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Defatted black soldier fly (*Hermetia illucens*) larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure

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ABSTRACT

A 59-days feeding trial was carried out to estimate the effects of fish meal replacement by defatted black soldier fly larvae meal (DBSFLM) on growth performance, antioxidate enzyme activities, digestive enzyme activities, hepatopancreas and intestinal morphology in Jian carp (*Cyprinus carpio* var. Jian) juveniles (initial mean body weight, 34.78 g). Five isolipidic (5.29 ± 0.04%) and isoprotein (40.69 ± 0.11%) diets were formulated by replacing 0%, 25%, 50%, 75%, 100% fish meal (FM) protein with graded DBSFLM levels of 0%, 2.6%, 5.3%, 7.9% and 10.6%. Each diet was randomly assigned to triplicate groups of 20 fish per aquarium. Fish were fed three times daily to apparent satiation. The results showed that the growth performance and nutrients utilization of fish in five groups were not different ($P > 0.05$). The hepatopancreas lipid and serum cholesterol content of treated groups was significantly lower than that of the control group ($P < 0.05$). With increasing dietary DBSFLM level, the activity of the CAT significantly increased. No significant differences in the activity of intestinal protease, lipase and diastase were observed among dietary groups ($P > 0.05$). The histological examination of intestine showed that when 75% or more FM protein was replaced, apparent pathological changes for example tissue disruption were observed in intestine, and relative gene expression of HSP70 in hepatopancreas significantly increased ($P < 0.05$). The histological examination of hepatopancreas sections showed less vacuolated with lipid deposits in treatment groups compared with control group. These results suggested that the growth of Jian carp was not affected by dietary DBSFLM, while it boosted antioxidant status of Jian carp by higher CAT activity. However, dietary stress and intestinal histopathological damage was observed when the replacement levels exceeded 75%. The study demonstrates that it is suitable to replace up to 50% of dietary FM protein with DBSFLM.

1. Introduction

Fishmeal is a major component in aquafeed due to its highly digestible protein, amino acids, as well as good palatability. However, an increasing demand and unstable production of fishmeal led to an increasing cost of aquaculture production. Therefore, there is a practical interest for partial or total replacement of fish meal with less expensive and protein-rich animal or plant ingredients has become a focus of research (Watanabe, 2002; Tacon and Metian, 2008). Insect is the largest organism community of ecosystem. In recent years, with constant exploration and use of insect resources, insect industry is gradually forms good ecological development pattern combined with planting industry and animal husbandry, such as processing feed protein (Sánchez-Muros et al., 2014). In China, one of the most promising insect species for commercial exploitation is the black soldier fly. Larvae of the black soldier fly have been reported to contain 42.1% crude protein and the defatted black soldier fly larvae contain 56.9% crude protein (Makkar et al., 2014), which comparable to that of soybean meal though slightly less than that of fish meal. Further, the black soldier fly larvae has a better amino acid profile and could be better substitutes of fish meal than soybean meal (Tran et al., 2015). Black soldier fly larvae oil has been investigated in aquafeed (S. Li et al., 2016). Because the fatty acid profile of black soldier fly is suboptimal for feed purposes, the oil must be extracted from the biomass before it is processed (Stamer, 2015). However, research on the application of defatted black soldier fly larvae was scarce in aquafeed.

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Jian carp (*Cyprinus carpio* var. Jian) is one of the economically important freshwater-cultured fish. In China, the production of Jian carp presents almost 50% of that of common carp every year (Zhou et al., 2008). Since its high meat content, deliciousness, high nutritional value, and cheap price, Jian carp is popular with consumers and the market demand is great.

Therefore, the aim of this study was to estimate the DBSFLM in the diet of Jian carp. We investigated the effects of DBSFLM on growth performance, feed utilization, serum biochemical parameters, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure in Jian carp, in order to provide reference information for the culture of carp using DBSFLM as protein source.

2. Materials and methods

2.1. Experimental diets

Five experimental diets were formulated to be isonitrogenous and isolipidic with approximately 41% crude protein, 5% crude lipid in the diets (National Research Council, 1993). Black soldier fly larvae provided by Jie mu Co Ltd. (Xi'an, Shaanxi Province, China) and the others purchased from Huaqin Agro-Tech Co Ltd. (Xi'an, Shaanxi Province, China). The defatted black soldier fly larvae meal was obtained by Soxhlet method added to the diets to replace fish meal (FM). The replacement levels were 0% (FM), 25% (DBSFLM25), 50% (DBSFLM50), 75% (DBSFLM75), and 100% (DBSFLM100), respectively. All diets were prepared and pelleted (2.5 mm pellet diameter) by the fish feed factory in Ankang Fisheries Experimental and Demonstration Station of Northwest A & F University (Ankang, Shaanxi Province, China). After drying in a cool and well-ventilated place at room temperature for 12 h, the pellets were collected and stored at − 20 °C until use. The ingredients and proximate compositions of the diets are given in Table 1. The amino acid composition (% fresh weight) of FM, DBSFLM, five experimental diets (1 sample per diets) was determined by amino acid analyzer (L-8900; Hitachi, Japan) and those results are presented in Table 2.

2.2. Fish feeding and management

Juvenile Jian carp (*Cyprinus carpio* var. Jian) were obtained from Ankang Fisheries Experimental and Demonstration Station of Northwest A & F University. In order to acclimate the rearing environment, the fish were cultured and fed a commercial diet (Huaqin feed factory, Yangling, Shaanxi Province, China) three times daily in a circulating water system for 2 weeks.

Before the beginning of the feeding experiment, the experimental fish were fasted for 24 h and weighed. A total of 300 fish (34.78 ± 3.03 g) were randomly distributed into 15 recirculating tanks (approximately 215 L; 80 cm in diameter; 70 cm in high), at a density of 20 fish per tank. The fish used in the experiment were equivalent size and weight. Water inflow was adjusted at 6 L min^{-1}, and supplemental aeration was provided via air stone diffusers. The fish were individually weighted at the beginning and end of the experiment with a 0.01 g sensitive electronic balance. The five experimental diets were randomly assigned to triplicate tanks. During the 59-days feeding, the fish were hand-fed to apparent satiation 3 times daily (at 8:30, 12:30 and 16:30). The water quality parameters were monitored on weekly basis, and the following parameters were recorded: the water temperature, dissolved O_2, pH and ammonia content maintained at 24.6 ± 2.55 °C, 6.13 ± 1.69 mg/L, 7.65 ± 0.42 and 0.11 ± 0.03 mg/L, respectively. Dead fish were weighed and the mortalities were recorded.

Table 1
Ingredients and proximate composition of the experimental diets.

	Experiment diets				
	FM[a]	DBSFLM25	DBSFLM50	DBSFLM75	DBSFLM100
Ingredients (g kg^{-1})					
Defatted black soldier fly larvae meal	0	2.6	5.3	7.9	10.6
Fish meal	10	7.5	5	2.5	0
Meat bone meal	5	5	5	5	5
Soybean meal	17	17	17	17	17
Full fat soybean	3	3	3	3	3
Rapeseed meal	22	22	22	22	22
Cottonseed meal	22	22	22	22	22
Wheat flour	12.4	12.2	11.9	11.8	11.5
Soya oil	2.6	2.7	2.8	2.8	2.9
Monocalcium phosphate	2	2	2	2	2
Bentonite	2	2	2	2	2
Mixture[b]	2	2	2	2	2
Proximate composition (g kg^{-1})					
Ash (%)	12.75	12.68	12.73	12.63	12.61
Moisture (%)	10.32	10.14	10.24	10.37	10.65
Lipid (%)	5.35	5.31	5.28	5.24	5.29
Crude protein (%, N% ∗ 6.25)	40.62	40.52	40.73	40.84	40.73

[a] FM = 100% fish meal; DBSFLM25 = 25% defatted black soldier fly larvae meal; DBSFLM50 = 50% defatted black soldier fly larvae meal; DBSFLM75 = 75% defatted black soldier fly larvae meal; DBSFLM100 = 100% defatted black soldier fly larvae meal.
[b] Contained 0.5% vitamin, 0.5% mineral and 1% Limestone carrier. Ingredients including/1 kg: VA 4000 IU, VD3 800 IU, VE 50 IU, VB1 2.5 mg, VB2 9 mg, VB6 10 mg, VC 250 mg, nicotinic acid 40 mg, pantothenic acid calcium 30 mg, biotin 100 μg, betaine 1000 mg, Fe 140 mg, Cu 2.5 mg, Zn 65 mg, Mn 19 mg, Mg 230 mg, Co 0.1 mg, I 0.25 mg, Se 0.2 mg.

2.3. Sampling

Fish were starved for 24 h prior to sampling, then anesthetized with tricaine methanesulfonate (MS-222). All fish were measured for final body weight (FBW) and 17 fish per tank were killed for collecting data on hepatopancreas, intraperitoneal fat (IPF), hepatosomatic index (HSI), viscera index (VSI), the remaining 3 fish were kept in − 20 °C until biochemical analysis. Among 17 sampling fish, 6 fish per tank were randomly selected for blood sampling from the caudal vein and the separated serum were removed by centrifuging (825 × *g*, 10 min) after keeping for 6 h at 4 °C, which were immediately frozen in liquid nitrogen and stored at − 80 °C for serum biochemical analysis. The hepatopancreas from 3 fish in each tank were collected into 1.5 ml tubes (RNase-Free; Axygen), frozen in liquid N_2 and then stored at − 80 °C until analysis for HSP70 gene. The hepatopancreas and muscle from another 3 fish were excised and then stored in − 20 °C for proximate composition analysis. All the procedures were based on the EU Directive 2010/63/EU for animal experiments.

Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF), viscera index (VSI), hepatosomatic index (HSI), intraperitoneal fat index (IFI), the relative gut length (RGL) and feed intake (FI) were calculated via the following formulae:

$$\text{Specific growth rate (SGR)} = (\text{Ln final weight} - \text{Ln initial weight}) \times 100/\text{days}.$$

$$\text{Feed conversion ratio (FCR)} = \text{amount of feed given (g)}/\text{weight gain (g)}.$$

$$\text{Weight gain rate (WGR)} = 100 \times [(\text{final weight} - \text{initial weight})/\text{initial weight}].$$

$$\text{Protein efficiency ratio (PER)} = \text{weight gain (g)}/\text{protein intake (g)}.$$