

Effects of Fermented Mulberry Leaf Supplementation in High-Fat Diet on Growth Performance, Body Composition, and Serum Biochemical Parameters of Hybrid Snakehead (Postprint)

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Abstract

This experiment aimed to investigate the effects of fermented mulberry leaves supplementation in high-fat feed on the growth performance, body composition, and serum biochemical indices of hybrid snakehead fish. A total of 360 healthy hybrid snakehead fish with an average body weight of 10 g were selected and randomly divided into 3 groups, with 4 replicates per group and 30 fish per replicate. Each group was fed experimental diets containing 0 (control group), 7.5%, and 15.0% fermented mulberry leaves in high-fat feed, respectively. The experimental period lasted for 8 weeks. The results showed: 1) Compared with the control group, the specific growth rate (SGR) and protein efficiency ratio (PER) of hybrid snakehead fish in the 15.0% fermented mulberry leaves group were significantly decreased ($P < 0.05$), while the feed conversion ratio (FCR) was significantly increased ($P < 0.05$); the SGR, PER, and FCR of hybrid snakehead fish in the 7.5% fermented mulberry leaves group showed no significant differences ($P > 0.05$). There were no significant differences in feeding rate (FR) among all groups ($P > 0.05$). 2) Compared with the control group, the whole-body crude fat content, hepatosomatic index, viscerosomatic index, and intraperitoneal fat ratio of hybrid snakehead fish in both the 7.5% and 15.0% fermented mulberry leaves groups were significantly decreased ($P < 0.05$), and the liver fat content of hybrid snakehead fish in the 15.0% fermented mulberry leaves group was significantly decreased ($P < 0.05$). There were no significant differences in whole-body crude ash, crude protein, or muscle fat content among all groups ($P > 0.05$). 3) Compared with the control group, the serum aspartate aminotransferase and alanine aminotransferase activities, as well as total cholesterol and triglyceride contents of hybrid snakehead fish in the 15.0% fermented mulberry leaves group were significantly decreased ($P < 0.05$); the blood glu-

cose content of hybrid snakehead fish in both the 7.5% and 15.0% fermented mulberry leaves groups was significantly decreased ($P < 0.05$). There were no significant differences in serum total protein content among all groups ($P > 0.05$). These results indicate that supplementation of 7.5% fermented mulberry leaves in high-fat feed does not affect the growth performance of hybrid snakehead fish, while 15.0% fermented mulberry leaves inhibits growth but benefits liver health and improves glucose and lipid metabolism.

Full Text

Effects of High-Fat Diet Supplemented with Fermented Mulberry Leaves on Growth Performance, Body Composition and Serum Biochemical Indexes of Hybrid Snakehead

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with fermented mulberry leaves in a high-fat diet on growth performance, body composition, and serum biochemical indices of hybrid snakehead. A total of 360 healthy hybrid snakehead with an average body weight of 10 g were randomly allocated into three groups, each consisting of four replicates of 30 fish. The three experimental groups were fed high-fat diets supplemented with 0% (control), 7.5%, and 15.0% fermented mulberry leaves, respectively, for eight weeks. The results showed that: (1) Compared with the control group, the 15.0% fermented mulberry leaves group exhibited significantly decreased specific growth rate (SGR) and protein efficiency ratio (PER) ($P < 0.05$), along with a significantly increased feed conversion ratio (FCR) ($P < 0.05$). No significant differences in SGR, PER, or FCR were observed between the 7.5% fermented mulberry leaves group and the control group ($P > 0.05$). Feeding rate (FR) did not differ significantly among all groups ($P > 0.05$). (2) The whole-body crude lipid content, hepatosomatic index (HSI), viscerosomatic index (VSI), and intraperitoneal fat ratio (IPF) were significantly lower in both the 7.5% and 15.0% fermented mulberry leaves groups compared with the control group ($P < 0.05$). Additionally, the 15.0% fermented mulberry leaves group showed significantly reduced liver lipid content ($P < 0.05$). No significant differences were detected among groups in whole-body crude ash, whole-body crude protein, or muscle

lipid content ($P > 0.05$). (3) Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, as well as total cholesterol (TC) and triglyceride (TG) concentrations, were significantly decreased in the 15.0% fermented mulberry leaves group compared with the control group ($P < 0.05$). Blood glucose content was significantly reduced in both the 7.5% and 15.0% fermented mulberry leaves groups ($P < 0.05$). Serum total protein (TP) content did not differ significantly among groups ($P > 0.05$). In conclusion, supplementation with 7.5% fermented mulberry leaves in a high-fat diet does not adversely affect growth performance of hybrid snakehead, whereas 15.0% supplementation inhibits growth but promotes liver health and improves glucolipid metabolism.

Key words: fermented mulberry leaves; high-fat diet; hybrid snakehead; growth; metabolism

Hybrid snakehead is an important freshwater aquaculture species in China. Currently, commercial feeds for intensively cultured hybrid snakehead (typically yielding 45,000–75,000 kg per hectare) contain more than 8% lipid, which often leads to metabolic disorders and excessive fat deposition in the liver, thereby compromising the healthy development of the hybrid snakehead industry. Nutritionists have been actively exploring nutritional strategies to ameliorate or regulate glucolipid metabolism disorders in farmed fish. In recent years, mulberry leaves have attracted considerable research attention due to their abundant bioactive components such as polysaccharides, flavonoids, and alkaloids, as well as their rich resource availability. Although reported effects of mulberry leaves on animal growth performance have been inconsistent, their hypoglycemic and hypolipidemic efficacy is widely recognized. Our previous research has also confirmed that mulberry leaves can effectively regulate lipid metabolism in fish. To further investigate the regulatory effects of mulberry leaves on fat metabolism, this study formulated a basal diet containing 14% lipid to examine the impacts of fermented mulberry leaf supplementation in a high-fat diet on growth performance, body composition, and serum biochemical indices of hybrid snakehead, aiming to accumulate data for the feed application development of mulberry leaves and provide new insights for optimizing aquafeed formulation techniques.

1.1 Experimental Diets

The nutrient composition of mulberry leaves was: dry matter 31.2%, crude protein 21.5%, crude lipid 4.6%, soluble sugar 18.6%, and crude fiber 21.8%. Fermented mulberry leaves were produced through solid-state fermentation of mulberry leaves using lactic acid bacteria, yeast, bacillus, and nitrogen-fixing bacteria (fermentation conditions: pH 7.0, temperature 28 °C, duration 72 h), with a nutrient composition of: dry matter 32.5%, crude protein 24.7%, crude lipid 10.3%, soluble sugar 11.5%, and crude fiber 17.9%.

A high-fat basal diet was formulated using fish meal, soybean meal, and cottonseed protein as primary protein sources, and fish oil and soybean oil as main

lipid sources, containing 14% crude lipid, 41% crude protein, and 19.5 MJ/kg gross energy. Three experimental diets were prepared by supplementing the basal diet with 0% (control), 7.5%, and 15.0% fermented mulberry leaves. The composition and nutrient levels are presented in Table 1. All dietary ingredients were ground to pass through an 80-mesh sieve, mixed thoroughly using the stepwise dilution method, and processed into 3.0 mm extruded floating pellets. The pellets were air-dried and stored at 4 °C until use.

1.2 Experimental Design and Husbandry

A total of 360 healthy hybrid snakehead from the same cohort, with uniform size and an average body weight of 10 g, were randomly distributed into three groups with four replicates each containing 30 fish. Fish were reared in an indoor freshwater culture system (tank effective volume: 260 L) for eight weeks. The daily feeding rate was 3%-5% of body weight, with three feedings daily at 08:00, 12:30, and 17:00. The culture water was aerated tap water. During the experimental period, water temperature was maintained at (24.5 ± 2.5) °C, pH at 7.4 ± 0.4 , dissolved oxygen at 6-7 mg/L, ammonia nitrogen < 0.10 mg/L, and nitrite nitrogen at 0.005-0.010 mg/L.

1.3 Sample Collection and Analysis

At the end of the feeding trial, fish were fasted for 24 h and weighed. Three fish per replicate were randomly selected as whole-body samples for body composition analysis. Another three fish per replicate were anesthetized with MS-222, and the viscera, hepatopancreas, and mesenteric fat were dissected for morphological parameter determination. Five fish per replicate were sampled via caudal vein using disposable medical syringes. A portion of the collected blood was used for whole blood glucose measurement, while the remainder was centrifuged at $4,000 \times g$ for 10 min at 4 °C to obtain serum, which was stored at -20 °C for serum biochemical analysis.

Feed ingredients and whole-body samples were dried at 105 °C to constant weight. Crude protein content was determined by the Kjeldahl method, crude lipid by Soxhlet extraction, crude ash by incineration at 550 °C, total phosphorus by molybdenum yellow spectrophotometry, and dietary gross energy by a GR3500 oxygen bomb calorimeter. Serum biochemical indices, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), triglycerides (TG), and total protein (TP), were measured using an automatic analyzer (Mindray BC-3000). Blood glucose (GLU) content was determined using a Johnson & Johnson glucometer.

1.4 Calculation Formulas

Specific growth rate (SGR, %/d) = $100 \times [\ln(W_t) - \ln(W_0)]/t$

Protein efficiency ratio (PER) = $(W_t - W_0)/(F \times F_p)$

Feed conversion ratio (FCR) = $F/(W_t - W_0)$

Feeding rate (FR, g/d) = $F \times 2 / [(W_t + W_0) \times t]$

Survival rate (SR, %) = $100 \times N_t / N_0$

Viscerosomatic index (VSI, %) = $100 \times W_v / W_t$

Intraperitoneal fat ratio (IPF, %) = $100 \times W_i / W_t$

Hepatosomatic index (HSI, %) = $100 \times W_h / W_t$

Where: W_t = final mean weight (g); W_0 = initial mean weight (g); t = feeding duration (d); F = total feed intake on dry weight basis (g); F_p = dietary crude protein content (%); N_t = initial fish number; N_0 = final fish number; W_v = viscera weight (g); W_i = intestinal fat weight; W_h = liver weight (g).

1.5 Statistical Analysis

Data were analyzed using one-way ANOVA and Tukey's multiple comparison test with SPSS 17.0. All data except survival rate are expressed as mean \pm standard error. Significance level was set at $P < 0.05$.

2.1 Effects of Fermented Mulberry Leaves on Growth Performance of Hybrid Snakehead

As shown in Table 2, the final body weight of hybrid snakehead in the 7.5% fermented mulberry leaves group was significantly higher than that in both the 15.0% fermented mulberry leaves group and the control group ($P < 0.05$). No significant differences were observed in specific growth rate, protein efficiency ratio, or feed conversion ratio between the 7.5% fermented mulberry leaves group and the control group ($P > 0.05$). However, the 15.0% fermented mulberry leaves group exhibited significantly lower specific growth rate and protein efficiency ratio ($P < 0.05$) and significantly higher feed conversion ratio compared with the control group ($P < 0.05$). Feeding rate did not differ significantly among all groups ($P > 0.05$), and survival rate was 100% in all groups.

2.2 Effects of Fermented Mulberry Leaves on Morphological Parameters and Body Composition of Hybrid Snakehead

As presented in Table 3, the whole-body crude lipid content, hepatosomatic index, viscerosomatic index, and intraperitoneal fat ratio were significantly reduced in both the 7.5% and 15.0% fermented mulberry leaves groups compared with the control group ($P < 0.05$). Additionally, the 15.0% fermented mulberry leaves group showed significantly decreased liver lipid content ($P < 0.05$). No significant differences were detected among groups in whole-body crude ash, whole-body crude protein, or muscle lipid content ($P > 0.05$).

2.3 Effects of Fermented Mulberry Leaves on Serum Biochemical Indices and Blood Glucose Content of Hybrid Snakehead

As shown in Table 4, serum aspartate aminotransferase and alanine aminotransferase activities, as well as total cholesterol and triglyceride concentrations, were

significantly lower in the 15.0% fermented mulberry leaves group compared with the control group ($P < 0.05$). Blood glucose content decreased significantly with increasing dietary fermented mulberry leaves levels ($P < 0.05$). No significant differences were observed in serum total protein content among all groups ($P > 0.05$).

3.1 Effects of Fermented Mulberry Leaves on Growth Performance of Hybrid Snakehead

The present results indicate that low-level supplementation of fermented mulberry leaves in a high-fat diet does not affect the specific growth rate, protein efficiency ratio, or feed conversion ratio of hybrid snakehead. These findings are consistent with studies on Indian catfish (*Heteropneustes fossilis*) [4], *Labeo bata* [5], and *Labeo rohita* [6], which reported that replacement of fish meal with fermented mulberry leaf meal did not impair growth performance or feed utilization. The current study also demonstrated that replacing 4% of soybean meal with fermented mulberry leaves was feasible. However, the optimal inclusion level of fermented mulberry leaves varies depending on animal species and dietary formulation. Notably, low-level fermented mulberry leaf supplementation significantly increased final body weight in hybrid snakehead, suggesting that an appropriate level can promote weight gain [7]. Fermentation improves the palatability and protein utilization efficiency of mulberry leaves [8], which may explain the enhanced weight gain at low inclusion levels. Conversely, high-level supplementation inhibited growth. Our previous research also found that high dietary levels of mulberry leaves reduced growth performance in tilapia [9], and similar results have been reported in laying hens [10]. Although fermentation effectively reduces antinutritional factors in mulberry leaves [11], the crude fiber content remains relatively high (17.9%), which can affect digestive enzyme activity and reduce nutrient digestibility [12]. The 15.0% inclusion level used in this study may be excessive for carnivorous hybrid snakehead, as the elevated crude fiber content likely decreased nutrient utilization and subsequently impaired growth. Unfortunately, feed digestibility was not measured in this study. Further research is needed to elucidate the mechanisms of mulberry leaf action in fish.

Plant protein sources often reduce feed intake due to poor palatability, thereby compromising growth performance [13]. However, fermented mulberry leaf supplementation did not affect feed intake in hybrid snakehead, indicating that fermentation does not impair palatability. This is consistent with findings in tilapia [10] and supports the conclusion that reduced digestibility rather than palatability was responsible for the growth inhibition observed at high inclusion levels.

3.2 Effects of Fermented Mulberry Leaves on Lipid Metabolism of Hybrid Snakehead

Numerous studies have demonstrated that appropriate dietary lipid levels can promote growth and improve protein utilization efficiency, thereby achieving protein-sparing effects [14-15]. However, excessive dietary lipid levels inhibit growth [16], cause excessive fat deposition [17], and compromise fish health [18]. The present results show that fermented mulberry leaf supplementation in a high-fat diet significantly reduced body fat deposition and viscerosomatic index. This may be attributed to reduced antinutritional factors and increased bioactive components after fermentation, which enhance hypoglycemic and hypolipidemic efficacy [19-20]. The dose-dependent reduction in serum total cholesterol and triglyceride concentrations further confirms that fermented mulberry leaves can regulate lipid metabolism and transformation in hybrid snakehead. These findings are consistent with studies on tilapia [10] and largemouth bass [21], indicating that fermented mulberry leaves can effectively modulate lipid metabolism and improve lipid deposition in fish.

The significant reduction in liver lipid content and serum transaminase activities observed with fermented mulberry leaf supplementation demonstrates effective protection against high-fat-induced hepatocellular damage and improved liver health in hybrid snakehead.

Fermented mulberry leaves also reduced blood glucose content in a dose-dependent manner. Bioactive components in mulberry leaves, such as alkaloids, 1-deoxynojirimycin (DNJ), and polysaccharides, competitively inhibit α -glucosidase in the small intestine, delaying or suppressing glucose absorption and thereby exerting hypoglycemic effects [22]. Other studies suggest that alkaloids and flavonoids can regulate key enzyme activities in hepatic glucose metabolism, thereby modulating glucose, lipid, and protein metabolism [20]. These findings indicate that different bioactive components in mulberry leaves may exert hypoglycemic effects through various mechanisms, collectively demonstrating significant glucose-lowering effects in fish. Given the limited capacity of fish to utilize carbohydrates, enhancing their carbohydrate tolerance has important practical implications.

In summary, supplementation with 7.5% fermented mulberry leaves in a high-fat diet does not compromise feed utilization efficiency and can promote growth in hybrid snakehead, whereas 15.0% supplementation significantly inhibits growth but benefits liver health. Serum total cholesterol, triglyceride, and blood glucose concentrations decreased progressively with increasing dietary fermented mulberry leaves levels.

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