Effects of Four Feeding Attractants on Growth and Serum Biochemical Indices of Hybrid Snakehead (Channa maculata × Channa argus) Postprint

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Abstract

To evaluate the feeding attractant effects of fish soluble paste, yeast extract, dimethyl-β-propiothetin (DMPT), and compound feeding attractant on hybrid snakehead, one basal diet (control group) and four experimental diets supplemented with 2.0% fish soluble paste, 2.0% yeast extract, 0.1% DMPT, and 0.1% compound feeding attractant in the basal diet were formulated to feed hybrid snakehead with initial average body weight of (17.30$±$0.03) g for 8 weeks. The five diets were isonitrogenous and isoenergetic (crude protein 42%, gross energy 18 MJ/kg). The results showed: the final average body weight (FABW), weight gain rate (WGR), and specific growth rate (SGR) of hybrid snakehead in the feeding attractant supplemented groups were significantly higher than those in the control group ($P<0.05$), while the FABW, WGR, SGR, and feed intake (FI) of hybrid snakehead in the yeast extract group and compound feeding attractant group were significantly higher than those in the fish soluble paste group and DMPT group ($P<0.05$). There were no significant differences in protein efficiency ratio (PER) and feed conversion ratio (FCR) among all groups ($P>0.05$). Compared with the control group, the hepatosomatic index (HSI) of hybrid snakehead in the fish soluble paste group, yeast extract group, and compound feeding attractant group decreased ($P<0.05$), while the splenosomatic index (SSI) increased significantly ($P<0.05$). The viscerosomatic index (VSI) of hybrid snakehead in the compound feeding attractant group was significantly lower than that in other groups ($P<0.05$). Compared with the control group, the serum cholesterol (CHO) and malondialdehyde (MDA) contents of hybrid snakehead in the fish soluble paste group, yeast extract group, and compound feeding attractant group decreased significantly ($P<0.05$), while the serum total protein (TP) content and superoxide dismutase (SOD) and catalase (CAT) activities in-

creased significantly (P<0.05). There were no significant differences in serum triglyceride (TG), globulin (GLOB) contents, and alkaline phosphatase (ALP) activity among all groups (P>0.05). These results indicate that dietary supplementation of feeding attractants can increase feed intake, reduce blood lipid levels, improve fish immunity and antioxidant capacity, and thereby promote growth, with yeast extract and compound feeding attractant showing superior effects.

Full Text

Effects of Four Feeding Attractants on Growth and Serum Biochemical Indices of Hybrid Snakehead

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Abstract: To evaluate the attractant effects of fish soluble, yeast extract, dimethyl-β-propiothetin (DMPT), and compound feeding attractant on hybrid snakehead (*Channa* spp.), five isonitrogenous and isoenergetic diets (crude protein 42%, gross energy 18 MJ/kg) were formulated. A basal diet served as the control, while four experimental diets were prepared by supplementing the basal diet with 2.0% fish soluble, 2.0% yeast extract, 0.1% DMPT, and 0.1% compound feeding attractant, respectively. Hybrid snakehead with an initial average body weight of (17.30$±$0.03) g were fed these diets for eight weeks. The results showed that fish fed diets containing attractants exhibited significantly higher final average body weight (FABW), weight gain rate (WGR), and specific growth rate (SGR) compared to the control group (P<0.05). Furthermore, the yeast extract and compound attractant groups demonstrated significantly higher FABW, WGR, SGR, and feed intake (FI) than the fish soluble and DMPT groups (P<0.05). No significant differences were observed in protein efficiency ratio (PER) or feed conversion ratio (FCR) among all groups (P>0.05). Compared with the control group, fish in the fish soluble, yeast extract, and compound attractant groups showed significantly reduced hepatosomatic index (HSI) and elevated splenosomatic index (SSI) (P<0.05). The compound attractant group exhibited the lowest viscerasomatic index (VSI), which was significantly lower than all other groups (P<0.05). Serum cholesterol (CHO) and malondialdehyde (MDA) contents were significantly decreased in the fish soluble, yeast extract, and compound attractant groups, while serum total protein (TP) content and superoxide dismutase (SOD) and catalase (CAT) activities

were significantly increased (P<0.05). No significant differences were detected in serum triglyceride (TG), globulin (GLOB) contents, or alkaline phosphatase (ALP) activity among groups (P>0.05). These findings indicate that dietary supplementation with feeding attractants can enhance feed intake, reduce serum lipid levels, improve immunity and antioxidant capacity, and consequently promote growth in hybrid snakehead, with yeast extract and compound attractant showing the most promising effects.

Keywords: feeding attractants; hybrid snakehead; growth; antioxidant ability; serum biochemical indices

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Hybrid snakehead (*Channa* spp.) has become an important rapidly developing freshwater aquaculture species worldwide due to its fast growth rate and high economic value. In recent years, hybrid snakehead aquaculture has expanded rapidly in China, with annual production reaching approximately 200,000 tons. However, the lack of reliable nutritional and feed parameters, combined with shortages of fishmeal resources and declining farming profits, has led to extensive use of plant proteins in feed formulations. This has resulted in poor feed palatability and feed rejection phenomena in practical farming operations. High levels of plant protein in aquafeeds typically reduce palatability, subsequently decreasing feed intake and impairing normal growth. Enhancing feed intake in aquatic animals represents a long-standing challenge for animal nutritionists and constitutes an important approach for improving plant protein utilization. Currently, feed manufacturers commonly incorporate exogenous feeding attractants to increase feed consumption in aquatic species. Research has confirmed that dietary attractants can improve feed palatability. Substances such as amino acids, betaine, nucleotides, fish soluble, yeast extract, dimethyl-β-propiothetin (DMPT), and taurine have proven effective as feeding stimulants for fish and shrimp. However, some studies suggest that single attractants are less effective than compound formulations. Research on improving feed palatability for hybrid snakehead remains limited, yet addressing feed intake issues is urgently needed in practical production. Therefore, based on preliminary maze screening tests of individual attractants, this study conducted growth trials with four effective attractants to provide theoretical support for hybrid snakehead aquaculture and feed formulation.

1.1 Experimental Diets

A basal diet was formulated using fishmeal, soybean meal, cottonseed protein, and rapeseed meal as protein sources (Table 1). Four experimental diets were prepared by supplementing the basal diet with 2.0% fish soluble, 2.0% yeast extract, 0.1% DMPT, and 0.1% compound feeding attractant, respectively. The compound attractant consisted of yeast nucleotides, taurine, betaine, and amino acids. All five diets were isonitrogenous and isoenergetic (crude protein 42%, gross energy 18 MJ/kg). Feed ingredients were ground to pass through a 245

m sieve, mixed thoroughly using the stepwise dilution method, and processed into 3.0 mm extruded pellets using a Yanggong TSE65S twin-screw wet extruder (Beijing Xiandai Yanggong Machinery Technology Development Co., Ltd.). The pellets were air-dried and stored at 4°C until use.

1.2 Experimental Fish and Feeding Management

Hybrid snakehead (*Channa maculata* × *C. argus*) were obtained from the Yongchuan District Fish Seed Farm in Chongqing. After 10 days of acclimation and adaptation, 450 healthy fish with uniform size and an initial average body weight of (17.30$±$0.03) g were randomly distributed into five groups with three replicates per group (30 fish per replicate). The control group received the basal diet, while the other four groups were randomly assigned to one of the four experimental diets containing attractants. Fish were reared in indoor freshwater recirculating aquaria (effective volume: 300 L) for eight weeks. The daily feeding rate was 3–5% of body weight, with three feedings daily at 08:30, 13:30, and 18:30. During the experimental period, water temperature was maintained at 26–29°C, dissolved oxygen at 7–8 mg/L, ammonia nitrogen below 0.48 mg/L, nitrite nitrogen below 0.07 mg/L, and pH at 7.2–7.6.

1.3 Sample Collection and Analysis

At the end of the feeding trial, fish were fasted for 24 hours before weighing and counting by replicate. Four fish were randomly selected from each replicate, anesthetized with MS-222, weighed, and then dissected to separate and weigh the viscera, liver, and spleen. An additional four fish per replicate were randomly selected for blood collection from the caudal vein. Blood samples were centrifuged at 3,000×g for 10 min at 4°C, and serum was harvested and stored at -20°C until analysis.

Feed intake, growth performance, and morphological indices were calculated using the following formulas:

- Weight gain rate (WGR, %) = 100 × (W -W_0) / W_0
- Specific growth rate (SGR, %/d) = 100 × (lnW -lnW_0) / t
- Feed intake (FI, g/d) = Wf × 2 / [(N + N_0) × t]
- Protein efficiency ratio (PER, %) = (W -W_0) / (Wf × Wp)
- Feed conversion ratio (FCR) = Wf / (W -W_0)
- Survival rate (SR, %) = 100 × N / N_0
- Viscerasomatic index (VSI, %) = 100 × W / W
- Hepatosomatic index (HSI, %) = 100 × W / W
- Splenosomatic index (SSI, %) = 100 × W / W

Where: t = feeding duration (d); N_0 = initial fish number; N = final fish number; Wp = crude protein content of diet (%); Wf = total dry weight of feed consumed (g); W = final average body weight (g); W_0 = initial average body weight (g); W = viscera weight (g); W = liver weight (g); W = spleen weight (g); W = body weight (g).

Serum superoxide dismutase (SOD), catalase (CAT), and alkaline phosphatase (ALP) activities, as well as cholesterol (CHO), triglyceride (TG), total protein (TP), globulin (GLOB), and malondialdehyde (MDA) contents were measured using an automatic biochemical analyzer (Hitachi 7100).

1.4 Data Processing and Analysis

Data were analyzed using SPSS 23.0 software. One-way ANOVA was performed, and when significant differences were detected ($P<0.05$), Tukey' s multiple comparison test was applied. All data are presented as means ± standard error, except for survival rate.

2.1 Feed Intake and Growth Performance

As shown in Table 2 , hybrid snakehead fed diets supplemented with attractants exhibited significantly higher final average body weight, WGR, and SGR compared to the control group ($P<0.05$). The yeast extract and compound attractant groups showed significantly higher final average body weight, WGR, SGR, and feed intake than the fish soluble and DMPT groups ($P<0.05$). No significant differences were observed in PER or FCR among all groups ($P>0.05$). Survival rate was 100% across all groups during the experimental period.

2.2 Morphological Indices

Table 3 presents the morphological indices of hybrid snakehead. Compared with the control group, fish in the fish soluble, yeast extract, and compound attractant groups showed significantly reduced HSI and elevated SSI ($P<0.05$). The DMPT group exhibited no significant differences in VSI or SSI compared to the control group ($P>0.05$). The compound attractant group had the lowest VSI, which was significantly lower than all other groups ($P<0.05$).

2.3 Serum Biochemical Indices

As shown in Table 4 , serum CHO content was significantly lower in the fish soluble, yeast extract, and compound attractant groups compared to the control group ($P<0.05$), while serum TP content was significantly higher ($P<0.05$). Serum MDA content was significantly reduced in the fish soluble, yeast extract, and compound attractant groups, whereas serum SOD and CAT activities were significantly increased compared to the control group ($P<0.05$). No significant differences were detected in serum TG, GLOB contents, or ALP activity among groups ($P>0.05$).

Discussion

The present study demonstrates that dietary supplementation with exogenous feeding attractants promotes growth in hybrid snakehead, consistent with findings in other fish and shrimp species. Yeast extract and compound attractant

exhibited the most effective performance. Previous research has also confirmed the beneficial attractant effects of yeast extract on gibel carp and *Litopenaeus vannamei*. Different animal species exhibit varying sensitivity to feeding stimulants. For instance, betaine has proven effective for Dover sole but shows limited response in Japanese flounder and largemouth bass. Similarly, different amino acids at the same concentration elicit varying feeding responses in rice field eel. Even within the same fish family, responses to identical stimulants show both similarities and differences. For example, sockeye salmon (*Oncorhynchus nerka*) exhibits strong gustatory and electrophysiological responses to proline but varying responses to alanine. Additionally, sockeye salmon shows strong reactions to phenylalanine, leucine, and betaine, while other fish species do not. Furthermore, most responses to animal extracts in fish and shrimp are based on complex reactions to multiple small molecules rather than sensitivity to just one or two substances. This may explain why single attractants are less effective than compound formulations in practical production, as single attractants typically provide only simple stimulation rather than creating a strong comprehensive feeding stimulus. The current results support this hypothesis. Studies on Japanese flounder, striped bass, and rice field eel have similarly demonstrated that components of compound attractants can act synergistically to compensate for deficiencies in individual ingredients. Research has shown that amino acids can enhance the feeding-stimulating effect of inosine-5' -monophosphate (IMP) on yellowtail amberjack larvae. These findings indicate that different fish species have distinct feeding habits and requirements for attractant types and active ingredient concentrations, and even the same species may show interest in different compounds.

Feed intake serves as an effective indicator for evaluating feed palatability. Long-term feeding growth trials represent a more effective approach for assessing feeding stimulants. The current results show that dietary supplementation with yeast extract and compound attractant significantly promoted feed intake in hybrid snakehead without compromising feed efficiency, thereby enhancing growth. This demonstrates that stimulating feed intake is an effective strategy for promoting growth in this species. Similar findings have been reported for Japanese flounder, striped bass, rice field eel, and gibel carp, where dietary attractants increased feed intake and consequently improved growth. Fish feeding behavior and feed preference represent complex phenomena involving intricate interactions among various internal and external factors, making the elucidation of feeding mechanisms challenging.

The present study indicates that dietary supplementation with exogenous attractants reduced serum CHO content in hybrid snakehead, with yeast extract and compound attractant showing the most pronounced effects. In contrast, studies on *L. vannamei* reported that attractants increased serum CHO and TG contents while maintaining good liver function. These discrepancies may be attributed to species differences or experimental objectives. Generally, demersal fish store fat in body cavities or visceral tissues, while pelagic fish store fat in muscle. In this study, all attractants except DMPT significantly reduced

HSI, and the compound attractant also significantly decreased VSI. These results suggest that attractants can improve fat deposition patterns in visceral tissues and enhance physiological function, consistent with previous findings in hybrid snakehead.

Blood biochemical parameters reflect the physiological status of animals. Serum TP content indicates immune stress status, as stress factors such as disease can reduce plasma TP levels in fish. The spleen plays an important role in immunity, and SSI directly reflects immune function. The current results demonstrate that dietary attractants significantly increased serum TP content and SSI in hybrid snakehead, with yeast extract showing the most prominent effect, indicating that exogenous attractants can enhance immune capacity. This immunostimulatory effect may be attributed to β-glucans and mannan oligosaccharides present in yeast extract, which have been shown to enhance disease resistance and immunity in aquatic animals.

Most serum enzymes originate from various tissues and organs, and their activities reflect metabolic status and substance transformation. SOD scavenges superoxide anion radicals generated during biological oxidation, while the resulting hydrogen peroxide (H_2O_2) can be eliminated by CAT to produce water and oxygen. MDA is a product of lipid peroxidation, and its concentration reflects the degree of lipid peroxidation in vivo. The present study shows that dietary supplementation with exogenous attractants increased serum SOD and CAT activities while decreasing MDA content, indicating that attractants significantly improve antioxidant capacity. However, research on the effects of attractants on antioxidant capacity remains limited, and the underlying mechanisms require further investigation.

In conclusion, dietary supplementation with feeding attractants can enhance feed intake, reduce serum lipid levels, improve immunity and antioxidant capacity, and consequently promote growth in hybrid snakehead, with yeast extract and compound attractant demonstrating the most effective performance.

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