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Effects of Dietary Carbohydrate Type and Level on Intestinal Digestive Enzyme Activities and Histology of Intestine and Liver in Songpu Mirror Carp (Postprint)

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Abstract

This study aimed to investigate the effects of different carbohydrate types and levels on intestinal digestive enzyme activities and intestinal and hepatic tissue structure in Songpu mirror carp. Using casein and fish meal as the primary protein sources, fish oil and soybean oil as lipid sources, and glucose and starch as carbohydrate sources, four isonitrogenous and isolipidic experimental diets with carbohydrate levels of 25.0% and 50.0% were formulated. Four hundred and twenty Songpu mirror carp with an initial body weight of (8.30$±$0.15) g were selected and randomly divided into 4 groups, with 3 replicates per group and 35 fish per replicate. The four groups of experimental fish were fed four isonitrogenous and isolipidic experimental diets containing two carbohydrate types and two carbohydrate levels, designated as the low starch (LS) group, high starch (HS) group, low glucose (LG) group, and high glucose (HG) group. The experimental period lasted 60 days. The results showed that intestinal amylase activity in the HS group was significantly higher than in all other groups ($P<0.05$), and protease activity was significantly higher than in the HG and LG groups ($P<0.05$); intestinal lipase activity in the HG and LG groups was significantly higher than in the LS group ($P<0.05$). The foregut villus height in the HG group was significantly lower than in the LG group ($P<0.05$), the midgut villus height was significantly lower than in the LS group ($P<0.05$), and the hindgut villus height was significantly lower than in all other groups ($P<0.05$). No significant differences were observed in muscular layer thickness of the foregut, midgut, and hindgut among all groups ($P>0.05$). The foregut and midgut villi in the HG group were wide and short, with villus density lower than in the LS and LG groups. Hepatocyte membrane rupture and vacuolation were observed in the HS and HG groups. In conclusion, diets containing 50%

starch increased intestinal digestive enzyme activities in Songpu mirror carp, diets containing 50% glucose decreased intestinal villus height, and a dietary carbohydrate level of 50% exerted certain negative effects on hepatic tissue structure in Songpu mirror carp.

Full Text

Effects of Different Carbohydrate Types and Carbohydrate Levels on Intestinal Digestive Enzyme Activities, Intestinal and Liver Histological Structure of Songpu Mirror Carp (Cyprinus carpio specularis)

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Abstract: This experiment was conducted to investigate the effects of different carbohydrate types and carbohydrate levels on intestinal digestive enzyme activities, intestinal and liver histological structure of Songpu mirror carp (Cyprinus carpio specularis). Four isonitrogenous and isolipidic experimental diets were formulated with casein and fish meal as primary protein sources, fish oil and soybean oil as lipid sources, and glucose and starch as carbohydrate sources at two levels (25.0% and 50.0%). A total of 420 Songpu mirror carp with initial body weight of (8.30$±$0.15) g were randomly divided into 4 groups with 3 replicates each and 35 fish per replicate. The four groups were fed the experimental diets and designated as low starch (LS), high starch (HS), low glucose (LG), and high glucose (HG) groups. The feeding trial lasted for 60 days. The results showed that intestinal amylase activity in the HS group was significantly higher than in other groups ($P<0.05$), and protease activity was significantly higher than in HG and LG groups ($P<0.05$). Intestinal lipase activity in HG and LG groups was significantly higher than in the LS group ($P<0.05$). The foregut fold height in HG group was significantly lower than in LG group ($P<0.05$), midgut fold height was significantly lower than in LS group ($P<0.05$), and hindgut fold height was significantly lower than in all other groups ($P<0.05$). No significant differences were observed in muscular thickness of foregut, midgut, or hindgut among groups ($P>0.05$). The foregut and midgut folds in HG group were wide and short with lower fold density compared to LS and LG groups. Hepatocyte membranes were ruptured with vacuolation observed in HS and HG groups. In conclusion, dietary carbohydrate at 50% as starch can enhance intestinal digestive enzyme activities in Songpu mirror carp, while 50% glucose decreases intestinal fold height, and 50% dietary carbohydrate level negatively impacts liver histological structure.

Keywords: Songpu mirror carp; glucose; starch; intestinal morphology; liver

Carbohydrates serve as essential nutrients that play important roles in fish growth [1-4], acting as both primary energy sources and structural components. In addition to providing energy for fish activities, excess dietary carbohydrates can be synthesized into glycogen (hepatic and muscular) or converted to fat for storage. As the most economical energy source in feed, appropriate carbohydrate levels can spare protein, reduce feed costs, increase ATP formation to facilitate amino acid activation and protein synthesis, and importantly, decrease nitrogen and phosphorus emissions to mitigate water pollution and promote sustainable aquaculture development [5-6]. However, high dietary carbohydrate intake can impair fish growth performance and disease resistance, increase hepatic glycogen content, and compromise normal liver function and metabolism [7-9].

Numerous studies have examined the effects of different carbohydrate types or levels on growth performance across fish species [10-12], but few have investigated the impacts of high carbohydrate levels on intestinal and liver histological structure. Jiang et al. [13] reported that dietary carbohydrate levels of 40% and 45% caused obvious lipid droplet vacuolation, nuclear displacement, and cytoplasm loss in liver tissue. Research on gibel carp showed that while they could tolerate 50% dietary carbohydrate, long-term consumption of high-carbohydrate diets caused functional and structural liver damage [1]. Songpu mirror carp, a new strain developed by Heilongjiang River Fisheries Research Institute from the German mirror carp (F4), exhibits characteristics including reduced scales, fast growth, high flesh content, and good meat quality, making it economically valuable. As an omnivorous fish with strong carbohydrate utilization capacity, carp can utilize starch better than glucose and tolerate up to 400 g/kg dietary carbohydrates [9]. This study used Songpu mirror carp as the model species, employing two carbohydrate sources (glucose and starch) at two levels (25.0% low and 50.0% high) to investigate the effects on intestinal digestive enzyme activities and histological structure of intestine and liver, building upon previous research on growth performance and hematological indices [14] to better understand carbohydrate metabolism mechanisms in fish.

1.1 Experimental Animals and Diets

Songpu mirror carp were obtained from Heilongjiang River Fisheries Research Institute. Four isonitrogenous and isolipidic experimental diets were formulated using casein and fish meal as primary protein sources, fish oil and soybean oil as lipid sources, and glucose and starch as carbohydrate sources at 25.0% and 50.0% levels. Diet composition and nutrient levels are shown in Table 1 . Feed ingredients were ground, mixed according to formulation, with minor components incorporated using the progressive enlargement method, then pelleted into 2 mm diameter pellets using a pellet mill and stored at -20 °C.

1.2 Experimental Design and Management

Four hundred twenty healthy Songpu mirror carp with uniform initial body weight of $(8.30\$\pm0.15)gwereselectedandacclimatedfor2weeks,thenrandomlydividedinto4groupsaccordingtoc$ $lowstarch(LS),highstarch(HS),lowglucose(LG),andhighglucose(HG).Eachgrouphad3replicateswith35fish$ $dayfeedingtrialwasconductedinindoortemperature-controlledrecirculatingaquariaat(24\pm\$1)$ °C with continuous 24 h aeration (dissolved oxygen >5 mg/L). Fish were fed 4 times daily to satiation without residual feed. Tanks were siphoned daily, and two-thirds of the water volume was replaced weekly with aerated water. Fish feeding and mortality were observed and recorded daily.

1.3 Intestinal Digestive Enzyme Activity Assay

At the end of the trial, fish were fasted for 24 h. Three fish per tank were randomly selected and dissected on an ice tray. Intestinal tissue was collected, fat and contents removed, washed with ice-cold 0.86% saline, blotted dry with filter paper, and weighed. Tissue was homogenized in saline at 1:9 mass-to-volume ratio using an FJ-200CL high-speed tissue homogenizer, centrifuged at 4,000 r/min for 10 min at 4 °C, and the supernatant collected as crude intestinal enzyme extract. The extract was aliquoted into 1.5 mL centrifuge tubes and stored at -20 °C for subsequent protease, amylase, and lipase activity assays using methods described in reference [15].

1.4 Intestinal and Liver Histological Preparation

At the end of the trial, three fish per group were randomly selected and rapidly dissected on an ice tray. Liver tissue and foregut, midgut, and hindgut segments were collected, rinsed with 0.86% saline, and fixed in Bouin' s solution. After 24 h fixation, samples underwent graded ethanol dehydration, xylene clearing, paraffin embedding, sectioning (5 m thickness), hematoxylin-eosin (HE) staining, dehydration, clearing, and neutral resin mounting. Liver and intestinal sections were observed and photographed using a Leica MD 4000B microscope. Images were analyzed using Motic Images Plus 2.0 software. More than 15 complete fold heights and muscular thicknesses were measured per intestinal segment, with averages calculated as the final values.

1.5 Statistical Analysis

Data were analyzed using SPSS 20.0 software for one-way and two-way ANOVA, followed by Duncan' s multiple comparison tests. Results are expressed as mean±standard error (mean±SE) with significance level set at $P<0.05$.

2.1 Effects of Different Carbohydrate Types and Levels on Intestinal Digestive Enzyme Activities

Intestinal digestive enzyme activities are presented in Table 2 . The HS group showed significantly higher intestinal protease activity than HG and LG

groups (P<0.05) and significantly higher amylase activity than all other groups (P<0.05). Intestinal lipase activity in HG and LG groups was significantly higher than in the LS group (P<0.05). No interactive effects between dietary carbohydrate type and level were observed on intestinal digestive enzyme activities (P>0.05).

2.2 Effects of Different Carbohydrate Types and Levels on Intestinal Morphology

As shown in Table 3 , no significant differences were found in muscular thickness of foregut, midgut, or hindgut among groups (P>0.05), and two-way ANOVA revealed no interactive effects of carbohydrate type and level on muscular thickness (P>0.05). The foregut fold height in HG group was significantly lower than in LG group (P<0.05), midgut fold height was significantly lower than in LS group (P<0.05), and hindgut fold height was significantly lower than in all other groups (P<0.05). Dietary carbohydrate level significantly affected foregut and midgut fold height (P<0.05), carbohydrate type significantly affected hindgut fold height (P<0.05), and interactive effects of carbohydrate level and type were observed on foregut and hindgut fold height (P<0.05).

2.3 Intestinal Tissue Observations

Foregut histological sections of Songpu mirror carp are shown in Figure 1 [Figure 1: see original paper]. The HS group exhibited lower foregut fold density compared to the LG group. LS and LG groups showed tall, slender, densely arranged folds, while HG group displayed short, wide foregut folds that were lower than those in LG group.

Midgut histological sections are presented in Figure 2 [Figure 2: see original paper]. Except for HG group, midgut folds were tall, slender, and densely arranged. The HG group showed wide, short, and sparsely arranged midgut folds.

Hindgut histological sections are shown in Figure 3 [Figure 3: see original paper]. Compared to LS and LG groups, HS and HG groups exhibited slightly shorter hindgut folds, though all groups showed dense, intact morphology.

2.4 Liver Tissue Observations

Liver histological sections of Songpu mirror carp are presented in Figure 4 [Figure 4: see original paper]. Compared to LS group, HS group showed hepatocytes with blurred edges, unclear contours, disrupted reticular stroma, and clustered nuclei. Histopathological changes occurred in 60-70% of samples per group, causing elevated biochemical indices. LG group showed neatly arranged hepatocytes with intact structure and clear, orderly cell contours. In contrast, approximately 60% of HG group samples displayed hepatocytes with blurred edges and disorganized arrangement.

The fish intestine is crucial for nutrient digestion and absorption, with digestive enzyme activities serving as important indicators of nutrient utilization [16,17]. Amylase secretion and activity directly determine carbohydrate digestion capacity [18]. While some studies suggest amylase activity is primarily genetically determined [19-20], others indicate that increasing dietary carbohydrate levels can enhance amylase activity [21], and that carbohydrate digestibility relates to molecular weight. Li et al. [22] reported that dietary starch supplementation increased intestinal amylase activity in large yellow croaker. Our results showed that HS group had significantly higher intestinal amylase activity than glucose groups (HG and LG) and LS group, with protease activity also highest in HS group. This synergistic effect on digestive enzyme activities was similarly observed in studies on carbohydrate nutrition of Babylonia areolata [23]. However, glucose groups showed significantly higher intestinal lipase activity than LS group. These results demonstrate that dietary carbohydrate type and level influence intestinal digestive enzyme activities in Songpu mirror carp, though specific mechanisms require further investigation.

As lower vertebrates, fish have intestinal structures similar to but less developed than terrestrial animals. Therefore, intestinal fold density, height, and arrangement reflect digestive and absorptive capacity [24-25]. Normal intestinal development significantly affects fish digestion [26]. Limited reports exist on carbohydrate effects on fish intestinal morphology. Our results showed that 50.0% glucose as carbohydrate source significantly reduced foregut, midgut, and hindgut fold height, while high starch had no significant effect, indicating severe impacts of high glucose levels. Histological observations confirmed markedly reduced fold height in HG group, with wide, short, sparsely arranged folds, particularly in foregut and midgut, severely impairing nutrient absorption. This corroborates our previous findings that high glucose diets reduced growth performance, with HG group showing lowest weight gain and specific growth rate, highest feed conversion ratio, and significantly lower protein efficiency [14].

The liver is a vital organ for carbohydrate metabolism in fish. Excessive dietary carbohydrate creates nutritional stress with evident histological manifestations. Cheng et al. [27] reported that dietary carbohydrate levels of 15% and 30% caused hepatocyte swelling, lipid droplets, ruptured cell membranes, and displaced nuclei in southern catfish. Xu et al. [28] observed nuclear displacement and vacuolation in largemouth bass fed diets containing 10% waxy corn starch, wheat starch, or cassava starch. Studies on Labeo rohita fed 51.7% and 42.43% carbohydrate diets also reported hepatocyte swelling and vacuolation [29-30]. Such changes may result from metabolic overload stress or glycogen accumulation [30]. In our study, HS and HG groups predominantly showed ruptured hepatocyte membranes with unclear contours, indicating high-carbohydrate diets can damage liver function and negatively affect growth, body composition, and serum biochemical indices [14,31].

In conclusion, dietary carbohydrate at 50% as starch can enhance intestinal digestive enzyme activities in Songpu mirror carp, while 50% glucose decreases

intestinal fold height, and 50% dietary carbohydrate level negatively impacts liver histological structure.

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