (U/ml) | 1.21±0.11 ^b | 1.56±0.22 ^{ab} | 2.48±0.20 ^a | 2.15±0.23 ^{ab} | 2.11±0.27 ^{ab} | 2.58±0.29 ^a |
|--------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|------------------------|

| | | | | | | |
|-------------------|-------------------------|--------------------------|-------------------------|------------------------|------------------------|-------------------------|
| Peroxidase | 7.83±0.46 ^{bc} | 10.06±0.58 ^{ab} | 11.83±0.89 ^a | 6.20±0.62 ^c | 6.43±0.47 ^c | 8.16±0.52 ^{bc} |
|-------------------|-------------------------|--------------------------|-------------------------|------------------------|------------------------|-------------------------|

| | | | | | | | |
|-----|------------|--------------------------|--------------------------|-------------------------|-------------------------|--------------------------|--------------------------|
| 830 | *C3 (g/dL) | 29.33±1.14 ^{cd} | 34.60±0.87 ^{ab} | 38.93±1.21 ^a | 24.70±0.94 ^d | 28.43±1.31 ^{cd} | 30.83±1.01 ^{bc} |
|-----|------------|--------------------------|--------------------------|-------------------------|-------------------------|--------------------------|--------------------------|

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|-----|-----------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|
| 831 | C4 (g/dL) | 15.16±1.01 ^a | 15.86±1.04 ^a | 16.80±0.69 ^a | 12.03±0.57 ^b | 12.70±1.20 ^{ab} | 13.16±0.88 ^{ab} |
|-----|-----------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|

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835 Table 5. The serum antioxidant enzyme activity in the common carp, *Cyprinus carpio* over 30 836 days feeding with experimental diets: T1: non-supplemented and non- abamectin exposed fish, T2: 837 non- abamectin exposed fish supplemented with 300 mg/kg curcumin, T3: non-abamectin exposed 838 fish supplemented with 300 mg/kg resveratrol, T4: non-supplemented fish exposed to 12.5 % LC50 839 of abamectin, T5: 300 mg/kg curcumin supplemented fish exposed to 12.5 % LC50 of abamectin, 840 T6: 300 mg/kg resveratrol supplemented fish exposed to 12.5 % LC50 of abamectin. Data are 841 presented as mean ± SE. Different letters in the same row show significant differences (P<0.05).

| Parameters | T1 (control) | T2 | T3 | T4 | T5 | T6 |
|--------------|--------------|---------------|--------------|--------------|--------------|---------------|
| CAT (U/ml) | 100.16±3.89a | 103.66±5.19a | 105.00±3.75a | 92.86±3.19a | 93.90±3.03a | 95.93±2.52a |
| SOD (U/ml) | 25.33±1.16ab | 26.43±1.65ab | 29.40±1.05a | 21.33±0.99b | 24.63±1.24ab | 25.46±1.31ab |
| MDA(nmol/ml) | 35.56±1.66b | 31.80±1.47b | 28.63±1.12b | 45.43±1.65a | 44.06±1.76a | 43.33±1.45a |
| GPx (U/ml) | 150.33±3.17b | 154.00±2.30ab | 165.50±3.04a | 142.50±2.35b | 148.33±2.89b | 152.83±2.45ab |

842 *CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde; GPx: glutathione peroxidase.

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852 Table 6. The activity of liver metabolic enzymes in serum of the common carp, *Cyprinus carpio* 853 over 30 days feeding with experimental diets: T1: non-supplementd and non- abamectin exposed 854 fish, T2: non- abamectin exposed fish supplemented with 300 mg/kg curcumin, T3: non-abamectin 855 exposed fish supplemented with 300 mg/kg resveratrol, T4: non-supplementd fish exposed to 856 12.5 % LC50 of abamectin, T5: 300 mg/kg curcumin supplemented fish exposed to 12.5 % LC50 857 of abamectin, T6: 300 mg/kg resveratrol supplemented fish exposed to 12.5 % LC50 of abamectin. 858 Data are presented as mean \pm SE. Different letters in the same row show significant differences 859 ($P < 0.05$).

| Parameters | T1 (control) | T2 | T3 | T4 | T5 | T6 |
|------------|---------------------|--------------------|--------------------|--------------------|---------------------|--------------------|
| ALT (U/l) | 21.53 \pm 1.01ab | 18.66 \pm 0.88bc | 17.10 \pm 0.51c | 24.90 \pm 0.60a | 20.50 \pm 0.76bc | 21.26 \pm 0.89ab |
| AST (U/l) | 80.40 \pm 1.53a | 78.16 \pm 2.16a | 80.16 \pm 2.20a | 86.10 \pm 1.93a | 81.20 \pm 1.74a | 84.40 \pm 1.81a |
| ALP (U/l) | 107.16 \pm 4.18ab | 98.83 \pm 4.20b | 95.50 \pm 2.59b | 116.23 \pm 3.03a | 104.50 \pm 2.59ab | 98.50 \pm 2.75b |
| LDH (U/l) | 287.00 \pm 3.78a | 274.00 \pm 2.30b | 270.83 \pm 1.58b | 292.20 \pm 1.74a | 288.83 \pm 2.74a | 290.83 \pm 1.48a |

860 *ALT: alanine aminotransferase; AST: aspartate transaminase; ALP: alkaline phosphatase; LDH: lactate
861 dehydrogenase.

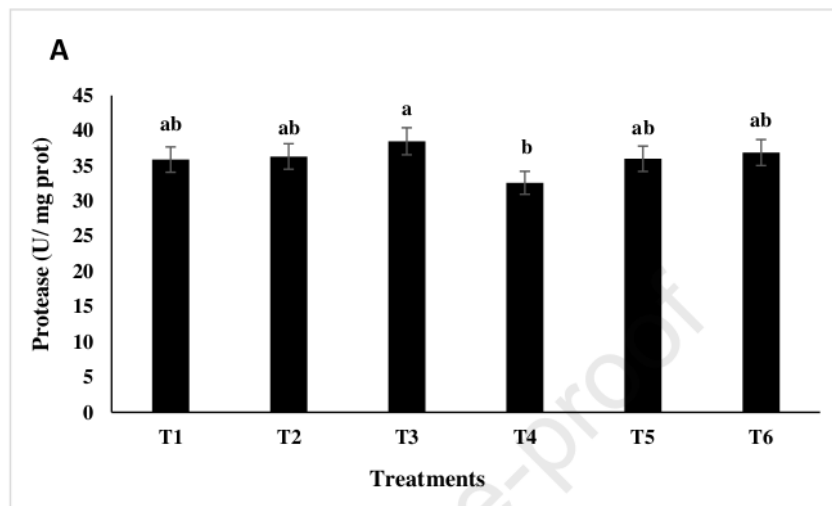
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864 Table 7. The biochemicals in the serum of the common carp, *Cyprinus carpio* over 30 days feeding 865 with experimental diets: T1: non-supplementd and non- abamectin exposed fish, T2: non- 866 abamectin exposed fish supplemented with 300 mg/kg curcumin, T3: non-abamectin exposed fish 867 supplemented with 300 mg/kg resveratrol, T4: non-supplementd fish exposed to 12.5 % LC50 868 of abamectin, T5: 300 mg/kg curcumin supplemented fish exposed to 12.5 % LC50 of abamectin, 869 T6: 300 mg/kg resveratrol supplemented fish exposed to 12.5 % LC50 of abamectin. Data are 870 presented as mean \pm SE. Different letters in the same row show significant differences ($P < 0.05$).

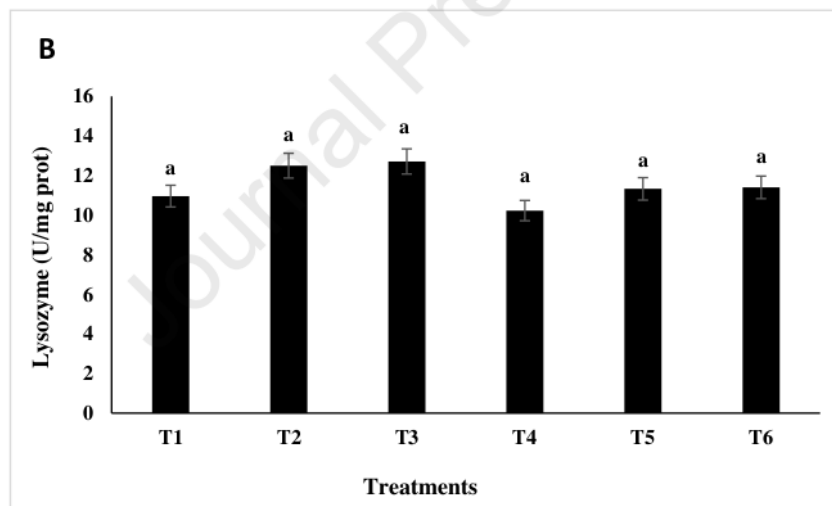
| Parameters | T1 (control) | T2 | T3 | T4 | T5 | T6 |
|--------------------------------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Total Protein (g/dL) | 3.00 \pm 0.17 ^a | 3.40 \pm 0.26 ^a | 3.95 \pm 0.27 ^a | 2.90 \pm 0.18 ^a | 2.86 \pm 0.20 ^a | 3.03 \pm 0.29 ^a |
| Albumin (g/dL) | 1.30 \pm 0.05 ^{ab} | 1.46 \pm 0.17 ^{ab} | 1.63 \pm 0.13 ^a | 1.05 \pm 0.10 ^b | 1.36 \pm 0.08 ^{ab} | 1.43 \pm 0.08 ^{ab} |
| Globulin (g/dL) | 1.70 \pm 0.11 ^a | 1.93 \pm 0.08 ^a | 2.31 \pm 0.18 ^a | 1.85 \pm 0.08 ^a | 1.50 \pm 0.28 ^a | 1.60 \pm 0.25 ^a |
| Cortisol (ng/ml) | 93.50 \pm 1.89 ^b | 86.76 \pm 1.29 ^{bc} | 81.26 \pm 1.75 ^c | 105.16 \pm 2.61 ^a | 93.50 \pm 1.32 ^b | 91.40 \pm 1.66 ^b |
| Glucose (mg/dL) | 81.76 \pm 1.67 ^{ab} | 74.16 \pm 2.45 ^{bc} | 67.50 \pm 2.17 ^c | 85.50 \pm 1.89 ^a | 83.10 \pm 1.93 ^{ab} | 76.86 \pm 1.27 ^{ab} |

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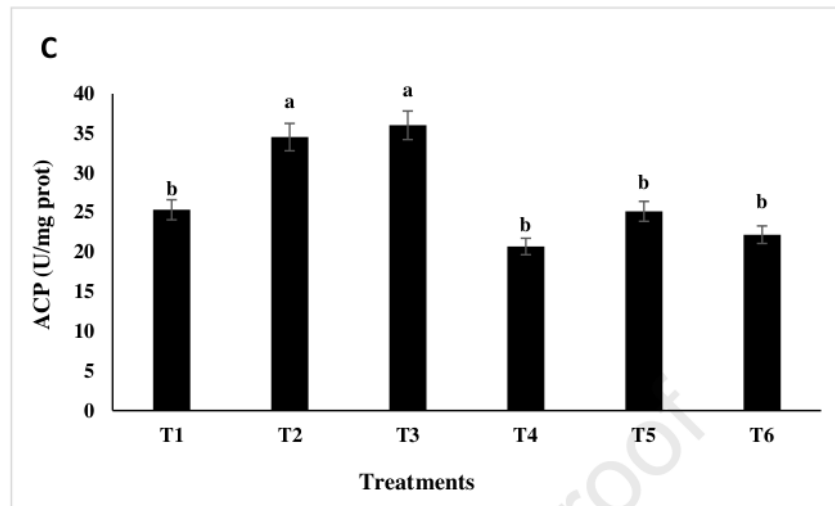
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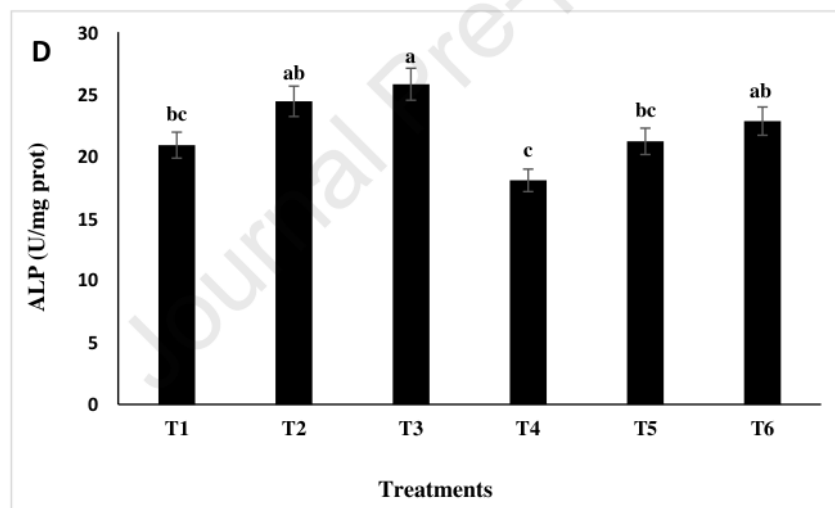
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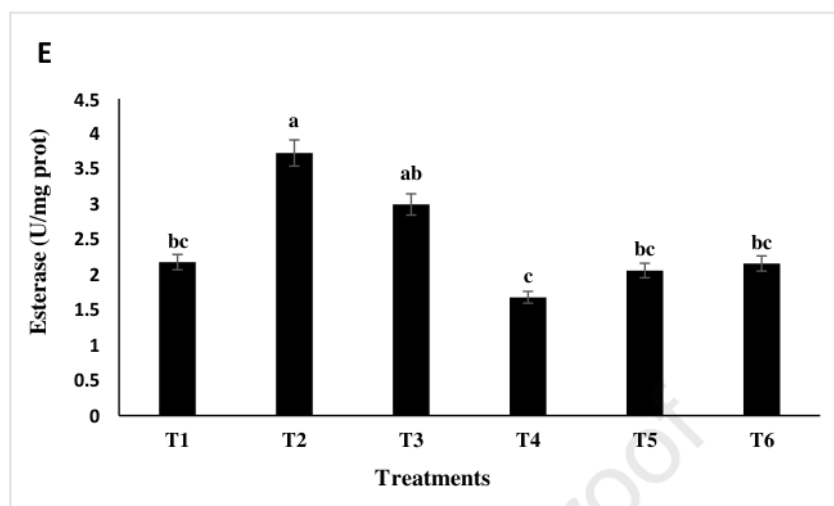
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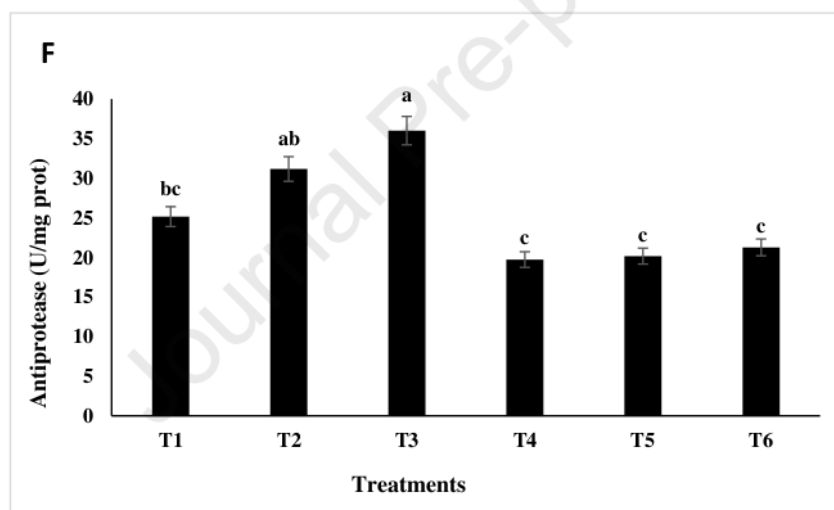
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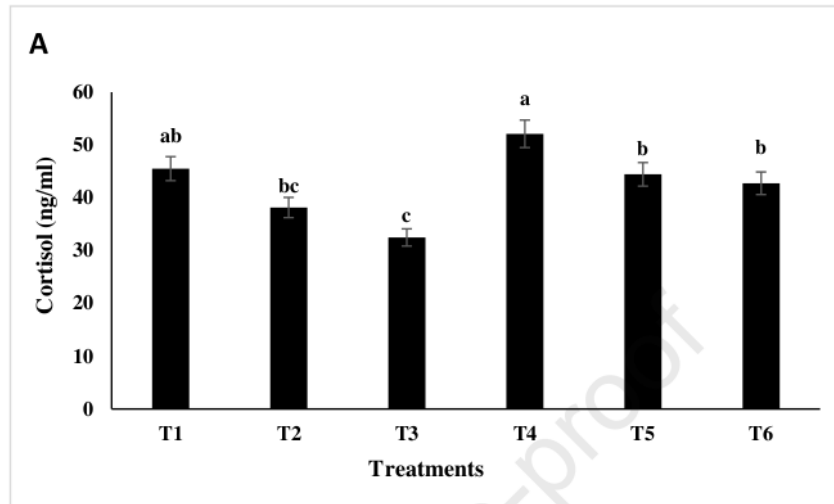
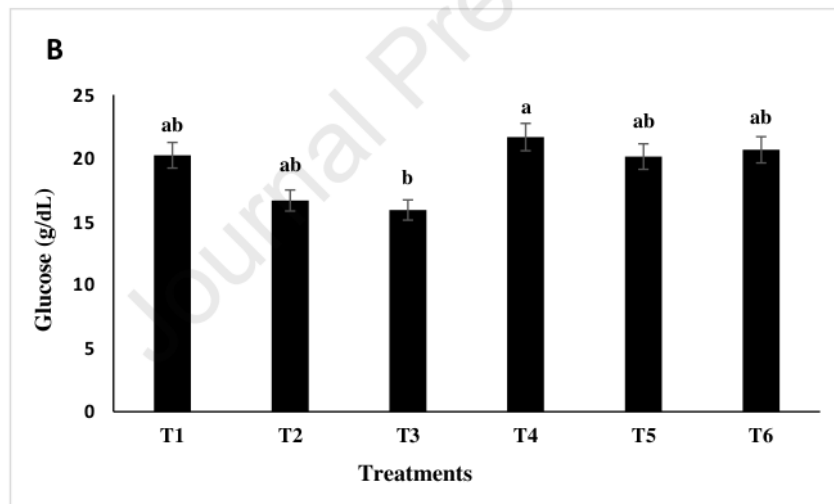


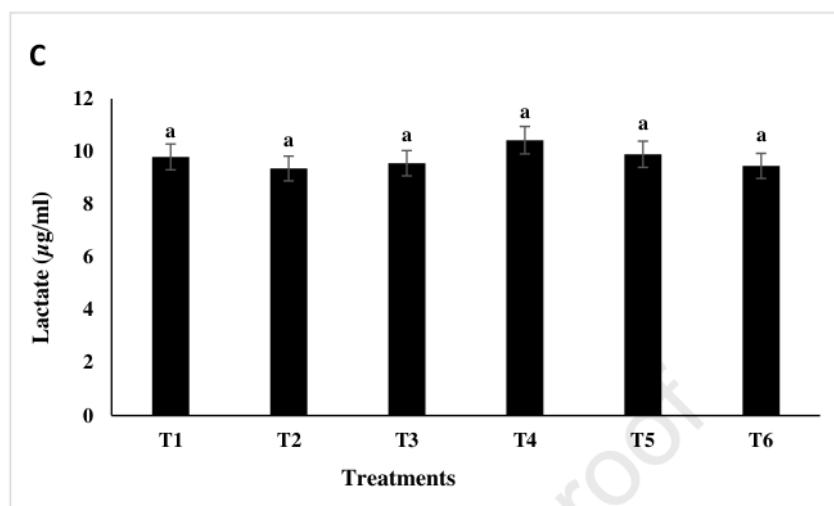
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894 Fig. 1. The immune components in the mucus of the common carp, *Cyprinus carpio* over 30 days 895 feeding with experimental diets: T1: non-supplemented and non-abamectin exposed fish, T2: non- 896 abamectin exposed fish supplemented with 300 mg/kg curcumin, T3: non-abamectin exposed fish 897 supplemented with 300 mg/kg resveratrol, T4: non-supplemented fish exposed to 12.5 % LC50 898 of abamectin, T5: 300 mg/kg curcumin supplemented fish exposed to 12.5 % LC50 of abamectin, 899 T6: 300 mg/kg resveratrol supplemented fish exposed to 12.5 % LC50 of abamectin. (ACP: acid 900 phosphatase; ALP: alkaline phosphatase). Data are presented as mean \pm SE. Different letters in the 901 same row show significant differences ($P < 0.05$).

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911 Fig. 2. The biochemicals in the mucus of the common carp, *Cyprinus carpio* over 30 days feeding 912 with
913 experimental diets: T1: non-supplemented and non-abamectin exposed fish, T2: non- 913
914 supplemented with 300 mg/kg curcumin, T3: non-abamectin exposed fish 914
915 supplemented with 300 mg/kg resveratrol, T4:
916 non-supplemented fish exposed to 12.5 % LC50 915
917 of abamectin, T5: 300 mg/kg curcumin supplemented fish exposed to
918 12.5 % LC50 of abamectin, 916 T6: 300 mg/kg resveratrol supplemented fish exposed to 12.5 % LC50 of abamectin. Data are
919 presented as mean \pm SE. Different letters in the same row show significant differences ($P < 0.05$). 918

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