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Effects of melamine on growth performance and skin color of darkbarbel catfish (*Pelteobagrus vachelli*)

Jipeng Xue, Qinghui Ai *, Kangsen Mai, Wei Xu, Yinghao Yang, Zhiguo Liufu

The Key Laboratory of Mariculture, Ocean University of China (Ministry Education of China), Qingdao, 266003, PR China

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ABSTRACT

A feeding experiment was conducted to investigate the effects of melamine (MEL) on growth performance and skin color of darkbarbel catfish (*Pelteobagrus vachelli*). Artificial diets with 0.2, 0.5 and 1% MEL supplementation were fed to juvenile darkbarbel catfish (initial weight 14.30 ± 0.10 g) for eight weeks, and MEL-nonsupplemented diet was used as a control. The results indicated that specific growth rate (SGR) of darkbarbel catfish fed the diets with MEL supplementation was significantly lower than that of the control group ($P<0.05$). Melanin content in dorsal skin decreased with increasing MEL supplementation. Dorsal skin melanin content of darkbarbel catfish fed the diets with 0.5 and 1% MEL supplementation was significantly lower than that of the control group ($P<0.05$). Lightness (CIE [1976], L^* tristimuli value) of dorsal skin increased with increasing supplementation of MEL. L^* of darkbarbel catfish fed the diet with 0.5% and 1% MEL was significantly higher than the control group ($P<0.05$). However, tyrosinase activity of dorsal skin was not significantly affected by dietary MEL ($P>0.05$). These results indicated that dietary MEL could decrease melanin content of darkbarbel catfish dorsal skin, and therefore increase its lightness. During this procedure, tyrosinase activity of dorsal skin was not significantly affected.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine, MEL) is a white, odorless, crystalline chemical, which is commonly used to make melamine–formaldehyde resin (Kandelbauer and Widsten, 2009), flame retardant materials (Wu et al., 2008) and widely used in the production of pesticides (Miller et al., 1996) and crop fertilizers (Hauck and Stephenson, 1964). MEL was previously considered as a non-protein nitrogen (NPN) source for cattle feed in 1958, but this use was discontinued in 1978, since it was incompletely hydrolyzed in ruminants. It has been reported to be illegally adulterated to milk, dairy products and animal feeds to boost the product's apparent protein content because of its high nitrogen content (66.7% by mass), and neither Kjeldahl (Benedict, 1987; Dintzis et al., 1988) nor Dumas (Thompson et al., 2002) tests can distinguish nitrogen from amino acids or other nitrogen derivatives. Previous toxicological studies demonstrated that MEL alone is of low toxicity in mammals (OECD, 2002), however, when combined with cyanuric acid (CYA), it could lead to crystal formation and subsequent kidney toxicity.

In 2007, numerous incidents of acute renal failure were reported in dogs and cats in North America, which were fed with MEL tainted pet foods. One year later, the Minister of Health of the People's Republic of China announced that 294,000 infants and young children were diagnosed with urolithiasis, who had MEL tainted dairy products (Chu et al., 2010). And the public was concerned with the detection and toxicity of MEL. In recent three years, many analytical methods have been developed to detect MEL content in food products (Sun et al., 2010). However, studies on the toxicity of MEL are limited. To the best of our knowledge, most of the research focused on the pharmacokinetics of MEL in pigs, goats (Baynes et al., 2008, 2010) and rats (Chu et al., 2010), or the formation of crystal in cats (Puschner et al., 2007), channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*) (Reimschuessel et al., 2010).

Darkbarbel catfish is one of the commercially important freshwater aquaculture species in China. Due to its delicious flavor and high market value, more and more people began to culture this species in recent years. But the skin color of cultured darkbarbel catfish is not comparable to that of the wild one. Since the coloration of the skin of this species is among the most important quality criteria, which is essential for its market price, the skin color of darkbarbel catfish is of primary concern. Some fisherman observed that there was no black color in the dorsal skin of darkbarbel catfish, and assumed that might be the results of feeds adulterated with MEL. The purpose of this study was to investigate the effects of dietary MEL on skin color of darkbarbel catfish. For the former purpose, L^* (through the color tristimuli analysis CIE [1976], lightness-L^*) of dorsal skin was recorded, and melanin content and tyrosinase activity in the dorsal skin were examined as well.

* Corresponding author. Tel./fax: +86 532 82031943.
E-mail address: qhai@ouc.edu.cn (Q. Ai).

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2. Materials and methods

2.1. Preparation of diets

Experimental diets (Table 1) were formulated with fishmeal, soybean powder and wheat meal to provide 40% crude protein, and the dietary lipid level was about 8%. The composition of vitamins and minerals were similar to those used by Ai et al. (2006). The diets were made with the machine in our lab.

MEL with a purity of no less than 99.5% was purchased from Tianjin Bodi Chemical Holding Co., Ltd., (Tianjin, China). MEL was incorporated into feed in the same way of vitamins at a ratio of 0.2, 0.5, 1% (w/w) to prepare diet 2, diet 3 and diet 4, respectively. Diet 1 without the supplementation of MEL was used as a control. All formulated diets were stored at −20 °C until used.

2.2. Animal rearing

Darkbarbel catfish (initial weight 14.30 ± 0.10 g) were purchased from Shanghai Quanjie Fishery (Xicen Town, Qingpu District, Shanghai). An eight-week feeding experiment was conducted in round PVC tanks (water volume 280 L) with aerated pond freshwater. Circulating water system was used, and the system water was replaced about 10% daily. Each diet was randomly assigned to triplicate groups of tanks, and each tank was stocked with 30 darkbarbel catfish. Prior to the initiation of the experiment, darkbarbel catfish were fed MEL-nonsupplemented diet (diet 1) for 2 weeks. Following this, the darkbarbel catfish were fed to satiation twice at 7:00 am and 6:00 pm. Every morning, feces were removed to maintain water quality. During the eight-week experiment, water temperature varied between 25 °C to 28 °C, pH between 7.2 to 7.6, and dissolved oxygen were no less than 6.0 mg l^{-1}.

Table 1
Formulation and proximate chemical composition of the experimental diets for darkbarbel catfish (*Pelteobagrus vachelli*) (% dry matter).

	Diet no. (MEL content)			
	Diet 1 (0% MEL)	Diet 2 (0.2% MEL)	Diet 3 (0.5% MEL)	Diet 4 (1% MEL)
Ingredient				
Fish meal[a]	24	24	24	24
Soybean meal[b]	35	35	35	35
Beer yeast	2.5	2.5	2.5	2.5
Menhaden oil	2.5	2.5	2.5	2.5
Soybean oil	2.5	2.5	2.5	2.5
Lecithin	1	1	1	1
Wheat meal	27	27	27	27
Vitamin premix[c]	2	2	2	2
Mineral premix[d]	2	2	2	2
Microcrystalline cellulose	1	0.8	0.5	0
Melamine[e]	0	0.2	0.5	1
Attractant[f]	0.5	0.5	0.5	0.5
Proximate composition (% dry matter)				
Crude protein,	40.2	40.6	40.4	40.9
Crude lipid	7.8	7.9	7.8	7.8
Ash	9.0	8.9	8.9	8.9

[a] Commercially available from Seafood Co., Ltd., Shandong, China. Chemical composition: moisture, 7.4%; crude protein, 72.5% of dry matter; crude lipid, 6.6% of dry matter; ash, 18.5%.

[b] Commercially available from Seafood Co., Ltd., Shandong, China. Chemical composition: moisture, 11.5%; crude protein, 49.4% of dry matter; crude lipid, 1.0% of dry matter; ash, 6.4%.

[c] Vitamin premix (per kg diets): vitamin A, 32 mg; vitamin D3, 5 mg; vitamin E, 80 mg; vitamin K, 10 mg; thiamin, 25 mg; riboflavin, 45 mg; pyridoxine, 20 mg; vitamin B12 0.1 mg; inositol, 800 mg; D-calcium pantothenate 60 mg; niacin, 200 mg; folic acid, 20 mg; biotin, 1.2 mg; ascorbic acid, 2000 mg; choline chloride, 2000 mg; microcrystalline cellulose, 14.012 g.

[d] Mineral premix (per kg diets): NaF 2 mg; $Ca(IO_3)_2$ 1.8 mg;$CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; $Ca(H_2PO_3)_2 \cdot H_2O$, 3000 mg; NaCl, 100 mg; zeolite, 15.446 g.

[e] Purchased from Tianjin Bodi Chemical Holding Co., Ltd., Tianjin, China, with a purity of no less than 99.5%.

[f] Attractant, betaine.

2.3. Sampling

At the end of the feeding trial, the darkbarbel catfish were not fed for two days. All the fish from each tank was weighed. Five darkbarbel catfish from each tank were randomly selected for determining L^* of left dorsal skin in less than 20 s using a Konica Minolta ChromaMeter CR 400 (Konica, Japan). The measurement was carried out in the same position of each fish. Two measurements were taken in each position, and the measuring head was rotated 90° between each measurement. Average values per fish were calculated. After that, the dorsal skin on the left side of the body was dissected and stored at −80 °C for subsequent analysis.

Growth was expressed as the specific growth rate (SGR). Formula is:

$$\mathrm{SGR}\left(\%\ \mathrm{day}^{-1}\right) = \frac{(\ln W_t - \ln W_i)}{t} \times 100$$

where W_t and W_i are the final and initial mean weights (g), and t is the feeding trial period (days).

2.4. Determination of melanin content in the skin

Skin samples pre-treatment were similar to that of Wilson and Dodd (1973), Sugimoto (1993) and Pavlidis et al. (2006), with several modifications. Briefly, skins were accurately weighed and fixed in 95% ethanol for 24 h and then incubated with 1% HCl (60 °C, 1 h) for decalcification. After that, the decalcified pieces were washed with distilled water three times and boiled in 0.2% NaOH for 1 h to extract the melanin. Melanin concentration was determined by measuring the absorbance of the supernatant at 402 nm against a sepia melanin synthetic standard (Sigma, M-2649). The melanin standard was solubilized in 1 ml 1 M NaOH and 10 μl 3% H_2O_2 by heating in a boiling water bath for 30 min. Results were presented as melanin (mg)/skin weight (g).

2.5. Determination of tyrosinase activity in the skin

Tyrosinase activity in the skin was determined according to Chen et al. (2005) with several modifications. Briefly, skins were accurately weighed and homogenated with 50 mM Na_2HPO_4–NaH_2PO_4 buffer (pH 6.8). After centrifugation (5000 *g*, 20 min), the supernatant was placed on ice for the assay of tyrosinase activity. One milliliter 0.5 mM L-DOPA (prepared immediately before use) was added to a 5 ml microcentrifuge tube, and 2 ml supernatant was added and mixed, the absorbance (OD_0) at 475 nm was immediately recorded. The mixture was placed in 28 °C water bath for accurate 10 min, and the absorbance (OD_{10}) at 475 nm was recorded again, calculating $\triangle OD_{475} = OD_{10} - OD_0$. The enzyme activity of tyrosinase is calculated as:

$$\text{Tyrosinase Activity(U)} = \frac{\Delta \mathrm{OD}_{475}}{0.001 \times V \times T}$$

where V is the volume of homogenate (ml), and T is the reaction time (min).

The tyrosinase activity in the skin was expressed as tyrosinase activity (U)/skin weight (g).