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EFFECTS OF TURMERIC RESIDUE ON HEMATOLOGICAL PARAMETERS, NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF HYBRID CATFISH *(Clarias gariepinus × Heterobranchus bidorsalis)*

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Abstract. Turmeric residue (TR), which is obtained from the extraction process of curcuminoids, may remain biological active compounds. This study was conducted to examine the effects of the TR on hematological parameters, nutrient digestibility, and growth performance in hybrid catfish *(Clarias gariepinus × Heterobranchus bidorsalis)*. Three experimental diets were formulated denoted as follows: BD (Basal diet), BTR1D (BD plus 1% TR powder), and BTR2D (BD plus 2% TR powder). Twelve juvenile hybrid catfish with an initial body weight of 180 g were allocated to each of the 6 concrete tanks (350-L holding capacity), resulting in two replicate tanks per dietary treatment. For 4 weeks, the fish were hand-fed the experimental diets to apparent satiation twice daily. The results showed that the final body weight and weight gain tended to increase in fish fed TR-supplemented diets, and significant differences were recorded in the BTR2D group as compared to the BD group ($P < 0.05$). In contrast, FCR values were decreased in fish fed BTR1D and BTR2D in comparison with those fed BD, and the FCR value of the BTR2D group was significantly lower than that of the BD group ($P < 0.05$). Hemoglobin concentration, white blood cell count and protein and lipid apparent digestibility coefficients were higher in fish fed TR-supplemented diets than those fed BD. These results indicated that dietary supplementation of the TR was beneficial to hematological parameters, nutrient digestibility, growth performance, and feed utilization in hybrid catfish. The positive effects of the BTR2D on fish performances in the present study suggest that supplementation of the TR at the ratio of 2% in the diet may be necessary for hybrid catfish practical culture.

Keywords: turmeric residue, blood, digestibility, growth performance, hybrid catfish.

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1. Introduction

Turmeric, which is derived from the rhizome of the medicinal plant *Curcuma longa* L, has been commonly consumed in the form of powdered rhizomes for coloring and flavoring foods. Turmeric reportedly contains several biologically active compounds, such as curcuminoids, turmerone, arturmerone, and zingiberene, that exhibit anti-oxidant, anti-microbial, immunomodulatory, and anti-stress effects [1-3]. Among the active compounds, curcumin has been known to possess diverse biological activities [4-6]. In fish, this compound has been reported to improve growth performance, feed utilization, nutrient digestibility, blood cell count, and immunological parameters in several species, including grass carp (*Ctenopharyngodon idells*) [7], large yellow croaker (*Pseudosciaene crocea*) [8], rainbow trout (*Oncorhynchus mykiss*) [9], tilapia (*Oreochromis mossambicus*) [10].

Turmeric is widely used for the extraction of curcuminoids and volatile oil. With this process, the extract accounts for only 6 - 7% of the original turmeric weight [11]. Consequently, a large amount of turmeric residue (TR) is generated and disposed as waste after the extraction. Moreover, curcumin and other active compounds may be remained in the TR due to insufficient extraction. Therefore, supplementation of the TR in the diet is possibly beneficial to growth performance, feed utilization, and physiological conditions of fish. Hybrid catfish *(Clarias gariepinus × Heterobranchus bidorsalis)* is an economically important species for aquaculture due to its fast growth rate and high market demand. To date, there have been no studies to use the TR in feed for this species. Therefore, the present study aimed to examine the effects of the TR on hematological parameters, nutrient digestibility, and growth performance in hybrid catfish.

2. Contents

2.1. Materials and methods

**** Turmeric residue***

The raw TR was provided by Tan Hoang Minh Co., Ltd. (Chi Tan, Khoai Chau, Hung Yen). The residue was dried by an oven at 50 ºC, then the dried residue was ground to below 400 µm mesh size. The proximate composition of the TR (dry matter basis) was presented as follows: protein, 4.8%; ether extract, 16.1%; fiber, 28.8%; nitrogen free extract, 46.9%; ash, 3.4%.

**** Experimental diets***

Three experimental diets were formulated with fish meal, soybean meal, corn gluten meal, and wheat flour as main ingredients. The diets were denoted as follows: BD (Basal diet), BTR1D (BD plus 1% TR powder), and BTR2D (BD plus 2% TR powder) (Table 1). Chromium oxide (5 g/kg diet) was added to all the experimental diets as an inert marker to estimate nutrient apparent digestibility coefficient (ADC). All of the powdered ingredients were manually mixed, then pollock liver oil was slowly added to the mixture. After the powdered ingredients were thoroughly mixed with pollock liver oil, water was added to produce a stiff dough. Finally, the dough was then pelleted using a laboratory pellet mill and stored at -20°C until use.

Table 1. Formulation and proximate composition of the experimental diets

Ingredients (g/kg)	BD	BTR1D	BTR2D
Fish meal	200	200	200
Soybean meal	350	350	350
Wheat flour	170	170	170
Pollock liver oil	40	40	40
Starch	100	100	100
Cellulose	105	95	85
Vitamin and mineral mixture*	15	15	15
CMC-Na**	15	15	15
Chromium oxide	5	5	5
Turmeric residue (TR)	0	10	20
Proximate composition (dry matter basis)			
Protein	30.4	30.5	30.6
Lipid	7.2	7.4	7.7
Fiber	15.0	13.9	12.8
Nitrogen free extract***	37.8	38.9	39.4
Ash	9.6	9.4	9.5

**Vitamin and mineral mixture (IU or mg/kg mixture): thiamine HNO_3, 1030; riboflavin, 3070; pyridoxine HCl, 1390; cyanocobalamin, 8.1; vitamin C (L-ascorbate-2-monophosphate), 18100; vitamin A acetate, 485000; vitamin D_3 (cholecalciferol), 172000; vitamin E (DL-α-tocopherol acetate, 7010; vitamin K_3 (menadione sodium bisulfite), 1850; folic acid, 550; nicotinamide, 5200; D-calcium pantothenate, 4250; D-biotin, 16.5; inositol, 15400; $ZnSO_4$, 2700; $MnSO_4$, 1730; $CuSO_4$, 1310; $FeSO_4$, 6250; $CoSO_4$, 156; potassium iodide, 175; sodium selenate, 38.1.*

***Sodium carboxymethyl cellulose.*

****Nitrogen free extract (NFE)=100 - [protein (%) + lipid (%) + ash (%) + fiber (%)]*

**** Fish and rearing conditions***

The experiment was carried out at the Faculty of Biology, Hanoi National University of Education in 2019. Twelve juvenile hybrid catfish with an initial body weight of 180 g were allocated to each of the 6 concrete tanks (350-L holding capacity), resulting in two replicate tanks per dietary treatment. The tanks were aerated and supplied with filtered fresh water at a rate of 2 L/min. For 4 weeks, the fish were hand-fed the experimental diets to apparent satiation twice daily (09:00 am and 16:00 pm). Feed intake in each tank was recorded daily.

**** Sampling, analytical methods and calculations***

At the end of the feeding trial, all fish were fasted for 48 h before sampling. The fish were anesthetized with 400 ppm 2-phenoxyethanol and weighed individually to determine the average final body weight (BW). Three fish in each tank were randomly selected for blood sampling. The blood samples were collected with heparinized syringes from the caudal vein, then used for analyses of hematological parameters. These fish were then returned to the original tanks for fecal collection. For this purpose, fish continued to be fed the experimental diets, and feces were collected by tripping at 5 h after feeding with the method described by Austrians (1978) [12].

Aliquots of the blood samples were used for quantifications of hematocrit and hemoglobin levels using a commercial automatic analyzer (Architect c16000, Abbott, Illinois, USA). The remainder of the blood was used for red blood cell and white blood cell counts using a Neubauer chamber. The red blood cells were counted with the method described by Natt and Herrick (1952) [13]. The white blood cells were stained with Giemsa (Sigma-Aldrich Corp., St. Louis, MO, USA) and counted in accordance with the method described by Hrubec et al. (2000) [14]. The proximate compositions of the TR, experimental diets and feces, and the digestibility marker were analyzed using the standard methods of the Association of Official Analytical Chemists (AOAC, 2005) [15].

Weight gain (WG), feed intake (FI), feed conversion ratio (FCR), and nutrient ADC were calculated using the following formulas:

WG (%) = 100 × (final mean BW - initial mean BW)/initial mean BW

FI (%BW/day)= 100 × total dry FI (g)/[total initial BW (g) + total final BW (g)]/2/feeding days

FCR = total dry FI (g)/[final total BW (g) - initial total BW (g)]

Nutrient ADC (%) = 100 × [100 - Cr_2O_3 in diet (%)/Cr_2O_3 in feces (%) × nutrient in feces(%)/nutrient in diet(%)].

**** Statistical analysis***

Data were analyzed and evaluated using one-way analysis of variance (ANOVA). Statistical differences between groups were assessed using Tukey-Kramer test, and significance was based on a 5% level of probability.

2.2. Results

2.2.1. Growth performance and feed utilization

As presented in Table 2, there were no significant differences in the initial BW among the treatment groups. However, the final BW and WG of fish fed the BTR2D were significantly higher than those of fish fed BD ($P < 0.05$). These parameters were slightly increased in the BTR1D group as compared to the BD group, although no significant differences were recorded. The FCR was the highest in fish fed BD, and a significant difference was observed between fish fed BD and BTR2D. The tested diets did not influence the FI of the experimental fish.

Table 2. Growth performance and feed utilization of hybrid catfish fed the experimental diets

Parameters*	**Dietary groups**		
	BD	***BTR1D***	***BTR2D***
Initial BW (g)	180,2 ± 2.5	180.4 ± 2.8	180.5 ± 2.1
Final BW (g)	289.6 ± 5.8^{a}	307.3 ± 7.1^{ab}	314.9 ± 8.2^{b}
WG (%)	60.7 ± 4.2^{a}	70.3 ± 5.1^{ab}	74.5 ± 4.7^{b}
FI (%BW/day)	4.7 ± 0.4	4.9 ± 0.3	4.8 ± 0.2
FCR	1.68 ± 0.12^{b}	1.45 ± 0.10^{ab}	1.36 ± 0.08^{a}

**Values are presented as mean ± standard deviation of two replicates. The values with different superscripts in the same row are significantly different (P < 0.05).*

2.2.2. Hematological parameters

The hematocrit level, hemoglobin concentration, red and white blood cell counts are presented in Table 3. There were no significant differences in hematocrit level and red blood cell count among the treatment groups, although these hematological parameters tended to increase in fish fed BTR1D and BTR2D in comparison with those fed DB. The hemoglobin concentrations of the BTR1D and BTR2D groups were was significantly higher than the BD group ($P < 0.05$). The white blood cell count was higher in fish fed BTR-supplemented diets than fish fed BD, and a significant difference was recorded between fish fed BTR2D and BD ($P < 0.05$).

Table 3. Hematological parameters of hybrid catfish fed the experimental diets

Parameters*	**Dietary groups**		
	BD	***BTR1D***	***BTR2D***
Hematocrit (%)	39.5 ± 3.8	42.8 ± 3.5	43.6 ± 2.7
Hemoglobin (g/dL)	10.4 ± 0.5^{a}	12.7 ± 0.4^{b}	13.2 ± 0.7^{b}
Red blood cells (10^6/mm^3)	2.3 ± 0.4	2.5 ± 0.3	2.6 ± 0.5
White blood cells (10^3/mm^3)	141.5 ± 6.7^{a}	152.9 ± 4.2^{ab}	159.3 ± 6.5^{b}

**Values are presented as mean ± standard deviation (n = 6). The values with different superscripts in the same row are significantly different (P < 0.05)*

2.2.3. Protein and lipid apparent digestibility coefficients

The protein and lipid ADCs are shown in Figure 1 and Figure 2. Both the protein and lipid ADCs were enhanced by TR supplementation. The protein ADC was significantly higher in the BTR1D and BTR2D groups as compared to the BD group ($P < 0.05$) (Figure 1). Fish fed BTR1D tended to have higher lipid ADC than those fed BD, although no significant difference was recorded (Figure 2). The lipid ADC was further increased in fish fed BTR2D, and a significant difference was observed between this group and the BD group ($P < 0.05$).

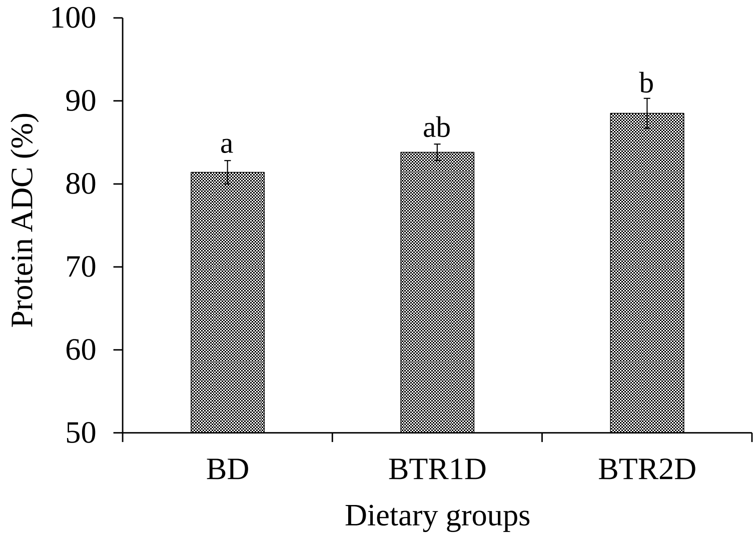

Figure 1. Protein ADC of hybrid catfish fed the experimental diets

Values are presented as the mean ± standard deviation of two replicates. Bars assigned with different superscripts denote significant differences (P < 0.05).

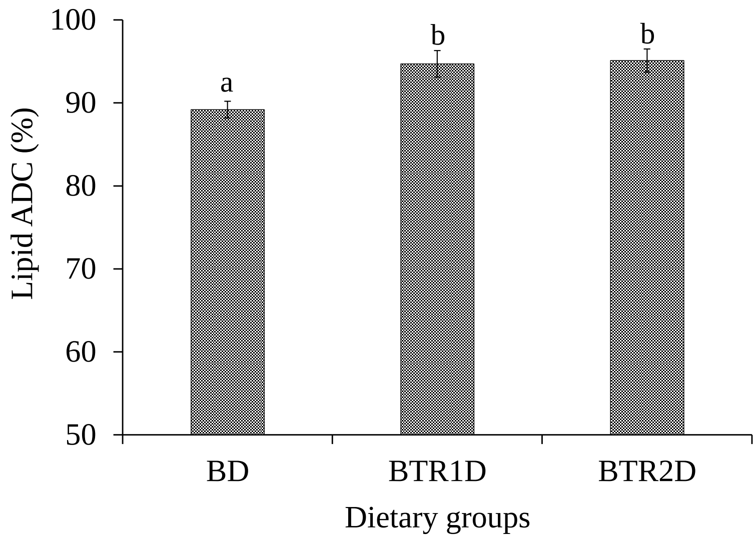

Figure 2. Lipid ADC of hybrid catfish fed the experimental diets

Values are presented as the mean ± standard deviation of two replicates. Bars assigned with different superscripts denote significant differences (P < 0.05).

2.3. Discussion

In this study, fish fed diets supplemented with the TR tended to increase the final BW and WG as compared to those fed BD, and significant differences were recorded between the BTR2D and the BD groups. In contrast, FCR value of fish fed BTR2D was significantly lower than that of fish fed BD. Moreover, hemoglobin level, white blood cell count, and protein and lipid ADCs were also improved by supplementation of the TR in the diet. These results indicated that the dietary TR supplementation was beneficial to growth performance, hematological parameters, and nutrient digestion and absorption of hybrid catfish. The findings of the present study support an earlier report of common carp (*Cyprinus carpio* L.), where diets supplemented with turmeric powder elevated the final BW, WG, and immunological parameters [16]. Although the TR had such positive effects on performances of fish, the final BW, WG, white blood cell count, protein digestibility of fish fed BTR1D were not significantly different from those of fish fed BD. Meanwhile, these parameters were dramatically improved in the BTR2D group as compared to the BD group. These findings suggest that supplementation of the TR at the ratio of 2% in the diet may be necessary to improve growth performance, feed utilization and hematological parameters in hybrid catfish.

Turmeric reportedly contains several active compounds, such as curcumin, turmerone, arturmerone, and zingiberene [1, 2, 3]. Among these compounds, curcumin has been known to possess diverse biological activities, including antioxidant [4], anti-inflammatory [5], anti-bacterial [6], anti-viral [17], and anti-stress effects [18]. Therefore, curcumin has been suggested to effectively improve growth performance, feed utilization, and immunological parameters in grass carp [7], large yellow croaker [8], rainbow trout [9], and tilapia [10]. There are at least three possible reasons for these improvements. Firstly, curcumin functions as a digestive enhancer through increasing lipase and trypsin activities in the pancreas and intestine, resulting in elevated growth performance [19]. Secondly, curcumin has the ability to activate some key enzymes located in the intestine that involve in nutrient transportation, such as Na^+/K^+-ATPase, intestinal alkaline phosphatase, gamma-glutamyl transpeptidase, and creatine kinase, thus, enhance nutrient digestion and assimilation [20]. Lastly, curcumin has anti-bacterial, anti-viral, and anti-stress effects, therefore, contributing to growth performance of fish [16]. In the present study, the TR was a produced by extraction of curcuminoids from turmeric. The result of proximate composition analysis of the TR showed that ether extract accounted for 16.1% of dry matter, suggesting that curcumin and other active lipid-soluble compounds might remain in the TR. Consequently, these compounds could be the factors responsible for improved hematological parameters and nutrient digestibility, thereby enhance the growth performance of the experimental fish.

3. Conclusions

Dietary supplementation of the TR increased hemoglobin concentration, white blood cell count, protein and lipid ADCs, final BW, and FCR of hybrid catfish. The findings of the present study suggest that supplementation of the TR at the ratio of 2% in the diet may be necessary for the practical culture of hybrid catfish to improve hematological parameters, nutrient digestion, and growth performance.

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