Effects of Dietary Cassava Starch Supplementation Levels on Growth Performance, Digestive Capacity, and Carbohydrate Metabolism in Common Carp (Postprint)

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Abstract

Using a practical feed formulation as the basal diet, 5% (control group), 10%, and 20% cassava starch were added to formulate three iso-lipidic (crude lipid content approximately 6.7%) and iso-energetic (gross energy approximately 16 MJ/kg) diets. Common carp with an average weight of (55.73$±$3.55) g were reared in cages for 8 weeks to investigate the effects of dietary cassava starch supplementation levels on growth performance, digestive capacity, and glucose metabolism, and to evaluate the fishmeal-sparing effect of cassava starch. Each diet was assigned three replicates, with 50 experimental fish per replicate. The results showed that weight gain rate (WGR), specific growth rate (SGR), protein efficiency ratio (PER), and condition factor (CF) were highest in the 20% cassava starch group, significantly higher than those in the 5% cassava starch group (P<0.05), whereas hepatosomatic index was lowest in the 20% cassava starch group, significantly lower than that in the 5% cassava starch group (P<0.05). With increasing dietary cassava starch levels, intestinal protease activity showed a decreasing trend, with the 5% cassava starch group being significantly higher than the 20% cassava starch group (P<0.05). Intestinal lipase activity in the 10% cassava starch group was significantly higher than that in both the 5% and 20% cassava starch groups (P<0.05), and intestinal amylase activity in the 10% cassava starch group was significantly higher than that in the 20% cassava starch group (P<0.05). Serum aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio, triglyceride, and total cholesterol contents all showed decreasing trends with increasing dietary cassava starch levels, whereas serum total protein and high-density lipoprotein cholesterol contents showed increasing trends. With increasing dietary cassava starch levels, hepatopancreatic AST and ALT activities both showed increasing trends, and hepatopancreatic AST

activity in the 20% cassava starch group was significantly higher than that in the 5% cassava starch group (P<0.05). Hepatopancreatic 6-phosphofructo-1-kinase and pyruvate kinase activities both showed increasing trends with increasing dietary cassava starch levels, and were significantly higher in the 20% cassava starch group than in the 5% cassava starch group (P<0.05). Hepatopancreatic glucose-6-phosphatase activity in the 20% cassava starch group was significantly lower than that in both the 5% and 10% cassava starch groups (P<0.05), and hepatopancreatic phosphoenolpyruvate carboxykinase activity in the 10% cassava starch group was significantly lower than that in both the 5% and 20% cassava starch groups (P<0.05). Based on the results obtained from this experiment and considering feed formulation costs, a cassava starch supplementation level of 10%–20% in common carp diets can meet the nutritional requirements for promoting fish growth, maintaining normal digestive capacity, protecting liver function, and effectively regulating the activities of key enzymes in glycolysis and gluconeogenesis.

Full Text

Effects of Dietary Cassava Starch Supplemental Level on Growth Performance, Digestive Ability and Glycometabolism of Common Carp

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Abstract: An 8-week feeding experiment was conducted to investigate the effects of dietary cassava starch supplemental level on growth performance, digestive ability and glycometabolism of common carp (*Cyprinus carpio*), and to evaluate the potential of cassava starch to spare fish meal in aquafeeds. Three iso-lipidic (crude lipid content ~6.7%) and iso-energetic (gross energy ~16 MJ/kg) experimental diets were formulated with 5% (control), 10% and 20% cassava starch based on practical feed formulation. Common carp with an average initial body weight of (55.73$±$3.55) g were stocked in cages and fed the experimental diets. Each diet was assigned to three replicates with 50 fish per replicate. The results showed that weight gain rate (WGR), specific growth rate (SGR), protein efficiency ratio (PER) and condition factor (CF) were highest in the 20% cassava starch group, significantly higher than those in the 5% cassava starch group (P<0.05). Conversely, hepatosomatic index (HSI) was lowest in the 20% cassava starch group, significantly lower than that in the 5% cassava starch group (P<0.05). Intestinal protease activity decreased with increasing dietary cassava starch level, with the 5% group being significantly higher than the 20% group (P<0.05). Intestinal lipase activity in the 10% cassava starch group was significantly higher than in both the 5% and 20% groups (P<0.05), while intestinal amylase activity in the 10% group was significantly higher than in the 20% group (P<0.05). Serum aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio and triglyceride and total cholesterol contents decreased with increasing cassava starch level, whereas serum total protein and high-density lipoprotein cholesterol contents increased. Hepatopancreas AST and ALT activities increased with dietary cassava starch level, with the 20% group showing significantly higher AST activity than the 5% group (P<0.05). Hepatopancreas phosphofructokinase-1 (PFK-1) and pyruvate kinase (PK) activities increased with cassava starch supplementation, with the 20% group being significantly higher than the 5% group (P<0.05). The 20% cassava starch group exhibited significantly lower glucose-6-phosphatase (G6Pase) activity compared to the 5% and 10% groups (P<0.05), while the 10% group showed significantly lower phosphoenolpyruvate carboxykinase (PEPCK) activity than the 5% and 20% groups (P<0.05). Based on these results and considering feed cost, dietary cassava starch supplementation at 10%–20% can promote fish growth, maintain normal digestive capacity, protect hepatic function, and effectively regulate key enzymes in glycolysis and gluconeogenesis.

Keywords: common carp; cassava starch; digestive ability; hepatic function; glycometabolism

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Carbohydrates serve as the primary energy source in aquafeeds due to their low cost, wide availability, and protein-sparing effects. However, fish exhibit relatively poor carbohydrate utilization compared to terrestrial mammals, attributed to insufficient insulin receptors, lack of hexokinase, and low glucokinase (GK) activity [1-2]. Previous studies have demonstrated that complex macromolecular carbohydrates like starch are more efficiently utilized by fish than simple sugars [3-4], stimulating active research on starch application in aquafeeds.

Common carp (*Cyprinus carpio*) is a dominant freshwater aquaculture species in China, characterized by rapid growth, strong adaptability, omnivorous feeding habits, tolerance to alkaline conditions and hypoxia, low disease incidence, and desirable meat quality. Previous studies have investigated optimal starch sources and levels for common carp, showing that 0-13% corn starch [5] or 10% wheat starch [6] in diets yielded favorable growth performance and digestive capacity. However, these studies focused on cereal starches, with limited research on tuber starches in common carp diets. Tuber starches generally have lower amylose/amylopectin ratios than cereal starches, containing more digestible amylopectin [7]. Cassava, known as the "king of starch," has been successfully applied in livestock feed, but its high linamarin content has restricted its use in aquafeeds [8-10]. Cassava starch, a processed product, overcomes this limitation and offers superior physicochemical properties compared to corn starch, including higher viscosity, penetrability, and film-forming capacity, along with lower crude protein and ash contents [11]. Recent studies have explored cassava and cassava starch in aquafeeds for Nile tilapia (*Oreochromis niloticus*) [8], largemouth bass (*Micropterus salmoides*) [9], and grass carp (*Ctenopharyngodon* *idella*) [10], demonstrating no growth inhibition and improved nutrient absorption and muscle quality. To date, no studies have examined cassava starch in common carp diets.

This study investigated the effects of dietary cassava starch levels on growth performance, digestive capacity, and glucose-lipid metabolism in common carp to determine optimal supplementation levels, providing data to support rational cassava starch application in aquafeeds and laying theoretical and practical foundations for optimizing common carp feed formulations and developing low-nitrogen, environmentally friendly diets.

1.1 Experimental Diet Preparation

Cassava starch was purchased from Dongguan Dongmei Food Co., Ltd. (Guangdong Province), meeting Grade 1 requirements of NY/T 875-2012 "Edible Cassava Starch" with moisture, crude protein, crude fat and crude ash contents (dry matter basis) of 12.30% (<14.0%), 0.13% (<0.3%), 0.11% (<0.2%) and 0.17% (<0.30%), respectively [12].

Three iso-lipidic (crude lipid ~6.7%) and iso-energetic (gross energy ~16 MJ/kg) experimental diets were formulated with fish meal (Peruvian, 64.0% crude protein), soybean meal (pre-pressed, 43.7% crude protein), rapeseed meal (pre-pressed, 36.2% crude protein), cottonseed meal (pre-pressed, 43.3% crude protein), and peanut meal (pre-pressed, 46.2% crude protein) as protein sources, soybean oil as lipid source, and cassava starch as carbohydrate source to contain 5% (control), 10% and 20% carbohydrate levels. Diet composition and nutrient levels are shown in Table 1 . All ingredients were ground to pass through a 60-mesh sieve, mixed thoroughly, then soybean oil was added. After complete mixing, the diets were processed into 3.00 mm sinking pellets using a MUZLM V4 pellet mill (Jiangsu Muyang Group) provided by Tianxiang Aquatic Co., Ltd., air-dried, sealed in plastic bags, and stored at -20°C.

Experimental diets were dried at 105°C to constant weight. Crude protein and crude lipid contents and gross energy were determined using the Dumas combustion method (GB/T 24318-2009), Soxhlet extraction method (GB/T 5009.6-2010), and a GR3500 oxygen bomb calorimeter, respectively.

1.2 Experimental Fish and Culture Management

The feeding trial was conducted at Tianxiang Aquatic Co., Ltd. in Tianjin. Experimental fish were obtained from the aquaculture base of Tianjin Agricultural University, disinfected, and acclimated in 3 m$×3*m*×$3 m cages for one week while being fed a commercial freshwater fish diet provided by Tianxiang Aquatic Co., Ltd.

After acclimation, 450 healthy common carp with uniform size (initial weight 55.73$±3.55*g*)*wereselectedandrandomlydividedinto*3*groupswith*3*replicatespergroup*(50*fishperreplicate*).*Th* *fedtwicedailyat*08 : 00*and*17 : 00*atafeedingrateof*4±$0.2, dissolved oxygen above 6.0 mg/L, ammonia nitrogen below 0.52 mg/L, and nitrite nitrogen below 0.08 mg/L.

1.3.1 Sample Preparation

At the start of the experiment, initial body weight was recorded. At the end, fish were fasted for 24 h before measuring final body weight. Fifteen fish were randomly selected from each replicate, weighed and measured for body length, then blood was collected via caudal vein. Blood from 3 fish was pooled into one centrifuge tube as a sample, anticoagulated with heparin sodium, centrifuged at 4,500 r/min for 15 min at 4°C to obtain serum, which was stored at -20°C. After blood collection, fish were dissected on ice to obtain hepatopancreas and intestine. Hepatopancreas weight was recorded for HSI calculation, and hepatopancreas and intestine samples were stored at -20°C.

Crude enzyme extracts were prepared by rinsing frozen hepatopancreas and intestine tissues with ice-cold 0.85% physiological saline, blotting dry, homogenizing in 9 volumes of ice-cold 0.85% saline, centrifuging at 4,500 r/min for 15 min at 4°C, and storing the supernatant at -80°C.

1.3.2 Index Determination and Methods

Serum biochemical indices and digestive enzyme activities in hepatopancreas and intestine were measured using assay kits from Nanjing Jiancheng Bioengineering Institute. Protease activity was determined by the Folin-phenol method, amylase activity by iodine-starch colorimetry, lipase activity by turbidimetry, and AST and ALT activities by the Reitman-Franke method. ELISA kits for glycometabolism key enzymes in hepatopancreas were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd.

1.3.3 Calculation Formulas

Weight gain rate (WGR, %) = 100 × (Wt - W0) / W0
Specific growth rate (SGR, %/d) = 100 × [ln(Wt) - ln(W0)] / t
Feed conversion ratio (FCR) = F / (Wt - W0)
Protein efficiency ratio (PER, %) = 100 × (Wt - W0) / (F × P)
Hepatosomatic index (HSI, %) = 100 × Wg / Wt
Condition factor (CF, %) = 100 × Wt / Lt

Where: W0 = initial body weight (g); Wt = final body weight (g); F = feed intake (dry weight, g); P = dietary crude protein content (%); Wg = hepatopancreas weight (g); Lt = final body length (cm); t = experimental duration (days).

1.4 Data Processing and Statistical Analysis

Data were analyzed using SPSS 17.0 software. One-way ANOVA was performed first, and if significant differences were detected among groups, Duncan’ s multiple range test was applied. Significance level was set at P<0.05. Data are presented as mean ± standard deviation.

2.1 Effects of Dietary Cassava Starch Level on Growth and Morphological Indices of Common Carp

As shown in Table 2 , WGR, SGR, PER and CF increased gradually with increasing dietary cassava starch level, while FCR and HSI decreased. The 20% cassava starch group exhibited the highest WGR, SGR, PER and CF, significantly higher than the 5% group (P<0.05), and the lowest FCR and HSI, significantly lower than the 5% group (P<0.05).

2.2 Effects of Dietary Cassava Starch Level on Intestinal Digestive Enzyme Activities of Common Carp

Table 3 shows that intestinal protease activity decreased with increasing cassava starch level, with the 5% group being significantly higher than the 20% group (P<0.05). Intestinal amylase and lipase activities increased first and then decreased, peaking at 10% cassava starch. Intestinal amylase activity in the 10% group was significantly higher than in the 20% group (P<0.05), while lipase activity in the 10% group was significantly higher than in both the 5% and 20% groups (P<0.05).

2.3 Effects of Dietary Cassava Starch Level on Serum and Hepatopancreas Transaminase Activities of Common Carp

Serum AST activity increased first and then decreased with increasing cassava starch level, peaking at 10% and being significantly higher than the 20% group (P<0.05) (Table 4). Serum ALT activity increased gradually, with the 20% group being significantly higher than the 5% group (P<0.05). The serum AST/ALT ratio decreased with increasing cassava starch level, with the 20% group being significantly lower than the 5% group (P<0.05).

As shown in Table 5 , hepatopancreas AST and ALT activities increased with dietary cassava starch level, peaking at 20%. Hepatopancreas AST activity in the 20% group was significantly higher than in the 5% group (P<0.05).

2.4 Effects of Dietary Cassava Starch Level on Serum Biochemical Indices of Common Carp

Serum total protein, glucose and high-density lipoprotein cholesterol contents increased with dietary cassava starch level, peaking at 20% and being significantly higher than the 5% group (P<0.05), except for glucose (Table 6). Serum triglyceride and total cholesterol contents decreased with increasing cassava starch level, with the 20% group being significantly lower than the 5% group (P<0.05). Dietary cassava starch level had no significant effect on serum urea nitrogen or low-density lipoprotein cholesterol contents (P>0.05).

2.5 Effects of Dietary Cassava Starch Level on Hepatopancreas Glycometabolism Key Enzyme Activities of Common Carp

Hepatopancreas PFK-1 and PK activities increased with dietary cassava starch level, peaking at 20% and being significantly higher than the 5% group ($P<0.05$) (Table 7).

Hepatopancreas G6Pase activity decreased with increasing cassava starch level, with the 20% group being significantly lower than the 5% and 10% groups ($P<0.05$) (Table 8). Hepatopancreas PEPCK activity decreased first and then increased, with the 10% group being significantly lower than the 5% and 20% groups ($P<0.05$).

3.1 Effects of Dietary Cassava Starch Level on Growth Performance of Common Carp

The results demonstrate that dietary cassava starch supplementation not only failed to negatively affect growth performance but also improved weight gain and feed utilization with increasing levels. These findings confirm that cassava starch can be applied in aquafeeds to spare fish meal, minimizing fish meal usage while optimizing fish growth. These results align with studies by Tian et al. [13] on cassava meal in tilapia and Olurin et al. [14] on cassava in African catfish (*Clarias gariepinus*), indicating that cassava products can promote growth across different feeding guilds. However, Ma et al. [15] reported decreased growth performance in snakehead (*Channa argus*) with increasing cassava meal, likely due to higher antinutritional factors (condensed tannins, linamarin) in their unprocessed cassava meal compared to the biotechnologically treated cassava starch used in this study.

Sun et al. [5] found that increasing corn starch levels decreased feed utilization but increased PER in common carp, suggesting that corn starch promoted efficient protein absorption despite limited feed efficiency. Our study showed similar trends, but with distinct differences in protein utilization. WGR, SGR and CF increased while HSI decreased with cassava starch level. HSI is sensitive to both long- and short-term nutritional status and serves as an indicator of lipid or glycogen accumulation in liver and viscera, while CF reflects health status and feeding intensity [16]. The improved CF without increased HSI indicates that enhanced growth resulted from improved feed conversion due to optimal carbohydrate levels rather than protein or carbohydrate conversion to liver/visceral fat or glycogen. This supports the protein-sparing effect of dietary carbohydrates. The contrasting results with corn starch are attributable to differences in amylose/amylopectin ratios, consistent with Yang [17]. Compared to terrestrial animals, fish have relatively low carbohydrate utilization capacity, with recommended dietary carbohydrate levels below 20% for marine fish and 40% for freshwater fish. The optimal performance at 20% cassava starch in this study warrants further investigation at higher inclusion levels.

3.2 Effects of Dietary Cassava Starch Level on Digestive Ability of Common Carp

Digestibility is the primary limiting factor for starch utilization in fish. Amylase activity reflects the capacity to digest dietary starch. Intestinal amylase activity increased then decreased with cassava starch level, peaking at 10%, consistent with Sun et al. [5] but at a different optimal level (6.5% for corn starch), indicating species-specific differences in starch utilization. Starch granule size and amylose/amylopectin ratio are key factors affecting starch digestion [18]. Amylopectin is more readily hydrolyzed, making low amylose/amylopectin ratios more favorable for fish [19-20]. Cassava starch contains more amylopectin than corn starch, with an amylose:amylopectin ratio of 20:80 [21], explaining the superior digestibility observed in this study.

Intestinal lipase activity increased then decreased, while protease activity decreased with cassava starch level. Qiang et al. [22] reported increased amylase and protease activities with carbohydrate intake in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), differing from our results possibly due to variations in dietary carbohydrate levels, fish species, feeding frequency, or developmental stage.

3.3 Effects of Dietary Cassava Starch Level on Hepatic Function of Common Carp

Transaminases play crucial roles in interconverting proteins, lipids and carbohydrates, with AST and ALT being the most important [23]. The decreasing serum AST/ALT ratio with cassava starch level indicates reduced liver damage, suggesting hepatoprotective effects. This aligns with Zhang et al. [24] in tilapia fed fermented cassava residue. Ma et al. [15] reported potential liver damage with high cassava meal inclusion in snakehead, likely due to higher antinutritional factors in their unprocessed material. Increased hepatopancreas AST and ALT activities with cassava starch level further support enhanced hepatic protein metabolism and amino acid utilization with reduced liver injury.

3.4 Effects of Dietary Cassava Starch Level on Serum Biochemical Indices of Common Carp

Serum biochemical indices reflect metabolic status and nutritional health [25]. Serum glucose increased with cassava starch level, consistent with studies on Japanese seabass (*Lateolabrax japonicus*) [26] and large yellow croaker (*Larimichthys crocea*) [27], confirming positive correlation between dietary starch and serum glucose. The lack of significant effect in snakehead [15] may reflect species differences or inclusion levels.

Blood lipids, including triglycerides, phospholipids and cholesterol, are transported as lipoproteins. Fish synthesize cholesterol primarily in the liver, with 70-80% of blood cholesterol originating hepatically; liver dysfunction rapidly elevates blood cholesterol [28]. Decreased serum triglycerides and total cholesterol with cassava starch indicate lipid-lowering and hepatoprotective effects. High-density lipoprotein cholesterol (HDL-C) functions as a lipid scavenger ("good" cholesterol), while oxidized low-density lipoprotein cholesterol (Ox-LDL-C) is considered "bad" cholesterol [29]. The increased HDL-C and decreased LDL-C with cassava starch suggest modulation of cholesterol fractions to suppress hyperlipidemia, though the molecular mechanisms require further investigation.

Serum total protein reflects dietary protein supply, absorption efficiency and protein metabolism. The increased total protein with cassava starch level (despite reduced dietary protein) indicates enhanced protein absorption efficiency, confirming the protein-sparing effect of carbohydrates. Decreased serum urea nitrogen with cassava starch level suggests reduced protein metabolic burden without compromising growth, consistent with studies on grass carp [30] and jundiá (*Rhamdia quelen*) [31].

3.5 Effects of Dietary Cassava Starch Level on Glycometabolism of Common Carp

Glycolysis is the primary glucose metabolic pathway in fish, converting one glucose molecule to two pyruvate molecules via three key regulatory enzymes: hexokinase, PFK-1 and PK. High-carbohydrate, low-protein diets increased hepatic PFK-1 activity in rainbow trout (*Oncorhynchus mykiss*) [32], consistent with our findings in common carp and with studies on gilthead seabream (*Sparus aurata*) [33]. Dietary carbohydrate effects on PK activity vary among species. The increased PK activity with cassava starch in this study aligns with grass carp [34] but contrasts with large yellow croaker [27], possibly due to species, starch type or inherent metabolic differences.

Gluconeogenesis converts non-carbohydrate precursors (lactate, glycerol, glucogenic amino acids) to glucose, but is not a simple reversal of glycolysis. Key regulatory enzymes include G6Pase, fructose-1,6-bisphosphatase and PEPCK. Increased cassava starch level decreased G6Pase and PEPCK activities, consistent with Panserat et al. [35] in common carp but differing from large yellow croaker [29], likely due to species, starch type or feeding habits. These results indicate adaptive enhancement of glycolysis and regulatory suppression of gluconeogenesis with high cassava starch, low-protein diets. However, the concurrent increase in serum glucose suggests that common carp' s capacity to maintain glucose homeostasis through metabolic regulation requires further improvement.

Under the conditions of this study, dietary cassava starch supplementation at 10%-20% promoted growth, enhanced starch and lipid digestion, protected hepatic health, improved glycometabolism, and achieved fish meal sparing in common carp.

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Note: Figure translations are in progress. See original paper for figures.