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The Effect of Dietary Organic Chromium on Specific Growth Rate, Tissue Chromium Concentrations, Enzyme Activities and Histology in Common Carp, *Cyprinus carpio* L.

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Abstract A 63-day feeding trial was carried out to investigate the effect of three levels of Cr yeast (0.5, 1.0 and 2.0 mg Cr/kg) on the utilization of diets containing 38.5 % of maize starch or dextrin in common carp, *Cyprinus carpio* L. (initial mean body mass 14±0.3 g) in an auto circulator system at 25±0.5 °C. A two-way analysis of variance (ANOVA) showed that the final body mass (FBM), percentage mass gain (%MG), specific growth rate (SGR) and feed conversion ratio (FCR) were significantly ($P<0.05$) affected by the two sources of variation (carbohydrate source and Cr level). In general, fish fed on a diet containing starch and fortified with 0.5 mg Cr/kg performed significantly higher FBM (47.23 g), %MG (225.11), SGR (1.91) and lower value of FCR (1.24) compared to fish fed on the other diets. Carp fed on 2.0 mg Cr/kg with maize starch and 1.0 mg Cr/kg with dextrin-based diet showed a significant reduction ($P<0.05$) in whole body lipid content as confirmed by a two-way ANOVA. Fish fed on a maize starch-based diet supplemented with 0.5 and 1.0 mg Cr/kg recorded the highest activities for hexokinase enzyme. Glucose-6-phosphate dehydrogenase activity was neither affected by Cr concentration nor by dietary carbohydrate source. Fish fed on dextrin-based diets accumulated higher Cr in the whole tissue compared to fish fed on starch-based diets. Normal histological structures in the liver and gut tissues were observed in all groups. The present data clearly showed that dietary Cr yeast was safe in the fish diet at the levels tested.

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Introduction

Regardless of species, fish do not have specific requirements for dietary carbohydrate per se, and they grow normally when fed on a diet free from carbohydrate [1]. The reason for this is due to their gluconeogenesis capacity whereby glucose is synthesized from non-glucose precursors such as amino acids [2]. However, from the economic and an environmental perspective, carbohydrate is considered to be a regular source of nutrients in fish diets with little negative impact on the ecosystem [3, 4]. Fish have a limited capacity for dietary carbohydrate digestion, and the efficiency of carbohydrate utilization by fish depends on the molecular complexity of carbohydrate [5, 6]. It has been reported that most fish species use complex carbohydrate (e.g. starch) for growth better than the more simple forms (e.g. glucose) [7–9]. On the other hand, other studies reported the opposite observation [10–12]. It seems that the variation in carbohydrate utilization by fish can be affected by different factors such as the differences in the digestive and physiology metabolic system of each species [13], carbohydrate source [9] and dietary carbohydrate level inclusion [14].

With the rapid expansion in aquaculture industries, carbohydrate utilization improvement in farmed fish is one of the major challenges. Hence, different studies have been carried out to enhance carbohydrate utilization in different fish species in order to reduce the cost of fish diets [15–18]. Creating transgenic fish is one of the possible strategies that can be used to achieve this goal [6]. As an attempt to improve the efficiency of carbohydrate metabolism in salmonid fish, human glucose transporter type 1 and rat

hexokinase (HK) type II cDNAs were cloned with viral (CMV) and piscine promoters and microinjected into rainbow trout (*Oncorhynchus mykiss*) and arctic charr (*Salvelinus alpinus* L.) eggs [19]. The latter study expected to obtain practical results and outcome in the second generation of the fish. However, it seems that the application of this kind of studies in aquaculture is limited because the improvement of growth performance by transferring the growth hormone gene (as an example) has been shown to affect other characters (could be negative or positive) such as body shape and composition, feed conversion efficiency and disease resistance [20]. Therefore, the enhancement of dietary carbohydrate utilization in fish by diet modification could be economic and more acceptable. In addition, consumer acceptance as well as potential negative views concerning the use of genetically modified organisms in aquaculture.

Chromium (Cr) has been distinguished as a vital element involved in glucose metabolism and subsequently growth improvement for different domesticated and farmed animals [21]. Several studies have provided a clear evidence of the positive effect of Cr as a growth inducer in fish [15, 22, 23]. The most interesting result of our previous study [17] demonstrated that different sources of Cr (Cr chloride, Cr picolinate and Cr yeast) at a level 0.5 mg Cr/kg significantly improved growth and starch utilization in mirror carp (*Cyprinus carpio*) compared to fish fed on the control diet. In addition, the Cr yeast-fed group showed the superior growth performance compared to fish fed on the other treatments. The results also suggested that the inorganic form of Cr (i.e. Cr chloride) at a level 2.0 mg/kg of diet negatively affected the normal structure of liver and gut tissues leading to health deterioration and growth reduction. This experiment was therefore designed to evaluate the effect of different levels of the organic form as Cr yeast at three levels (i.e. 0.5, 1.0 and 2.0 mg Cr/kg) on growth, the utilization of two sources of carbohydrate (i.e. starch and dextrin) and impact of these treatments on plasma glucose, tissue Cr level as well as the impacts on the histological structure of liver and gut tissues in common carp, *C. carpio*.

Materials and Methods

Diet Ingredients and Preparation

Two sources of carbohydrate [maize starch (M): Sigma-Aldrich Ltd., UK and dextrin (D): Roquette, Frêres, France] were used to formulate the experimental diets. Three diets were formulated for each carbohydrate source to contain 0.5, 1.0 and 2.0 mg Cr/kg as Cr yeast and labelled as follows: 0.5 M, 1.0 M, 2.0 M, 0.5 D, 1.0 D and 2.0 D. All dietary ingredients were well mixed and dry pelleted using a PTM Extruder system (model P6; Plymouth, UK) to give small pellets size (2 mm). Diets were dried at 45 °C for 24 h and stored in plastic containers until use. Ingredients and proximate composition of the experimental diets are presented in Table 1.

Experimental Animals and Design

This experiment was performed at the Aquaculture and Fish Nutrition Research Aquarium, Plymouth University, UK under the Home Office project licence #30/2644 and personal licence #30/8746. Common carp (*C. carpio*) were purchased from Hampshire Carp Fisheries, UK and acclimated to the laboratory condition for 4 weeks. Before the experimental phase, groups of 15 fish with an initial mean body mass 14±0.3 g stocked in 28×44×55-cm aquaria. The flow rate of water for each tank was 3 l/min. Fish were reared under a natural photoperiod of 12-h light to 12-h dark throughout the trial duration. The feeding regimen was 3 % biomass at a frequency of four times a day. Each experimental diet was fed to triplicate groups of fish.

Determination of Water Quality Parameters

Fresh water from the Plymouth supply network was used in the rearing system. During the trial (63 days), water temperature was maintained at 25±0.5 °C. The pH (6.48–7.03) and dissolved oxygen (94–97 %) were measured using an HQ40d pH and dissolved oxygen multiparameter meter (HACH Company, Loveland, USA). Ammonia (0.000–0.109 mg/l), nitrite (0.000–0.009 mg/l) and nitrate (11.22-36.92 mg/l) were measured weekly using a Nutrient Analyser (SEAL AQ2-Automated Discrete Analyzer, Ltd. UK). The concentration of Cr in the system (0.02 and 0.07 μg/l) was measured weekly using an ICP-OES (Varian 725-ES, Australia). A large water change was conducted every day to maintain suitable water quality in the system.

Proximate Composition of Diets and Whole Fish Tissue

Chemical composition of the experimental diets and fish carcass (initial and final) was determined according to the Association of Official Analytical Chemists (AOAC) protocols [24].

Cr Analysis of Diets and Fish Tissues

Before starting the experiment, sufficient amount of the experimental diets was collected for Cr analysis. At the end of the experiment, two fish per tank (six per treatment) were sampled to determine Cr in whole fish tissue. Liver and gut samples were collected for Cr analysis. Samples were acid digested and Cr concentration determined by an inductively coupled plasma mass spectrometry (X Series 2;

Table 1 Formulation and chemical composition of experimental diets

Ingredients (g/kg)	Diets					
	0.5 M	1.0 M	2.0 M	0.5 D	1.0 D	2.0 D
Herring meal LT 94[a]	250	250	250	250	250	250
Maize starch or dextrin[b]	382.2	382.2	382.2	382.2	382.2	382.2
Pea protein concentrate[c]	164.5	164.5	164.5	164.5	164.5	164.5
Maize gluten[d]	150	150	150	150	150	150
Sunflower oil	33.3	33.3	33.3	33.3	33.3	33.3
Vitamin/mineral premix[e]	20	20	20	20	20	20
Cr added (mg/kg)[f]	0.5	1.0	2.0	0.5	1.0	2.0
Cr analysed (mg/kg)	0.62	0.97	1.60	0.59	0.99	1.59
Proximate analysis (% dry matter)						
Moisture	5.67	5.31	5.52	5.42	5.26	5.15
Protein	38.11	38.47	39.66	38.85	38.23	38.23
Lipid	6.55	6.85	6.58	6.26	6.45	6.30
Ash	5.23	5.52	5.14	5.16	5.34	5.27

[a] Hearing meal: United Fish Products, Aberdeen, Scotland, UK

[b] Maize starch (Sigma-Aldrich Ltd., UK) and dextrin (Roquette Frêres, France)

[c] Pea protein concentrate: Roquette Frêres

[d] Maize gluten: Roquette Frêres

[e] Vitamin/mineral premix: Premier Nutrition Products (PNP Ltd.) Rugeley, Staffordshire (UK). Vitamin/mineral premix contains 121 g/kg calcium, vitamin A 1.000 μg/kg, vitamin D_3 0.100 μg/kg, vitamin E (as alpha tocopherol acetate) 7.0 g/kg, copper (as cupric sulphate) 250.0 mg/kg, magnesium 15.6 g/kg, phosphorus 5.2 g/kg

[f] Cr yeast or Biochrome™: Alltech, USA

Thermo Scientific, Hemel Hempstead, UK, following the procedure of AOAC [24] as described in our previous study [17]).

Plasma Glucose Level Measurement

The anaesthetisation of fish for sampling purposes was carried out using 0.20 g/l of tricaine methanesulfonate (MS-222; Pharmaq Ltd., Hampshire, UK). Plasma glucose concentrations were measured at the end of the experiment after 24 h of feed deprivation by the glucose oxidase method [25] on six individual fish per treatment. Whole blood was collected via the caudal vein into 1-ml heparinized syringes. The blood was centrifuged at 4,000×*g* for 5 min, and the plasma was collected in Eppendorf tubes, labelled and stored at −20 °C until analysis. A VersaMax Microplate Reader (Molecular Devices Sunnyvale, CA, USA) was used to read the absorbance at 505 nm.

Enzyme Activity Measurements

Enzyme activity measurement was performed on liver samples of two individuals taken from each tank (six fish per treatment) at the end of the experiment. The activity of HK (2.7.1.1) was determined as described by Bergmeyer et al. [26], and glucose-6-phosphate dehydrogenase (G6PD; 1.1.1.49) activity was measured according to the Barman protocol [27]. The assays for two enzymes were performed in triplicate monitoring the absorbance at 340 nm using a VersaMax Microplate Reader (Molecular Devices). The assay temperature was maintained at 37 °C. Protein was determined for each sample according to the Bradford assay [28]. Enzyme activity is expressed as specific activity in terms of milliunits per milligram protein. One unit of enzyme activity is defined as the amount of enzyme that catalysed the hydrolysis of 1 μmol of substrate/min at the assay temperature.

Histological Assessment

Six samples per treatment were collected following random sacrifice of fish at the end of the trial for the histological assessment. Briefly, the liver and the midsection of the intestine were collected from each fish and fixed in 10 % formaldehyde for a week and processed using an automatic tissue processor (Leica Microsystem TP 1020, Germany). Later, the tissue were embedded in paraffin blocks, cut into 7-μm slices using a Leica Microsystem microtome (RM 2235, Germany) and finally stained with haematoxylin and eosin stain by Leica Microsystem auto strainer XL, Germany. The slides were mounted with DPX and analysed under a light microscope at the final magnification of ×400.

Statistical Analysis

Data were analysed using SPSS statistics version 18 for Windows (SPSS Inc., Chicago, USA). Two-way analysis of variance (ANOVA) was used to compare means of the main effects followed by Tukey's test to detect the significant differences between the means. All data are presented as mean± standard deviation (SD).

Results

Growth Performance

The growth parameters recorded in the current study are presented in Table 2. The survival rate of the experimental fish was 100 % for all groups fed on different diets. In general, fish fed on 0.5 M diet showed significantly ($P<0.05$) higher response in terms of final body mass (FBM; 47.23), percentage mass gain (%MG; 225.11), specific growth rate (SGR; 1.91), feed conversion ratio (FCR; 1.24), and protein efficiency ratio (PER; 2.02) than those fed on the other experimental diets. The two-way ANOVA revealed a significant interaction ($P<0.05$) between the two variables (carbohydrate source and Cr level) for all growth parameters except the PER, although fish fed 0.5 M showed significantly ($P<0.05$) higher protein utilization compared to the other groups.

Proximate Body Composition

The proximate composition of whole fish body (final and initial) is presented in Table 3. The results of proximate analysis of whole fish carcass showed that moisture, protein and ash content were similar in different groups, while lipids were significantly affected ($P<0.05$) by the two variables (carbohydrate source and Cr level). Two experimental treatments (2.0 M and 1.0 D) were able to reduce the lipid content of whole fish tissue at the end of feeding trial.

Cr Accumulation in Liver, Gut and Whole Fish Tissue

Cr accumulation data are presented in Table 4. No significant differences ($P>0.05$) were detected in Cr content in the liver and gut tissues of fish fed different levels of Cr and two sources of carbohydrate despite an increasing trend reflecting increased dietary Cr levels. On the other hand, the accumulation of Cr in whole fish tissue increased with increasing dietary Cr supplementation with significant differences ($P<0.05$) between the different groups. In general, fish fed on dextrin-based diet accumulated higher Cr in the whole carcass and the organs tested compared to fish fed on starch-based diet.

Plasma Glucose Concentration

Plasma glucose data at the end of the experiment are presented in Table 5. The different diets had no significant effect ($P>0.05$) on the plasma glucose concentration.

Enzyme Activity

The activity of HK and G6PD of fish fed on the experimental diets is presented in Table 5. The results

Table 2 Growth performance and feed utilization of common carp fed diets containing maize starch (M) or dextrin (D) with three levels of Cr yeast over 63 days

Parameters	Experimental diets						Interaction (P value)
	0.5 M	1.0 M	2.0 M	0.5 D	1.0 D	2.0 D	
Initial body mass (g)	14.53±0.18	14.47±0.09	14.13±0.28	14.07±0.47	14.53±0.18	14.23±0.14	–
FBM (g)	47.23±1.74 a	42.13±0.09 b	38.97±0.04 c	38.87±1.22 c	41.30±1.83 bc	40.00±0.23 bc	0.001
%MG[a]	225.11±16.22 a	191.29±5.04 b	175.37±4.01 b	176.34±10.57 b	184.12±3.43 b	189.25±4.53 b	0.006
SGR[b]	1.91±0.08 a	1.72±0.02 b	1.64±0.03 b	1.71±0.03 b	1.68±0.06 b	1.72±0.04 b	0.007
FCR[c]	1.24±0.05 a	1.38±0.01 b	1.47±0.04 c	1.44±0.06 bc	1.41±0.02 b	1.38±0.01 bc	0.004
PER[d]	2.02±0.08 a	1.81±0.02 b	1.70±0.095 b	1.73±0.07 b	1.77±0.03 b	1.81±0.01 b	ns

Data are means±SD, $n=3$ groups per treatment. Significant differences ($P<0.05$) are indicated by different letters (i.e. a, b, c)

ns not significant

[a] Mass gain (in percent)=[(Final body mass−Initial body mass)/(Initial body mass)]×100

[b] SGR=[(ln Final body mass−ln Initial body mass)/Time (days)]×100

[c] FCR=Feed intake (in grams)/Mass gain (in grams)

[d] PER=Mass gain (in grams)/Crude protein fed (in grams)

Table 3 Whole body composition (percentage wet mass) of common carp fed experimental diets for 63 days

Parameters	Initial fish	Diets						Interaction
		0.5 M	1.0 M	2.0 M	0.5 D	1.0 D	2.0 D	
Moisture	72.6±0.3	72.2±0.3	72.3 ±0.6	72.4±0.4	72.6±0.9	72.6±0.4	72.2±0.3	ns
Protein	14.5±0.2	14.4±0.9	14.2±0.4	14.4±0.3	14.6 ±0.3	14.1±0.6	14.5±0.3	ns
Lipid	11.1±0.2	11.0±0.7 a	12.1±0.3 b	9.4±0.1 c	10.3±0.2 d	9.6±0.6 c	10.9±0.1 ad	0.000
Ash	2.5±0.3	2.6±0.10	2.6±0.6	2.5±1.0	2.6±0.8	2.7±1.8	2.5±1.19	ns

Values are means±SD (n=3). Means followed by different letters (i.e. a, b, c, d) in each row are significantly different (P<0.05)
ns not significant

suggested that different treatments had no significant effect (P>0.05) on the activity of HK. However, fish fed 0.5 M and 1.0 M diets recorded the highest HK activity (6.36 and 6.06 mU/mg protein), respectively. G6PD activity was neither affected by Cr concentration nor dietary carbohydrate source, and there were no significant differences (P>0.05) between different experimental fish fed on different diets.

Histological Assessment

At the end of feeding trial, normal histological structures in the liver tissues (Fig. 1) were observed in all groups fed on different diets for 63 days. Similarly, different treatments did not change the normal structure of gut tissues of the experimental fish as presented in Fig. 2.

Discussion

Trivalent chromium is often claimed to affect carbohydrate metabolic pathway [29], and the source of carbohydrate could alter the metabolism of Cr [30]. In this study, growth parameters (except the PER) were significantly affected by the two variables in the experimental diets (the source of carbohydrate and dietary Cr level). Fish fed the starch diet and supplemented with 0.5 mg Cr/kg achieved higher mass gain and SGR with the best FCR and PER value. The possible explanation for this result is that complex carbohydrates need more time for digestion and absorption, whereas simple sugars are readily absorbed soon after administration which can lead to hyperglycaemia and relatively growth reduction [31]. The other possible explanation is that fish do not possess the adequate capacity to deal with the high level of the absorbed glucose [32]. Therefore, the excess amount of the absorbed glucose may be excreted from the blood before the utilization process occurs in the cells [14]. Similarly, common carp fed on a diet containing 42 % maize starch grew better than those fed a diet containing the same level of dextrin [33]. The same observation has also been reported for catfish (*Mystus nemurus*), an omnivorous species [34]. Cr has been described as an insulin-mimetic agent, facilitating insulin binding to its receptors, leading to an increase in the rate of glucose uptake by cells and thereby affecting growth performance [21, 29]. Since 0.5 mg Cr/kg meets the requirement of carp [35], it was not surprising that this level with starch-based diet produced the best growth performance in the current study.

In this trial, different treatments did not change the moisture, protein and ash content of whole fish body, and this finding supports previous investigations carried out on carp species [15, 17]. Dietary Cr supplementation was able to decrease carcass fat in Japanese quails [36], rats [37] and chicks [38]. This decrease may be due to inhibition of the lipogenesis pathway [39]. In addition, the source of carbohydrate has been shown to affect the deposition of lipid in whole fish tissue [40]. The two-way analysis of variance in the current investigation elucidated that both Cr concentration and carbohydrate source significantly affected the final lipid content of the whole fish tissue. Two out of six

Table 4 Chromium content in liver and gut tissues (in micrograms Cr per kilogram) and whole fish carcass (in milligrams Cr per kilogram) of fish fed different diets for 63 days

Treatment	0.5 M	1.0 M	2.0	0.5 D	1.0 D	2.0 D	Interaction
Liver Cr content	12.88±2.30	14.88±2.90	15.48±2.09	14.91±1.93	15.55±1.86	17.71±2.26	ns
Gut Cr content	42.17±4.3	46.19±2.01	48.88±6.80	45.52±4.62	47.04±2.14	48.14±6.25	ns
Carcass Cr content	0.44±0.12 a	0.55±0.14 a	0.75±0.12 b	0.55±0.12 a	0.85±0.11 bc	0.97±0.10 c	ns

Values are presented as means±SD, n=2 fish per tank, 6 per treatment. Significant differences (P<0.05) are indicated by different letters (i.e. a, b, c)
ns not significant

Table 5 Enzyme activity profile (in milliunits per milligram protein) and glucose concentrations (in millimoles per litre) in response to different treatments at the end of feeding trial

Parameters	Diets						Interaction
	0.5 M	1.0 M	2.0 M	0.5 D	1.0 D	2.0 D	
HK	6.36±1.90	6.06±1.50	5.14±2.02	4.65±1.38	4.86±1.02	4.40±0.92	ns
G6PD	87.50±9.34	82.37±9.34	75.49±7.97	81.83±7.15	73.68±14.08	83.79±4.32	ns
Glucose	2.84±0.46	3.15±0.32	2.81±0.40	2.73±0.53	2.65±0.38	2.92±0.43	ns

Values are means±SD, n=6 per treatment

ns not significant

experimental groups showed the lowest lipid content (9.49 and 9.44 %) for 2.0 M and 1.0 D diet, respectively. It is noteworthy that these groups recorded the lowest G6PD activity. This finding is in line with Liu et al. [15] who reported that grass carp fingerlings fed high level of Cr picolinate (1.6 and 3.2 mg Cr/kg) had significantly lower whole body lipid content. In addition, a previous study reported that different sources of carbohydrate in the European eel (*Anguilla anguilla* L.) diet significantly affected the lipid deposition in whole fish tissue [40].

In the current trial, the concentrations of Cr were measured in two organs (liver and gut) for specific reasons. The liver of teleost fish is considered to be an important organ of heavy metal accumulation [41], while the intestinal tract is the most effective organ for dietary Cr absorption and excretion. In this study, Cr levels in both liver and gut tissues were similar in different groups despite an increasing trend reflecting increased dietary Cr levels. These results may indicate that Cr tends to accumulate in other organs too. For example, it has been reported that bone tissue is the greatest depot of Cr in higher animals [42]. In contrast, Cr carcass content increased with increasing dietary Cr supplementation in case of starch- and dextrin-based diets with significant differences. The same increasing level has been recorded in carp fed on dietary Cr chloride [17]. Interestingly, fish fed with dextrin diets were able to accumulate higher Cr in whole carcass than fish fed on a starch-based diet. The possible explanation for this result is that dextrin has a relatively higher bioavailability and higher absorption rate from the gut compared to starch, and this may increase the rate of Cr absorption and accumulation. It has been suggested that the source of carbohydrate altered Cr absorption and retention in mice fed on Cr chloride with different sources of carbohydrate including starch, sucrose, fructose and glucose [30]. It is worth mentioning that the latter study demonstrated that mice fed on starch diet accumulated more Cr in their bodies. It is likely that the absorption and retention processes in fish are different from that of mice. In general, the efficiency of mineral absorption by the animal is affected by different factors such as sex, genetic variables, general health, nutritional status in addition to the absorption coefficient or the bioavailability of the compound in which the metal is present [43, 44].

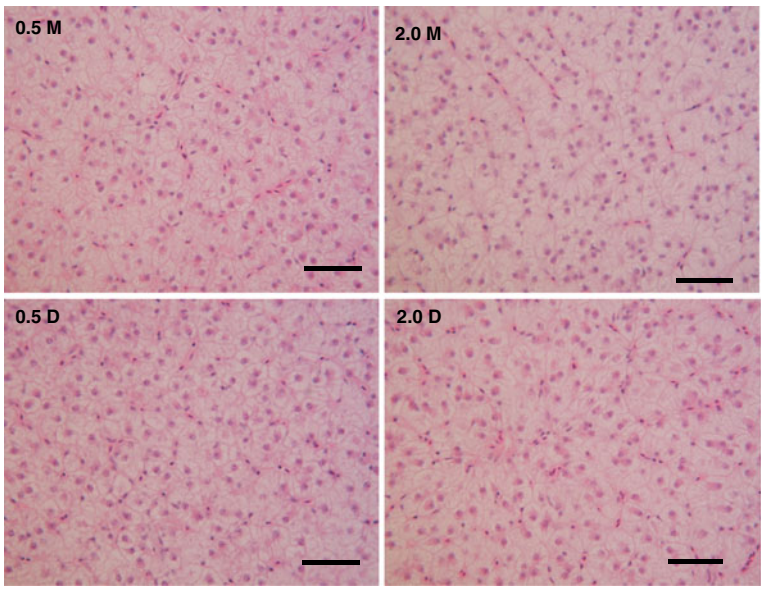

Fig. 1 Liver histology of carp fed maize starch (*M*) or dextrin (*D*) diets supplemented with low or high levels of Cr yeast (0.5 and 2.0 mg Cr/kg) showing the normal liver structure. *Scale bar* 50 μm. Sections were 7 μm in thickness and stained with H&E. Images for 1.0 M and 1.0 D treatments are not included

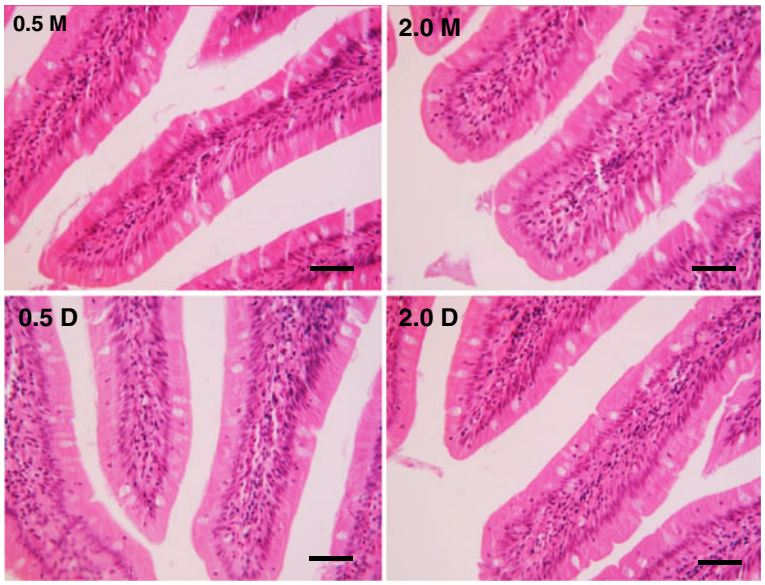

Fig. 2 Gut histology of carp fed maize starch (*M*) or dextrin (*D*) diets supplemented with low and high levels of Cr yeast (0.5 and 2.0 mg Cr/kg) depicting the normal gut structure. *Scale bar* 50 μm. Sections were 7 μm in thickness and stained with H&E. Images for 1.0 M and 1.0 D treatments are not included

In the current study, after 1 day of feed deprivation, plasma glucose levels were identical for all the experimental groups. It is likely that the peak phase of plasma glucose concentration was missed or may be that carp (as an omnivorous fish) has an adequate ability to regulate blood glucose when fed on carbohydrate diets as reported in gibel and common carp [9, 45]. Similarly, no significant differences have been detected in carp species fed organic and inorganic Cr [15, 17] despite the significant difference in growth performance recorded in these studies as a result of dietary Cr supplementation

Glycolysis is the major carbohydrate catabolic pathway taking place in the cytoplasm of organisms. Hexokinase catalyses the first step of the glycolysis pathway where glucose is phosphorylated by ATP to produce glucose-6-phosphate which passes through a sequence of reactions for energy production [46]. A previous investigation has indicated that the poor ability in rainbow trout to regulate the blood glucose concentration could be due to the lack of any betterment in glucose phosphorylation capacity in the liver [47]. This refers to the stability of HK level involved in glucose phosphorylation step while increasing the glucose uptake by the fish. In this experiment, no significant differences were recorded in HK activity between the experimental groups. However; the starch-based diet supplemented with 0.5 and 1.0 mg Cr/kg recorded the highest HK activity compared to the other treatments. It is likely that the activation of insulin by dietary Cr supplementation increases the glucose uptake and may stimulate the HK activity which subsequently improves glycolysis. It has been suggested that glucose-6-phosphate produced by overexpression of HK in rat hepatocytes may stimulate the glycolysis pathway [48].

Under in vivo conditions, the synthesis of G6PD is regulated by dietary composition and hormones and the regulation may happen as a direct response of the liver to the diet composition or indirectly by stimulating a specific endocrine response [49]. In the present study, the activity of G6PD was not affected by the two variables and there were no significant differences ($P>0.05$) between the experimental groups fed starch or dextrin with different levels of Cr yeast. However, fish fed (1.0 M and 2.0 D) recorded the lowest activity of G6PD which may indicate that Cr at certain levels was able to inhibit the lipogenesis pathway [39].

High level of Cr supplementation (2.0 mg Cr kg) as inorganic Cr (Cr chloride) to the carp diet had an adverse impact at the structural level of the liver and midsection of the intestine which negatively affected fish health and growth performance [17]. On the contrary, the same level of Cr supplementation as Cr yeast did not show any signs of abnormalities in these organs.

In conclusion, a starch-based diet supplemented with 0.5 mg Cr/kg showed beneficial effects on growth performance under the conditions of the current experiment. Based on the results presented in this trial especially those related to the histological assessment of liver and gut tissues, we can conclude that dietary Cr yeast is safe for fish at all the levels tested (0.5, 1.0 and 2.0 mg Cr/kg). Nevertheless, the long-term effects of dietary Cr yeast on the body composition, Cr tissue content (especially for carp of marketable size) and other biological responses are yet to be evaluated which will require further investigations.

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