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The effect of adding two types of organic chromium to the diet of common carp *Cyprinus carpio* L. on some blood and biochemical traits.

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A B S T R A C T

This research was carried out in the fish laboratory of the Department of Animal Production/College of Agriculture and Forestry/University of Mosul. 147 common carp, *Cyprinus carpio* L., were used with an average initial weight of 27.60 ± 2 gm/fish, distributed over seven experimental treatments, with three replicates for each treatment. The fish were acclimatized before the experiment for twenty-one days to the aquarium environment and food intake. Two types of organic chromium were added, namely chromium picolinate, at an amount of 0.3, 0.4, and 0.5 mg/kg feed (T2, T3, and T4), and 0.3, 0.4, and 0.5 mg/kg feed of chromium nicotinate (T5, T6, and T7), besides the control diet without additives (T1), which was balanced in terms of crude protein and metabolic energy. Results of the statistical analysis showed that the fish fed the experimental diets T3 and T7 were significantly superior ($P \leq 0.05$) in terms of hemoglobin and PCV to the rest of the experimental diets, including the control diet. Fish fed the T6 diet recorded a significant superiority over the rest of the other experimental treatments in the total blood protein and globulin standards, while no significant differences were observed between the different experimental treatments with the exception of the control diet, which was significantly behind the rest of the treatments in the albumin standard. Fish fed the control diet outperformed the parameters of blood sugar, triglycerides, AST, and ALT significantly more than the rest of the experimental treatments. It turns out that adding both types of organic chromium (organic chromium picolinate and nicotinate) at a rate of 0.5 mg/kg feed gave the best results in most of the criteria studied.

Introduction

An important nutrient for both people and animals is chromium (Cr). The minerals Cr^{+3} (trivalent) and Cr^{+6} (hexavalent), which are both found in nature, are vital nutrients for vertebrates, but the latter is poisonous and carcinogenic[1]. Protein, lipid, carbohydrate, and nucleic acid metabolisms are all impacted by chromium. Cr increases insulin's effectiveness and lowers insulin resistance [2,3,4]. There are two types of chromium: inorganic forms like chromic oxides and chromium chloride and organic forms like chromium picolinate, chelated chromium (nicotinate), chromium-amino acid complexes, and yeast-incorporated Cr [5]. Cr may also decrease plasma levels of total cholesterol and low-density lipoprotein (LDL) while raising those of high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) by inhibiting the activity of the hepatic hydroxymethylglutaryl-CoA reductase, an enzyme involved in cholesterol synthesis [6,7].

The current study intends to investigate the impact of supplementing blood with two forms of organic chromium, picolins and nicotinates, on hemoglobin and packed cell volume, as well as the biochemical properties of blood, which are represented by total protein, globulin, albumin, glucose, triglycerides, and aminotransferase enzymes.

Materials and methods

Ethical Approve

The Scientific Ethical Committee approved the research at the College of Veterinary Medicine at the University of Mosul, with its letter numbered UM.VET. 2023.095.25/2/2023.

Experience site

The Department of Animal Production Sciences at the University of Mosul's College of Agriculture and Forestry conducted this study from 23/10/2023 to 18/12/2023.

Experience fish

148 common carp, Cyprinus carpio L., were bought from the Tajran area in the Nineveh Governorate's Al-Kawir district for the purpose of growing and hatching fish from one of the nearby fish farms. To both sterilize and eradicate any external parasites that might have been present, the experimental fish were treated with a 3% solution of table salt. In the glass aquariums, 147 carp were arranged in three replicates for each treatment, weighing an average of 27.65 ± 2 g/fish. There were seven fish per tank. A three-week period of acclimation to the pond habitat and feed intake preceded the 56-day feeding trial.

Prepare a diet.

There were seven test diets developed for common carp.

The components of the experimental diet, which included

both organic chromium tablets, were ground into a powder using an electric grinder before being added. The pellets were continually mixed and exposed to three concentrations of chromium

picolinate and chromium nicotinate. They were also provided the control diet, which contains the ingredients mentioned in Table 1 and is explained below.

Table1. Food components and chemical composition (%DM) of the trial diets with varying diet percentages.

Treatments / Ingredients	T1	T2	T3	T4	T5	T6	T7
Animal protein*	12	12	12	12	12	12	12
Soybean meal	30	30	30	30	30	30	30
Local Barley	20	20	20	20	20	20	20
Yellow corn	16.5	16.5	16.5	16.5	16.5	16.5	16.5
Wheat bran	19	19	19	19	19	19	19
Vits. & Minerals	1	1	1	1	1	1	1
Salt food	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lime stone	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Bentonite	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chromium pic	-	0.3	0.4	0.5	-	-	-
Chromium nic	-	-	-	-	0.3	0.4	0.5

* mg/kg, **Calculated according to Smith's equation: ME (MJ\Kg) = Protein x 18.8+ Fat x 33.5 + NFE x 13.8.

Chemical composition of experimental diets

The chemical composition of the seven balanced experimental meals that were supplied to the fish utilized in the experiment is displayed in Table 2.

Table 2. Chemical composition (%) of the experimental diets.

Criteria	%
Dry matter	92.42
Moisture	7.58
Crude protein	25.60
Either extract	6.34
Ash	5.66
Nitrogen Free Extract (NFE)	54.80
*ME (Mega gol /Kg)	14.36

* Based on the Smith's equation (1971): Fat × 33.5+ Protein × 18.5 + NFE × 13.8.

The feeding rate of fish was changed according to their weight every fourteen days. The fish were fed on the basis of live weight at a rate of 3% from the beginning of the experiment, with three meals every day, and the percentage of feed provided was increased to 4% after the third week until the end of the research according to the weight gain that accompanied the fish.

Food additives:

The first type of organic chromium that was used was chromium picolinate, also known as Cr-nicotinate; it was produced as tablets by the Canadian company Isura and had 500 micrograms per tablet; the second type was produced as tablets by the American company Now and was referred to as chelated chromium, also known as chromium nicotinate; additionally, tablets containing 200 milligrams of the substance were produced. **The** two types of organic chromium were provided by one of the scientific offices of the Baghdad Governorate.

Blood parameters

After the study was over, five milliliter plastic syringes were used to take blood samples from six fish per treatment group and two fish each aquarium. The caudal vein is situated behind the caudal fin. According to the procedure outlined **[8],** a fraction of the blood was put in glass tubes devoid of anticoagulation and left at room temperature for two hours while angled. To do tests on additional blood parameters, the leftover blood was inserted into glass tubes that contained a blood anticoagulant.

Hemoglobin and packed cell volume

Hemoglobin and agglutinated blood cells were estimated using an American-made CBC Mindry BS-230 device. The device contains the reagents and their solutions necessary to conduct the examination of the aforementioned standards, which are the diluent solution, the lytic reagent, the detergent reagent, the calibration solution, and the bleach solutions.

Biochemical blood parameters

Biochemical blood parameters in the serum were estimated using the American-made Genotek Chemistry Analyzer Smart-150 device by taking the serum after separating it from the blood and placing it in plastic tubes inside the device. The tests took 15 minutes for each test to estimate the following characteristics.

Total protein measurement

The measuring equipment for total protein measurement was used by the used by the French company Biolabo for medical reagents, based on the use of a kit for total protein using a colorimetric method, and the wavelength is estimated at 550 nm.

Albumin measurement

The albumin level was measured using special analysis measurement equipment from the Italian company Giesse Diagnostics. The mechanism of action of albumin blocks is in a dilute solution with a pH of 3.8, a wavelength of 628 nanometers, and a temperature of 25, 30, and 37 °C. The unit of concentration is g/100 ml of blood serum.

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Globulin measurement

The globulin concentration was **measured** by calculating the difference between the concentrations of protein and albumin in the blood serum, and the unit that **expresses** the globulin level is g/100 ml of serum.

Measuring the level of the amino acid transporter enzymes ALT and AST

The level of the ALT enzyme in the serum was measured using an analysis kit belonging to the Italian company Giesse Diagnostics, which measures 340 nm at a temperature of 37 °C, proportional to the concentration of ALT and AST in the blood sample.

Glucose measurement

Serum glucose was measured using Giesse Diagnostics measuring equipment for a blood sample at a temperature of 37 °C and a wavelength of 510 nm.

Measurement of triglycerides

Serum triglycerides were estimated based on measuring equipment produced by Giesse Diagnostics for medical reagents. The blood serum sample was at 37 °C and at a wavelength of 546 nm.

Statistical analysis

In order to examine the impact of experimental factors on the criteria under study, the data were statistically analyzed using a complete randomized design (CRD) and the ready-made statistical application Statistical Package for Social Science (SPSS, 2017), version 25. Duncan's multiple range test (Duncan, 1955) was used to determine whether there were any significant variations between the means of the qualities under study.

Results and discussion

Hematological and biochemical criteria

Blood and biochemical parameters are vital indicators through which the health and physiological condition of fish can be diagnosed, in addition to their importance in drawing a clear picture of some positive and negative cases of the influence of environmental and nutritional factors to which fish may be exposed [9].

Hemoglobin and packed cell volume

The results of the statistical analysis of the blood hemoglobin
parameter (g/100 ml), shown in Table (3), indicated that there were significant differences (P≤ 0.05) between the different treatments, as T3 (14.98) and T7 (14.96) were significantly superior to the control diet (T1) (11.90) and the rest of the other experimental treatments. The results also showed a clear moral superiority for T2 (14.30) and T4 (14.28) over T1, T5 (12.90), and the T6 (13.21). Even though the control treatment's reported values in this criterion are significantly higher than those of any other experimental treatments, Table 3 shows the PCV blood count (%) results, which showed that T3 and T7 (46.00 and 46.00, respectively) were significantly better (P ≤ 0.05) than the T1, T2, T4, T5, and T6, with respective values of 36.38, 42.16, 42.50, 39.66, and 39.33. In terms of PCV, the results likewise demonstrated a substantial difference between the T2 and T4 compared with other treatments, which included the control diet (T1).

Table 3: The effect of organic chromium picolinate and nicotinate on parameters of Hb and PVC of common carp fish (Means ±SE).

Parameters Treatments	Hemoglobin (g/100 ml)	PVC (%)
T1 (control)	11.90 ± 1.03 e	36.38 ± 0.70 d
T2 (0.3 chr-pic.)	14.30 ± 0.40 b	42.16 ± 0.47 b
T3(0.4 chr-pic)	14.98 ± 0.90a	46.00 ± 0.51 a
T4 (0.5 chr-pic)	14.28 ± 1.10 b	42.50 ± 0.67 b
T5 (0.3 chr-nic)	12.90 ± 0.44 d	39.66 ± 0.88 c
T6 (0.4 chr-nic)	13.21 ± 0.90 c	39.33 ± 0.01 d
T7(0.5 chr-nic)	14.96 ± 0.42 a	46.00 ± 0.25 a

*Significant differences are present when there are different letters in a column at P ≤ 0.05.

These positive results of adding chromium picolinate led to an increase in hemoglobin and PCV. This may be due to the fact that organic chromium has the ability to preserve red blood cells from decomposition and thus enhance these values. Also, the enhancement of these two criteria may indicate the role of chromium in stabilizing red blood cells against cellular changes caused by peroxides [10].[11] hypothesized in their study on marikal fish that the fish may experience gill injury due to chromium toxicity, and this in turn leads to hypoxia, which enhances the production of red blood cells to reduce this negative effect.

A study on Nile tilapia fish by [12] suggests that organic chromium protects tissues from oxidation and maintains normal levels of Hb and PCV. According to [13] and [9], organic chromium serves to keep red blood cell membranes more stable and shields them from free radicals, which may damage the membranes and break down blood, which can result in anemia. In our current work, we added organic chromium to enhance the PCV % and hemoglobin level in common carp. The results that we achieved were consistent with the findings of [14] in silver carp *Hypophthalmichthys molitrix*, as well as in a study on Indian carp conducted by [15], while the results of our study did not agree with a study conducted by [16], who did not obtain significant differences in blood parameters when adding organic chromium to the diet of common carp compared to the control diet.

Total protein, albumin and globulin

The results shown in Table 4 showed that there was a significant superiority (P≤ 0.05) for the fish fed the T6 over the rest of the other experimental treatments in the total protein parameter, including the control treatment (T1), as their values reached 15.40 and 9.41 g/100 ml, respectively. The fish were superior in all experimental diets containing both types of organic chromium were significantly higher than in the control diet (Table 4). Within the same context, the results for the globulin parameter showed that fish fed T6 (5.80 g/100 ml) were significantly superior to all other experimental treatments, including the T1 (0.61 g/100 ml), and T4 (4.40 g/100 ml)
was significantly superior to all other experimental diets (except the sixth trial).

Table 4: The effect of organic chromium picolinate and nicotinate on the total protein, albumin, and globulin for common carp fish (Means ±SE).

Criteria T.	Total protein (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)
T1	9.41 ± 0.39 d	8.80 ± 0.44b	0.61 ± 0.01 e
T2	10.13 ± 0.35 c	9.71 ± 0.33 a	0.41 ± 0.01 e
T3	12.33 ± 0.36 c	9.44 ± 0.36 a	3.73 ± 0.06 c
T4	13.66 ± 0.44 b	9.71 ± 0.28 a	4.40 ± 0.14 b
T5	10.13 ± .023 c	9.55 ± 0.13 a	0.51 ± 0.01 e
T6	15.80 ± .020 a	10.08 ±0.12a	5.80 ± 0.08 a
T7	11.50 ± .020 c	9.93 ± 0.10 a	1.80 ± 0.04 d

* Significant differences are present when there are different letters in a column at P ≤ 0.05.

Total protein, albumin, and globulin play an important role in immune responses and are considered the main proteins in blood serum. A high level of these proteins in the serum is an indicator of good health and resistance to disease and stress. In our current experiment, we noticed that the parameters of total protein and globulin increased significantly when organic chromium was added. This indicates the positive role of these additives in achieving better health and nutritional status than fish fed a control diet. It is believed that the increase in total protein levels in the serum is due to increased levels of globulin, as chromium enhances the

immune system response and improves the production of antibodies [7]. The results of our current study agreed with a study by [17], in which he pointed to the role of organic chromium and its positive effect on enhancing the immune response of fish through its positive effects on white blood cells and lymphocytes. These results are related to those that were indicated about the role of chromium. Improving physiological and immune responses in Mozambique tilapia, Oreochromis mossambicus [18], and trout [19**].** While our results did not agree with what was indicated by [20], who did not obtain significant differences in the total protein standard in blood serum when adding organic chromium to the diets of trout or hybrid tilapia, Oreochromis niloticus x O. aureus. [21].

Glucose and triglycerides

The results of the statistical analysis of the blood sugar parameter (mg/100 ml) shown in Table 5 recorded that fish fed the first diet (T1) were significantly (P≤ 0.05) superior to all fish in the other experimental treatments, where their value reached 119.43, coming next in superiority. T5 (115.98), which differed significantly from all other experimental treatments (except the T1). The results also indicated that the fish fed T2 (97.33) were significantly superior to the T3, T4, T6, and T7, whose values reached 83.46, 53.76, 90.08, and 66.30. Respectively, the seventh treatment lagged significantly behind all other experimental treatments in this criterion.

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Table 5: The effect of organic chromium picolinate and nicotinate on parameters of glucose, and triglyceride of common carp fish (Means ±SE).

T.	Criterion	Glucose (g/100 ml)	Triglyceride (mmol/L)
	T1	119.43 ± 0.3**6** a	1.96 ± 0.02 a
	T2	97.33 ± 0.18 c	1.65 ± 0.06 b
	T3	83.46 ± 1.09 e	1.86 ± 0.0**6** a
	T4	53.76 ± 0.32 g	1.43 ± 0.04 c
	T5	115.98 ± 0.43 b	0.96 ± 0.04 d
	T6	90.08 ± 0.64 d	1.10 ± 0.06 d
	T7	66.30 ± 0.40 f	1.35 ± 0.11 c

* Significant differences are present when there are different letters in a column at P ≤ 0.05.

[19] indicated that there was a significant decrease in the concentration of glucose in the blood of trout with an increase in chromium when using diets supplemented with chromium picolinate. The results of our current study agreed with the results reported by [17] in Nile tilapia fish, where he indicated a decrease in blood glucose and triglyceride levels when adding chromium picolinate to the experimental diets, who mentioned that chromium is an integral structural component of the glucose tolerance factor (GTF), as it is believed that GTF was initially identified as the compound chromolenicotinic acid, which is the bioactive form of trivalent chromium. In addition, it is believed to enhance the action of insulin by increasing the binding and number of insulin receptors, and its function is to reduce glucose and fats in the blood, thus regulating glucose uptake in cells. Our results were consistent with what was reported by [25] on low sugar and triglycerides in Indian mackerel, as well as [26] and [27] on Indian carp fingerlings, while our results were not consistent with what was indicated by [7] when adding chelated chromium to Nile tilapia diets, where no significant differences were obtained in blood glucose and triglyceride parameters, as well as [28] in silver carp fish, where they noticed an increase in triglyceride levels when adding chromium picolinate to the experimental diets. .

Alanine aminotransferase enzymes (ALT) and aspartate (AST).

The results of the statistical analysis for the ALT standard (U/L) showed that there was a significant superiority in favor of the fish fed the control diet, amounting to 212.16 significant ($P \leq 0.05$) over the rest of the other experimental diets that included the diets for adding organic chromium with both types of picolins and nicotines, represented by the diets(Table 6). The T2, T3, T4, T5, T6, and T7 reached 195.33, 176.75, 129.00, 153.33, 80.03, and 121.73, respectively. The AST standard took the same direction as the aforementioned standard, as shown in Table 6, where the results indicated the superiority of the control method. (490.75) significantly higher than the experimental diets represented by T2, T3, T4, T5, T7, and T7, which amounted to 235.00, 395.00, 476.50, 325.16, 461.75, and 391.66 international units/liter, respectively.

Table 6. The effect of organic chromium picolinate and nicotinate on parameters of ALT and AST of common carp fish (Means ±SE).

Criterion T.	ALT (U/L)	AST (U/L)
T1	212.16 ± 1.16 a	490.75 ± 0.47 a
T2	195.33 ± 1.83 b	235.00 ± 0.93 f
T3	176.75 ± 2.02 c	395.00 ± 3.16 d
T4	129.00 ± 1.46 e	476.50 ± 1.81 b
T5	153.33 ± 1.05 d	325.16 ± 0.54 e
T6	80.03 ± 0.20 g	461.75 ± 2.56 c
T7	121.73 ± 1.46 f	391.66 ± 0.76 d

* Significant differences are present when there are different letters in a column at $P \leq 0.05$.

Adding both types of organic chromium reduced the concentrations of the liver enzymes ALT and AST in the experimental diets compared to the control diet, which had the highest levels of these two enzymes. Increased activities of the liver enzymes ALT and AST are indicators of stress in the organism [29] and can also be considered an indicator of decreased catabolism of amino acids for energy, leading to better muscle growth [26], as these enzymes work to use acids. Amina is for the purpose of perpetuation rather than construction [30]. This increase in blood serum may be due to damage to liver tissue as a result of physiological, nutritional, and pathological conditions [31], as the levels of serum enzymes ALT and AST are related to liver and kidney health [32]. Our current results agree with other studies in that there is a significant decrease in the level of these enzymes when adding different sources of chromium, and this is [33] indicated that there was a significant decrease in AST and ALT in the experimental treatments when adding different types of chromium to Nile tilapia fish, as well as with [34], who indicated that the decrease in ALT and AST due to the addition of chromium enhanced the ability of fish to protect themselves from stress, and this is what he observed in catfish. [26] did not obtain significant differences in ALT and AST parameters when adding organic chromium yeast to Indian carp fish diets. [35] did not obtain significant differences in the values of ALT and AST parameters when adding different types of chromium to Indian carp fish diets.

Conclusion

The results showed that feed additives represented by organic chromium have positive effects on the blood characteristics and parameters studied, as it turns out that adding both types of organic chromium (organochrome picolinate and nicotinate) at a rate of 0.5 mg/kg feed gave the best results in most of the parameters studied.

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Conflict of interest

The authors declare that there is no conflict of interest.

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