Effects of Cassava Meal on Nutrient Apparent Digestibility, Intestinal and Hepatopancreatic Digestive Enzyme Activities, and Serum Non-specific Immune Indices in Tilapia (Postprint)

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Abstract

This experiment aimed to investigate the effects of different dietary inclusion levels of cassava flour on the apparent digestibility of nutrients, digestive enzyme activities in the intestine and hepatopancreas, and serum non-specific immune indices in tilapia. A total of 540 healthy tilapia with an average body weight of (7.50±0.25) g were randomly divided into 6 groups (3 replicates per group, 30 fish per replicate) and fed isocaloric and isonitrogenous experimental diets containing cassava flour at inclusion levels of 0 (control), 4%, 8%, 12%, 16%, and 20% for 8 weeks. The results showed that: compared with the control group, dietary cassava flour inclusion at 4%-20% had no significant effect on the apparent digestibility of crude fat and calcium in tilapia ($P > 0.05$); dietary cassava flour inclusion at 4%-16% had no significant effect on the apparent digestibility of dry matter, crude protein, crude ash, and total phosphorus ($P > 0.05$); dietary cassava flour inclusion at 4%-12% had no significant effect on protease activity in the intestine and hepatopancreas of tilapia ($P > 0.05$); cassava flour inclusion levels up to 20% could increase ($P > 0.05$) or significantly increase ($P < 0.05$) the activities of lipase and amylase in the intestine and hepatopancreas; cassava flour inclusion levels of 4%-12% could increase the activities of serum lysozyme and superoxide dismutase, but the difference was not significant ($P > 0.05$), and had no significant effect on other serum non-specific immune indices ($P > 0.05$). Based on comprehensive evaluation of the results, at the dietary nutrient levels used in this experiment, cassava flour inclusion levels of 4%-12% in tilapia diets would not produce adverse effects on the apparent digestibility of nutrients, digestive enzyme activities in the intestine and hepatopancreas, or serum non-specific immune indices in tilapia, and could reduce feed costs.

Full Text

Preamble

Effects of Cassava Meal on Nutrient Apparent Digestibility, Digestive Enzyme Activities in Intestine and Hepatopancreas, and Serum Non-specific Immune Indices of Tilapia

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Abstract: This study aimed to investigate the effects of different dietary supplementation levels of cassava meal on nutrient apparent digestibility, digestive enzyme activities in the intestine and hepatopancreas, and serum non-specific immune indices of tilapia. A total of 540 healthy tilapia with an average body weight of (7.50±0.25) g were randomly allocated into 6 groups (3 replicates per group, 30 fish per replicate) and fed isoenergetic and isonitrogenous experimental diets containing 0% (control), 4%, 8%, 12%, 16%, or 20% cassava meal for 8 weeks. The results showed that compared with the control group, dietary inclusion of 4%–20% cassava meal had no significant effect on the apparent digestibility of ether extract and calcium (P>0.05). Inclusion of 4%–16% cassava meal did not significantly affect the apparent digestibility of dry matter, crude protein, ash, or total phosphorus (P>0.05). Dietary cassava meal at 4%–12% had no significant effect on protease activities in the intestine and hepatopancreas (P>0.05). Cassava meal inclusion up to 20% improved (P>0.05) or significantly improved (P<0.05) the activities of lipase and amylase in both intestine and hepatopancreas. When dietary cassava meal supplementation ranged from 4% to 12%, serum lysozyme and superoxide dismutase activities were enhanced, though not significantly (P>0.05), and other serum non-specific immune indices remained unaffected (P>0.05). Based on comprehensive evaluation under the dietary nutrient levels employed in this trial, cassava meal supplementation at 4%–12% does not adversely affect nutrient apparent digestibility, digestive enzyme activities in the intestine and hepatopancreas, or serum non-specific immune indices of tilapia, and can reduce feed costs.

Keywords: tilapia; cassava meal; digestibility; digestive enzyme; non-specific immune

Cassava is a crop primarily grown in tropical and subtropical regions, characterized by drought tolerance, adaptability to poor soils, and high yield. Together with sweet potato and potato, it is known as one of the world' s three major tuber crops. In recent years, the proportion of grains in livestock and poultry feeding has increased substantially, and rising grain prices have gradually elevated production costs. Consequently, reducing grain usage in animal production has become a research priority. Cassava meal, with its high starch and

low protein content, has emerged as a research focus for unconventional energy feed ingredients. Studies have demonstrated the feasibility of using cassava meal in dairy cows [1], meat ducks [2], and shrimp [3], while microbial fermentation can improve product quality [4]. Research has shown that fermented unpeeled cassava meal can improve metabolizable energy in meat cockerels [5], and that inclusion of cassava meal in weaner rabbit diets does not adversely affect growth performance or economic returns [6]. Although studies on cassava meal in aquatic animals such as tilapia [7], snakehead [8], and shrimp [3] exist, most have focused on feeding effects rather than providing in-depth investigation of its application in aquaculture. Building on previous research, this trial primarily examined the effects of different inclusion levels of cassava meal on nutrient apparent digestibility, digestive enzyme activities, and non-specific immune indices in tilapia to provide a theoretical basis for its application in tilapia culture.

1.1 Experimental Animals and Diets

Experimental tilapia were purchased from the Wuchan Base of Zhanjiang Guolian Aquatic Products Development Co., Ltd., and were GIFT (Genetically Improved Farmed Tilapia) strain. Cassava meal was purchased from Hainan Province, with laboratory analysis showing dry matter, crude protein, ether extract, crude fiber, ash, and nitrogen-free extract contents of 87.45%, 2.34%, 1.05%, 2.22%, 1.82%, and 80.02%, respectively. Six isoenergetic and isonitrogenous experimental diets were formulated with cassava meal inclusion levels of 0% (Group D0, control), 4% (Group D4), 8% (Group D8), 12% (Group D12), 16% (Group D16), and 20% (Group D20). Diet composition and nutrient levels are presented in Table 1 .

Table 1 Composition and Nutrient Levels of Experimental Diets

Item	Groups
Ingredients (air-dry basis)	
Fish meal	
Soybean meal	
Colza meal	
Cottonseed meal	
Cassava meal	
Wheat middling	
Wheat bran	
Soybean oil	
Fish oil	
Premix[1])	
Total	
Nutrient levels (DM basis)[2])	
Crude protein (CP)	
Ether extract (EE)	

Item	Groups
Ash	
Nitrogen-free extract (NEF)	

[1]) The premix provided the following per kilogram of diets: VA 7,500 IU, VD 3,600 IU, VK 180 IU, VE 150 IU, VC 450 mg, VB 30 mg, VB 30 mg, VB 60 mg, VB 0.45 mg, nicotinic acid 120 mg, folic acid 15 mg, calcium pantothenate 60 mg, inositol 450 mg, biotin 0.6 mg, MgSO ·7H O 150 mg, KCl 35 mg, KI 0.75 mg, ZnSO ·7H O 7 mg, MnSO ·4H O 1.5 mg, CuCl 2.5 mg, CoCl ·6H O 0.25 mg, FeSO ·7H O 7.5 mg, KH PO ·H O 2,250 mg, CaCl 1,400 mg.
[2]) Nutrient levels were measured values.

1.2 Feeding Management

A total of 540 healthy tilapia with an average body weight of (7.50±0.25) g were randomly divided into 6 groups, with 3 replicates per group and 30 fish per replicate. The experiment was conducted in 200 L tanks with continuous aeration. Water was exchanged every 3 days (using aerated tap water) at one-third of the total volume. Prior to the trial, all fish were acclimated for 2 weeks using the control diet. During the 8-week experimental period, fish were fed 4 times daily at 08:00, 12:00, 16:00, and 20:00 at 8% of total body weight per feeding.

1.3 Sample Collection and Processing

1.3.1 Feces Collection and Processing

Residual feed was removed 1 hour after feeding. Fresh, well-formed feces were collected by siphoning 4 hours post-feeding, then placed on dry filter paper and oven-dried at (65±5)°C for 24 hours to prepare air-dried samples for analysis.

1.3.2 Intestine and Hepatopancreas Collection and Processing

At the end of the trial, 3 fish per replicate (9 fish per group) were randomly selected and dissected on ice. Intestines and hepatopancreases were isolated, adipose tissue was removed, and intestinal contents and hepatopancreases were quickly collected into centrifuge tubes, labeled, and stored at -20°C.

1.3.3 Serum Collection and Processing

At the end of the trial, 3 fish per replicate (9 fish per group) were randomly selected. Five milliliters of blood were collected from the caudal vein of each fish, allowed to clot at 4°C for 1.0–1.5 hours, then centrifuged at 3,000 r/min for 1 minute. The supernatant serum was collected and stored at -20°C.

1.4 Analytical Methods

1.4.1 Nutrient Apparent Digestibility Analysis

Apparent digestibility of dry matter, crude protein, ether extract, ash, calcium, and total phosphorus in diets containing different cassava meal levels was determined using the endogenous indicator method with 4N-hydrochloric acid insoluble ash (4N-AIA) as the indicator [9].

4N-AIA Content Determination: Approximately 10–12 g (weighed to 0.0001 g precision) of dried, ground sample (Ws, corrected to dry weight) was placed in a 500 mL Erlenmeyer flask in duplicate. 4N-HCl was added at a ratio of 10 mL per gram of sample. The mixture was slowly boiled for 30 minutes on a hot plate in a fume hood with a reflux condenser attached to prevent loss of HCl and H O. The solution was filtered through rapid quantitative filter paper (120 mm diameter) and washed with 85–100 mL distilled water until neutral. The filter paper containing residue was transferred to a pre-weighed 100 mL crucible (We) and ashed in a muffle furnace at 650°C for 6 hours. After ashing, the crucible was cooled to room temperature in a desiccator and reweighed (Wf). 4N-AIA content was calculated as:

4N-AIA content (%) = 100 × (Wf - We) / Ws

where Wf = weight of crucible + 4N-AIA (g), We = weight of empty crucible (g), and Ws = sample weight (g).

Nutrient Apparent Digestibility Calculation:

Apparent digestibility of a nutrient (%) = 100 - 100 × [4N-AIA in diet (%) / 4N-AIA in feces (%)] × [Nutrient in feces (%) / Nutrient in diet (%)].

1.4.2 Digestive Enzyme Activity Analysis

Frozen intestine and hepatopancreas samples stored at -20°C were sent to Zhanjiang Nongken Hospital for determination of protease, lipase, and amylase activities.

1.4.3 Serum Non-specific Immune Index Analysis

Serum superoxide dismutase activity was measured by xanthine oxidase method, lysozyme activity by turbidimetry, alkaline phosphatase activity by p-nitrophenyl phosphate method, and catalase activity by spectrophotometry. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.4 Statistical Analysis

Data were initially processed using Excel 2007, then subjected to one-way ANOVA using SPSS 13.0 software. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as mean ± standard deviation (mean±SD). Differences were considered significant at $P<0.05$.

2.1 Effects of Cassava Meal on Nutrient Apparent Digestibility of Tilapia

As shown in Table 2 , the apparent digestibility of dry matter in groups D4, D8, D12, and D16 did not differ significantly from the control group (P>0.05), while group D20 showed the lowest dry matter digestibility, significantly lower than the control, D4, and D8 groups (P<0.05). The control group exhibited higher crude protein digestibility than all treatment groups, with significant differences observed in D16 and D20 compared to the control (P<0.05), while D4, D8, and D12 showed no significant differences (P>0.05). For ether extract digestibility, no significant differences were detected between the control and any treatment groups (P>0.05), though D4 and D12 groups were 0.97% and 1.40% higher than the control, respectively. The apparent digestibility of ash in the control and D4 groups was significantly higher than in D12, D16, and D20 groups (P<0.05). Calcium digestibility did not differ significantly between the control and any treatment groups (P>0.05), although D12 was higher than the control. The control group showed significantly higher total phosphorus digestibility than D16 and D20 groups (P<0.05), with no significant differences from D4, D8, and D12 groups (P>0.05).

Table 2 Effects of Cassava Meal on Apparent Digestibility of Nutrients of Tilapia (%)

Groups	Dry Matter	Crude Protein	Ether Extract	Ash	Calcium	Total Phosphorus
Control	60.12±1.66a	73.38±2.30a	79.46±1.26	37.11±3.82a	71.72±1.36	60.16±1.43a
D4	58.81±2.32a	72.55±1.66ab	80.43±1.77	36.91±3.87a	74.03±1.55	59.01±0.71ab
D8	59.22±1.48a	71.16±2.53ab	79.02±2.23	35.98±2.70ab	72.42±0.86	60.98±0.82ab
D12	57.69±2.61ab	71.70±1.79ab	80.86±1.46	34.87±3.98b	74.81±0.92	58.22±2.36ab
D16	57.86±2.29ab	70.22±1.99b	78.46±1.06	34.00±3.73b	73.78±0.65	57.98±1.36b
D20	55.11±1.68b	70.56±0.76b	79.11±2.61	34.68±3.78b	73.86±0.85	57.31±2.25b

In the same column, values with the same or no letter superscripts indicate no significant difference (P>0.05), while different lowercase letters indicate significant difference (P<0.05). The same applies below.

2.2 Effects of Cassava Meal on Digestive Enzyme Activities in Intestine and Hepatopancreas of Tilapia

As shown in Table 3 , the control group exhibited the highest intestinal protease activity, with no significant differences from D4, D8, and D12 groups (P>0.05), while D16 and D20 groups were significantly lower than the control (P<0.05). Except for D20, all treatment groups showed higher intestinal amylase activity than the control, though no significant differences were observed among groups (P>0.05). All treatment groups demonstrated higher intestinal

lipase activity than the control, with D12 and D16 groups showing significant differences (P<0.05).

Table 3 Effects of Cassava Meal on Digestive Enzyme Activities in Intestine of Tilapia

Groups	Protease (U/g)	Amylase (U/g)	Lipase (U/mg)
Control	561.16±24.08a	0.25±0.03	25.33±1.88b
D4	532.07±19.62ab	0.27±0.04	26.97±1.42ab
D8	542.41±33.04ab	0.27±0.05	27.00±1.14ab
D12	537.25±25.48ab	0.29±0.02	28.25±0.62a
D16	513.77±17.69b	0.29±0.02	28.44±0.72a
D20	515.68±23.17b	0.25±0.02	25.81±1.41b

As shown in Table 4 , different dietary cassava meal levels affected hepatopancreatic protease activity, though no significant differences were observed (P>0.05). The highest hepatopancreatic protease activity was observed in D8 group, while the lowest was in D4 group. Hepatopancreatic amylase activity in the control group was comparable to D4 and D8 groups (P>0.05), while D16 group showed the highest activity, significantly higher than the other five groups (P<0.05). Compared with the control, all cassava meal inclusion levels increased hepatopancreatic lipase activity. When inclusion levels were within 16%, lipase activity increased progressively with supplementation level, with enhancement rates of 6.47% (P>0.05), 6.60% (P>0.05), 9.00% (P<0.05), and 12.28% (P<0.05) in D4, D8, D12, and D16 groups, respectively. Activity declined at 20% inclusion, with D12 and D16 groups showing significantly higher activity than D20 group (P<0.05).

Table 4 Effects of Cassava Meal on Digestive Enzyme Activities in Hepatopancreas of Tilapia

Groups	Protease (U/g)	Amylase (U/g)	Lipase (U/mg)
Control	45.59±5.58	0.08±0.01b	25.88±1.63b
D4	43.80±2.74	0.08±0.01b	26.00±1.67ab
D8	49.24±3.70	0.08±0.02b	28.55±1.98ab
D12	44.69±3.88	0.09±0.01b	29.77±1.64a
D16	45.26±4.05	0.11±0.02a	31.20±2.35a
D20	47.54±3.87	0.07±0.01b	26.59±1.11b

2.3 Effects of Cassava Meal on Serum Non-specific Immune Indices of Tilapia

As shown in Table 5 , serum lysozyme activity in D12, D16, and D20 groups increased by 5.56%, 1.81%, and 5.38% compared with the control, respectively,

though no significant differences were observed among groups (P>0.05). Serum superoxide dismutase activity in D4 and D12 groups increased by 3.05% and 2.90% compared with the control, while other treatment groups showed lower activity, but no significant differences were detected (P>0.05). No significant differences in serum alkaline phosphatase activity were observed among groups (P>0.05), though all treatment groups showed higher activity than the control, with D4 group exhibiting the highest activity (10.61% above control) and D16 the lowest (4.80% above control). Serum acid phosphatase activity in D4 and D12 groups was higher than the control, while other groups were lower, but no significant differences were observed (P>0.05). All cassava meal inclusion levels reduced serum catalase activity, though no significant differences were detected (P>0.05).

Table 5 Effects of Cassava Meal on Non-specific Immune Indices in Serum of Tilapia

Groups	Lysozyme (U/mL)	Superoxide Dismutase (U/mL)	Alkaline Phosphatase (U/L)	Acid Phosphatase (U/mL)	Catalase (U/mL)
Control	81.26±5.71	102.17±7.72	35.62±5.63	22.69±2.71	3.05±0.17
D4	74.43±7.98	105.29±5.79	39.40±4.01	22.09±3.50	2.87±0.14
D8	80.26±4.22	101.90±12.38	37.51±3.67	23.72±4.66	2.80±0.23
D12	85.78±5.45	105.14±13.17	37.85±6.49	22.84±3.05	2.88±0.22
D16	82.73±2.78	99.94±13.68	37.33±2.42	20.75±2.56	2.83±0.28
D20	85.63±4.65	96.89±3.76	38.14±6.68	21.23±2.49	2.83±0.19

3.1 Effects of Cassava Meal on Nutrient Apparent Digestibility of Tilapia

Cassava meal has high starch content and can produce high-quality food after special processing [10]. Wu et al. [1] reported that replacing corn with cassava meal in dairy cow diets increased milk yield and milk fat percentage while reducing production costs and improving economic benefits. Lü et al. [2] found that inclusion of 10%–20% cassava meal in diets significantly improved apparent metabolizable energy and dry matter apparent digestibility in meat ducks. Deng and Wu [3] observed that 3% replacement of high-gluten wheat flour with cassava meal yielded the highest weight gain in Pacific white shrimp, with weight gain decreasing as cassava meal inclusion increased. Tian et al. [11] reported that Nile tilapia showed significantly higher apparent digestibility of dry matter and ash from cassava meal than from wheat middling, comparable to that from fish meal and soybean meal, while apparent digestibility of crude protein and ether extract did not differ significantly from soybean meal but was significantly lower than from fish meal. Our results indicated that cassava meal inclusion up to 20% had no significant effect on apparent digestibility of ether extract and calcium in tilapia, and inclusion of 4%–12% did not significantly affect appar-

ent digestibility of crude protein, ash, or total phosphorus. However, further increasing inclusion levels significantly reduced apparent digestibility of crude protein, ash, and total phosphorus. Tian et al. [7] fed GIFT tilapia diets containing different cassava meal levels and found that cassava meal improved protein utilization, with 40% inclusion significantly increasing apparent digestibility of crude protein and dry matter. These findings differ from our results, possibly due to different dietary nutrient levels. Our diets contained 30% crude protein, whereas Tian et al. [7] used 32% crude protein. Additionally, Tian et al. [7] used higher proportions of fish meal and wheat middling without wheat bran, while our diets contained lower proportions of fish meal and wheat middling with some wheat bran. Research indicates that tilapia utilize animal protein more efficiently than plant protein. Ma et al. [8] reported that cassava meal inclusion up to 12% in snakehead diets did not adversely affect growth performance or liver health, consistent with our findings.

3.2 Effects of Cassava Meal on Digestive Enzyme Activities in Intestine and Hepatopancreas of Tilapia

Tilapia, native to Africa, is characterized by rapid growth, broad feeding habits, good meat quality, and strong disease resistance, and has been designated a priority freshwater aquaculture species by China' s Ministry of Agriculture [12-13]. Fish digestive enzymes primarily include protease, amylase, lipase, and cellulase, which are secreted by the digestive system for nutritional and digestive functions. In fish, protease secretion sites vary among species, while lipase and amylase are mainly secreted by the hepatopancreas [14]. Zhang et al. [15] demonstrated that cassava meal diets resulted in higher amylase, protease, and lipase activities in sipunculid larvae compared to potato starch and corn starch diets, indicating that cassava meal can enhance these enzyme activities. Our study also found that cassava meal inclusion at 4%–16% increased amylase and lipase activities but showed no trend of increasing protease activity, possibly because we used isonitrogenous diets with similar protein levels across all groups. Lu et al. [16] reported that dietary protein level affects intestinal protease activity in yellow drum larvae. Our results showed that as cassava meal inclusion increased from 4% to 16%, intestinal and hepatopancreatic amylase and lipase activities gradually increased, suggesting that appropriate cassava meal supplementation can improve tilapia' s ability to digest starch and lipid. Although different cassava meal levels reduced intestinal protease activity, 8% and 20% inclusion increased hepatopancreatic protease activity.

3.3 Effects of Cassava Meal on Serum Non-specific Immune Indices of Tilapia

The non-specific immune system operates independently of the specific immune system. As fish have incompletely developed specific immune systems, they rely primarily on non-specific immunity for defense. Lysozyme, as a non-specific immune factor, plays an important role in fish immunity by clearing bacterial

debris remaining after the action of other antibacterial factors, enhancing sensitivity of other immune factors to bacteria, and synergistically resisting bacterial invasion [17]. Our results showed that different cassava meal inclusion levels had no significant effect on serum lysozyme activity, indicating that inclusion up to 20% does not adversely affect tilapia' s immune mechanisms. Superoxide dismutase is an important antioxidant in animals with anti-inflammatory, anti-aging, anti-radiation, and anti-viral functions, and is clinically used as a non-specific auxiliary diagnostic indicator. Its primary mechanism involves catalyzing superoxide anions into hydrogen peroxide and oxygen, thereby scavenging oxidative free radicals [18]. Our results showed that serum superoxide dismutase activity in the 4% and 12% inclusion groups was higher than the control, suggesting that appropriate cassava meal levels can enhance tilapia' s immune function. Alkaline phosphatase and acid phosphatase are phosphomonoesterases and important detoxification systems in animals. Widely distributed in the biological world, they are essential components of animal lysosomes and play important roles in immune responses [19]. Alkaline phosphatase is closely associated with calcium and phosphorus absorption and bone formation, serving as an important regulatory enzyme in metabolic processes. In our trial, all treatment groups showed higher serum alkaline phosphatase activity than the control, indicating that cassava meal can improve tilapia' s immune function. Catalase, like superoxide dismutase, has antioxidant functions. After superoxide dismutase dismutates superoxide anion radicals to hydrogen peroxide, catalase primarily catalyzes hydrogen peroxide to water and oxygen, with both enzymes working synergistically to protect the organism [19]. Our results showed that serum catalase activity in the control group was higher than all treatment groups, suggesting lower superoxide anion levels and less cellular damage in treatment groups.

Based on our results, under the dietary nutrient levels employed in this trial, cassava meal inclusion at 4%–12% does not adversely affect nutrient apparent digestibility, digestive enzyme activities in the intestine and hepatopancreas, or serum non-specific immune indices of tilapia, and can reduce feed costs.

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