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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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Running title: Defatted black soldier fly larvae meal in diets for juvenile Jian carp

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.

Keywords: defatted insect meal; *Cyprinus carpio* var. Jian; black soldier fly; histology; Oxidative stress; Fishmeal

Introduction

Fishmeal is a major component in aqua feed 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 (Li et al., 2016b). 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.

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 analyser (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 recircularing 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.

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 weights (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:

Specific growth rate (SGR)= (Ln final weight – Ln initial weight)× 100 / days.

Feed conversion ratio (FCR)= amount of feed given (g)/ weight gain (g).

Weight gain rate (WGR)=100 × [(final weight – initial weight) / initial weight]

Protein efficiency ratio (PER)= weight gain (g)/protein intake (g)

Condition factor (CF)= body weight (g) × 100/ body length^3 (cm).

Viscera index (VSI, %)= viscera weight × 100/ body weight.

Hepatosomatic index (HSI, %)= hepatopancreas weight × 100/ body weight.

Intraperitoneal fat index (IFI, %)= intraperitoneal fat weight× 100/ body weight.

Feed intake (FI, %/fish/d) = 100 × total amount of the feed consumed (g) / [(W_0 + W_t)/2]/t

RGL= [intestinal length (cm)/body length (cm)] × 100

2.4 Proximate composition of diets and fish

Dry matter, crude protein, crude lipid, and ash were analyzed for ingredients, experimental diets and fish samples. Dry matter was analyzed by drying the samples to constant weight at 105℃. Crude protein was determined using the Kjeldahl method (AOAC, 2000) and estimated by multiplying nitrogen by 6.25. Crude lipid was measured by ether extraction using Soxhlet method. Ash was examined by combustion in a muffle furnace at 550℃ for 6h. Triple analyses were conducted for each sample.

2.5 Determination of serum biochemical parameters

Serum samples of two fish were randomly pooled for assay using an automatic

biochemical analyzer (Hitachi 7180, Tokyo, Japan) (n=2 fish per tank, n=3 replicates per treatment). The serum biochemical parameters included alanine aminotransferase (ALT, U/ ml), aspartate aminotransferase (AST, U/ ml), total protein (TP, g/ l), albumin (ALB, g/ l), globulin (GLO, g/ l), glucose (Glc, mmol/ l), cholesterol (Chol, mmol/ l), triglyceride (TG, mmol/ l), high- density lipoprotein cholesterol (HDL- c, mmol/ l) and low- density lipoprotein cholesterol (LDL- c, mmol/ l).

2.6 Digestive enzymes activities

In this assay, most of the procedures of the activities of protease, lipase, and amylase were adopted from previous research. Briefly, the fish gut was divided into three sections: anterior, mid and posterior intestines. The midgut of the intestine of nine individuals from each group (three fish/tank) were carefully weighed and homogenized in 0.01M Tris buffer, PH 7.4, at a ratio of 1:9 (tissue : buffer) with a Teflon pestle of a motor driven tissue cell disruptor under an ice bath. The extract was later centrifuged at 3000×g at 4 ℃ for 10 min, and the supernatant was used as the enzyme source. The activities of protease (A080-2), lipase (A054) and amylase (C016) in the intestine were assayed by the Colorimetric method using commercial kits (Jiancheng Biotech. Co., Nanjing, China).

2.7 Serum anti-oxidation indices

The serum superoxide dismutase (SOD) activity, catalase (CAT) activity and the malondialdehyde (MDA) contant were measured using SOD (A001-1), CAT (A007-1) and MDA (A003-1) assay kits (Jiancheng Biotech. Co., Nanjing, China) respectively.

2.8 Histological observation of intestine and hepatopancreas

The fixed intestine and hepatopancreas (3 individuals/ tank) were wrapped in gauze and rinsed in running water for 24 h. Subsequently, the samples were dehydrated in ethanol, infiltrated in xylene and embedded in paraffin, according to the standard histological procedures. The samples were cut serially (5μm thick) using a rotary microtome, stained with hematoxylin and eosin, and photographed with an inverted microscope (OPTEC, China).

2.9 RNA extraction and real-time quantitative polymerase chain reaction(qRT-PCR)

RNA was extracted from the hepatopancreas tissue by homogenization in TRNzol reagent (Tiangen, Beijing, China). To avoid genomic DNA amplification during real-time qPCR, the extracted RNA was treated with RNase-free DNase (TaKaRa,Dalian, China), and the RNA integrity was checked by electrophoresis on 2% agarose gels prior to reverse-transcription PCR. First-strand cDNA was synthesized from total RNA in pooled biological replicates using 200 U of SuperScript III RT (TaKaRa). All cDNAs were prepared by reverse transcription of 1 μg of column-cleaned total RNA in a 20 μL reaction volume. All cDNAs were further diluted 10 times with nuclease-free water prior to qPCR. Real-time qPCR was performed using a CFX 96 Real-time PCR Detection System (Bio-Rad,Hercules,CA,USA) with SYBR® Premix Ex Taq™ II (TaKaRa). The total volume of the PCR reaction was 20μL, comprising 0.6 μL of each primer (10μM), 1μL of cDNA (10^{-1} dilution of the first-strand synthesis product), 10μL of 2×SYBR® Premix Ex Taq™ II and 7.8μL of sterile double distilled water. The PCR program comprised an initial 30 s denaturation at 95 °C followed by 40 cycles of 95 °C for 15s

and 57 °C for 15 s. The forward and reverse primers of target genes are listed in Table 3. Following PCR, melting curve analyses were performed to confirm the presence of single products in these reactions. The relative quantification method described by Livak and Schmittgen (2001) was used to calculate the gene expression values.

2.10 Statistical analysis

The results are expressed as means ± standard deviation (S.D.). All data were analysed using one-way analysis of variance, followed by Duncan's post hoc tests. Differences were considered statistically significant when $P<0.05$. All analyses were conducted using PASW Statistics 18 (SPSS, Chicago, IL, USA).

3.Results

3.1 Growth performance and somatic indices

The effects of experimental diets on growth performance and biological parameters are presented in Table 4. There were no significant differences in IBW of the fish at the start of the trial ($P>0.05$). After the 56 days feeding, fish grew approximately by triple of the initial body weight (IBW). No mortality was observed during the feeding trial. There was no significant difference on final body weight (FBW), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), condition factor (CF), hepatosomatic index (HSI), weight gain ratio (WGR), protein efficiency ratio (PER), viscera index (VSI), intraperitoneal fat body index (IFI) and relative gut length (RGL).

3.3 Proximate compositions

The proximate compositions of the feeding fish are shown in Table 5. There were

no significant differences in moisture and crude protein in hepatopancreas, muscle and whole body among treatments (P>0.05). In the hepatopancreas, the lipid content of DBSFLM treated groups was significantly lower than that of the control group (P<0.05), while there was no difference in muscle and whole body among DBSFLM treated groups (P>0.05).

3.4 Serum biochemical indices

The serum biochemical index values are shown in Table 6. The Chol level of treated groups was significantly lower than that of the control group (P<0.05). Also, the content of ALT, AST, TP, GLO, A/G, TG and GLB in treated groups were lower than that of the control group, and there was no significant difference among dietary treatments (P>0.05). The density of Glc were not influenced significantly among all dietary treatments (P>0.05).

3.5 Digestive enzymes

Alpha-amylases, lipases and trypsin in the intestine were assayed (Fig. 1). Alpha-amylases, trypsin and lipases was not influenced among the groups (P>0.05).

3.6 serum anti-oxidation indices

The results of measurements of serum SOD and CAT activity and MDA content are shown in Fig. 2. The CAT activity showed increased trend and was significantly higher levels in DBSFLM75 and DBSFLM100 groups compared to that in the FM (P<0.05). The SOD activity and the MDA content showed no significant difference among all groups.

3.7 Morphology of intestinal and hepatocytes

The intestinal microvilli in the FM, DBSFLM25 and DBSFLM50 groups were most regular in shape; those in the DBSFLM75 and DBSFLM100 groups were associated with more debris (Fig. 3). The hepatocytes in the FM, DBSFLM25, DBSFLM50 and DBSFLM75 groups were polygonal in shape and had centrally located nuclei and clear cell boundaries. It was difficult to clearly distinguish the cell boundaries in the BSF100 group. Moreover, in the DBSFLM100 group, the hepatocytes were of irregular shape, and apoptotic cells with small pyknotic nuclei were evident (Fig. 4).

3.8 Expression of HSP70 gene

Based on the hepatic mRNA content, the HSP70 gene was up-regulated with increasing dietary DBSFLM content, and were significantly higher in DBSFLM75 and BSF100 groups (P<0.05). There was no significant difference among the FM, DBSFLM25 and DBSFLM50 groups (Fig. 5).

4. Discussion

In the present study, both all-FM and DBSFLM diets were equally consumed by Jian carp throughout the 59-days experiment, indicating that voluntary feed intake was not affected by substitution of FM by DBSFLM, even at the highest DBSFLM inclusion level (106 g kg^{-1} diet). The current study showed that replacement of dietary FM by DBSFLM had no significant effect on the growth performance of fish, suggesting that it is possible to substitute up to 100% fishmeal by DBSFLM in diets for Jian carp without negative effect on growth performance and feed utilization. A similar result was found in Atlantic salmon that dietary replacement FM by black soldier fly larvae did not result in the difference of the growth performance (Lock et al., 2015). Moreover, dietary FM partially replaced by housefly maggot meal showed

no effects on weight gain, feed intake and feed efficiency (Lin and Mui, 2016). However, another study in Nile tilapia has found that dietary replacement of FM by black soldier fly larvae had significantly reduced weight gain (WG) and Specific growth rate (SGR), while significantly improved feed intake (FI) and Feed conversation ratio (FCR) (Webster et al., 2015). Kroeckel et al. (2012) have reported that dietary replacement of FM by black soldier fly larvae meal had significantly reduced growth performance and feed utilization of turbot. These varied results might be due to the difference in fish species, fish size, insect species and the substrates and processing for insect (Tschirner and Simon, 2015).

In addition, this study showed that feeding Jian carp diets with DBSFLM, affected hepatopancreas fat deposition. The crude lipid contents of hepatopancreas in DBSFLM-fed fish groups significantly lower than that in FM-fed fish group. Correspondingly, histological examination of hepatopancreas showed that the lipid hepatocytes accumulation in fish fed the DBSFLM diets was lesser than in fish fed the fishmeal diets (Fig. 4). Similar results have been reported in turbot by Kroeckel et al. (2012) who found that body lipid decreased with increasing black soldier fly pre-pupae meal inclusion levels. Besides, Belforti et al. (2015) found that compared to control, the lipid contents of fillets were decreased significantly following *Tenebrio molitor* meal inclusion in the rainbow trout diet. This may result from the chitin exist in insect meal. Chitin and its derivatives were reported to play a significant role on decreasing fatty acids synthesis and increasing hydrolysis of lipoproteins and triglyceride in the liver (zhang et al., 2008; Li et al., 2016a). In this study, no effect of dietary DBSFLM levels on the content of crude protein, moister and crude ash in whole body, hepatopancreas and muscle of Jian carp. Similar results have been reported in rainbow trout by Gasco et al. (2014). Differently, Belforti et al. (2015)

found that compared to control, the protein contents of fillets were increased significantly following *Tenebrio molitor* meal inclusion in the diet. Besides, Ji et al. (2015) have reported that the replacement of dietary FM with silkworm pupae meal significantly reduced muscle crude ash of Jian carp. This inconsistence could be related to different diet formulations, insect species and fish species.

The present study showed that serum biochemical indices except for Chol were not affected by dietary DBSFLM treatments. The Chol level of treated groups was significantly lower than that of the control group ($P<0.05$) (Table 6). Also, Ji et al (2015) reported that after 8 weeks feeding experiments, silkworm pupae meal significantly decreased Jian carp's serum Chol. In fish, the hypocholesterolemic effect of plant proteins has been well documented (Shafaeipour et al., 2008), but little information about animal proteins was reported. Here, we can suppose that a reduced level of Chol was attributed to the chitin, a poly-beta-1,4-N-acetylglucosamine (GlcNAc), which is the main component of arthropod exoskeletons, tendons, and the linings of their respiratory, excretory, and digestive systems (Clark & Smith, 1936; Herring, 1979). Chitin can be degraded by chitinase, which is widely distributed amongst fish (Lindsay, 1984). Chitosan as the major product of chitin enzyme hydrolysis, which several studies have reported, has a hypocholesterolemic action in animal models (Sugano et al., 1980). Also, the level of cholesterol in diets may partly be attributed to the level of cholesterol in serum (Kaushik et al., 1995). In this study, the defatted black soldier fly larvae meal may contain less cholesterol than fishmeal, and resulted in the lower cholesterol contents in DBSFLM-fed fish groups. Cholesterol metabolism in fish is different from that of terrestrial animal models, and

there is a multiplicity of mechanisms involved in the control of cholesterolemia which needs further elucidation (Shafaeipour et al., 2008). The activities of serum AST and ALT are generally related to liver damage, as a mark of liver necrosis when they increase (Hyder et al., 2013; Sheikhzadeh et al., 2012; Song et al., 2014; Wang et al., 2014). The results of no influence on the serum ALT and AST activities by dietary DBSFLM suggested that DBSFLM might not cause negative effects to hepatopancreas health. The concentration of TP, involved in ALB and GLO, increases in the diseases of immune disorders, liver dysfunction and impaired kidney activity (Banaee et al., 2011; John, 2007). GLO is also proved to modulate the immune response (EI-Kamary et al., 2009). In this study, no significant differences were detected in serum TP, ALB and GLO among treatments, indirectly suggesting that dietary DBSFLM might not influence the immune system ability of the fish.

Oxidative stress occurs when there is a disproportion of ROS production and the volume of antioxidant systems to control their damaging effects (Monaghan et al., 2009). ROS are essential by-products of normal metabolic process that could result in damage to DNA in the cell nucleus and is harmful to other proteins and lipids within cell membranes if not suppressed by the antioxidant mechanism (Pamplona and Costantini, 2011). The first line of defense against ROS is represented by the CAT-SOD enzyme mechanism. Hence, SOD catalyses the reduction in superoxide anions into hydrogen peroxide, which is later decomposed by CAT at intra- and extracellular levels (Nordberg and Arner, 2001). Catalase activity is correlated with an increasing concentration of H_2O_2 (Wilhelm Filho et al. 2005). In the present study,

we detected a significantly higher activity of this enzyme in the serum of Jian carp fed with DBSFLM75 and DBSFLM100 diets (Fig. 2). This situation was in accordance with Taufek et al. (2016) who found that CAT activities in African catfish liver produced a similar trend when including cricket meal in their diets. This situation was also supported by Ogunji et al. (2011) who observed that CAT activities in carp liver increased when including maggot meal in their diets. Insect were known to contain a significant amount of chitin in their exoskeleton. Chitin and its derivatives were reported to have antioxidant properties that could prevent deleterious effects in various diseases (Khoushab and Yamabhai, 2010; Ngo and Kim, 2014). The data (Fig. 2) obtained during this experiment demonstrated that defatted black soldier fly larvae meal could boost antioxidant status when given to Jian carp in a formulated diet.

Digestive enzyme activities demonstrate the potential impact on feed utilization and growth performance, especially protease, amylase, and lipase, which play a pivotal role in the digestive process. In the present study, we observed no significant differences in levels of trypsin, alpha-amylases, and lipases between treated groups and the control group (Fig. 1), indirectly suggesting that dietary DBSFLM might not influence the feed utilization ability of the fish. This was in line with the growth performance in this study.

In the present study, histopathological examination revealed mild hepatic necrosis of the liver only in DBSFLM75 and DBSFLM100 groups. The effect was mild enough that serum parameters indicated no significant differences in liver enzymes (Table 6). The Hepatocytes is highly sensitive to the nutritional status of the

fish and to the quality of the diet (Brusle and Anadon, 1996), and particularly the hepatocytes size varies with the digestible carbohydrate content of the diet (Kaushik et al., 1989). In this study, liver cells were partially atrophied in DBSFLM75 and DBSFLM100 groups, and those diets mediated effect on liver as already been noticed (Yamamoto et al., 2007). At the primary site of food digestion, and nutrient uptake and transformation, the intestine is central to physiological functioning, and optimum utilization of dietary nutrients depends on their functional effectiveness (Caballero et al., 2003). As protein nutrients will particularly affect the construction of intestinal microvilli and lots of histological studies have been investigated the impact of various dietary protein sources in fish including rainbow trout (Caballero et al., 2002), gilthead sea bream (Caballero et al., 2003) and common carp (Ostaszewska et al., 2010). Partial amino acid deficiency in plant protein-based diets adversely affect fish intestinal epithelium (Ostaszewska et al., 2010). But, Amino acid composition of the experimental diets showed slightly difference among groups (Table 2). Therefore, the histological effects on hepatocytes and intestine observed in this study maybe partly explained by chitin consist in DBSFLM meal. Chitin and its derivatives were reported to decreased dietary nutrients absorption in intestine (Li et al., 2016a; Alegbeleye et al., 2012).

The heat shock protein (HSP) family is a group of cellular proteins present in most life forms (Lindquist 1986). Based on their molecular mass, HSPs are classified into several families including the HSP90s, HSP70s, HSP60s and low molecular mass HSPs (Cui et al., 2010; Zhang et al., 2011). HSP70 is expressed at very low levels

under normal conditions, but is induced rapidly in response to various stressors (Cellura et al., 2006). Therefore, HSP70 plays an important role in marine carb exposed to environmental stressors and the occurrence of disease caused by feed factors (Zhang et al., 2009). It has also been found to respond to diets of suboptimal composition (Hemre et al., 2004). The current experiment showed that with increasing dietary substitution of FM with more than 75% DBSFLM, there was enhanced hepatic HSP70 gene expression, suggesting that the diets containing higher DBSFLM levels induced stress on Jian carp. The similar result was reported by Ji et al. (2015), while differs from that of Hansen et al. (2006). This may be due to the different experimental conditions, such as difference in species, ages, feed factors, etc.

In conclusion, this study demonstrated that at least 50% of FM protein could be replaced with DBSFLM without any adverse effects on growth performance, carcass composition，antioxidant enzyme activities and digestive enzyme activities of juvenile Jian carp. However, dietary stress and intestinal histopathological damage was observed when the replacement levels exceeded 75%.

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Tables

Table 1 Ingredients and proximate composition of the experimental diets

	Experiment diets				
	FM[1]	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[2]	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

[1] 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.

[2] Contained 0.5% vitamin, 0.5% mineral and 1% Limestone carrier. Ingredients including/1 kg: VA 4 000 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 100μg, 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.

Table 2 Amino acid composition (% fresh weight) of the experimental diets and defatted black soldier fly larvae meal.

	Diet groups					DBSFLM
Amino acids	FM	DBSFLM25	DBSFLM50	DBSFLM75	DBSFLM100	
Aspartic acid	3.05	3.07	3.14	3.14	3.11	5.28
Threonine	1.31	1.34	1.34	1.34	1.32	2.24
Serine	1.44	1.46	1.48	1.49	1.46	2.02
Glutamic acid	5.81	5.88	5.87	5.83	5.68	5.57
Proline	3.88	3.90	3.93	3.99	4.03	5.55
Glycine	1.75	1.72	1.73	1.71	1.71	2.63
Alanine	1.55	1.57	1.60	1.58	1.58	3.34
Valine	1.47	1.52	1.56	1.59	1.62	3.78

Methionine	0.45	0.46	0.53	0.45	0.34	1.16
Isoleucine	1.28	1.30	1.31	1.30	1.30	2.23
Leucine	2.33	2.35	2.38	2.35	2.32	3.61
Tyrosine	0.93	0.99	1.07	1.07	0.98	3.40
Phenylalanine	1.55	1.57	1.59	1.60	1.59	2.35
Lysine	1.85	1.88	1.83	1.86	1.86	3.72
Histidine	0.88	0.90	0.89	0.91	0.90	1.62
Arginine	2.58	2.58	2.66	2.59	2.55	2.65
SAA[1]	32.36	32.73	33.20	33.06	32.59	51.14

SAA[1]: sum of amino acids in the material and diets (%)

Table 3 Primers used in real-time PCR

Target gene	Genbank accession no.	Forward (5’-3’)	Reverse (5’-3’)
HSP70	AY120894	TCAGTCTGCCCTTGTCATTGGTGA	TTTGAGCTGACAGGAATCCCACCT
β -actin	M24113	AGTTGAGTCGGCGTGAAGTGGTAA	TCCACCTTCCAGCAGATGTGGATT

Table 4 Effect of DBSFLM on growth performance and biological indices of the experimental fish[1]

Index	Dietary groups				
	FM	DBSFLM25	DBSFLM50	DBSFLM75	DBSFLM100
FBW(g)	110.38±3.34	106.86±4.83	107.80±1.26	111.33±1.07	109.30±4.11
WGR(%)	217.36±9.61	211.79±7.91	204.91±10.16	220.10±3.08	214.27±11.82
SGR(%/d)	2.06±0.05	2.03±0.05	1.99±0.06	2.08±0.02	2.04±0.07
FI(%/d)	2.79±0.08	2.84±0.05	2.87±0.06	2.77±0.01	2.80±0.06
FCR	1.50±0.07	1.55±0.06	1.59±0.08	1.48±0.02	1.52±0.07
PER	1.64±0.08	1.60±0.13	1.59±0.07	1.65±0.11	1.62±0.08
HSI(%)	1.31±0.02	1.28±0.07	1.27±0.01	1.29±0.06	1.27±0.07
VSI(%)	13.58±0.31	13.47±0.46	13.44±0.17	12.78±0.59	12.53±0.34
IFI(%)	0.39±0.04	0.38±0.02	0.33±0.06	0.33±0.07	0.33±0.04
CF	2.44±0.10	2.57±0.20	2.46±0.14	2.38±0.02	2.35±0.01

RGL(%)	1.55±0.10	1.56±0.15	1.48±0.06	1.42±0.07	1.47±0.05

[1]FBW, final body weight; WGR, weight gain rate; SGR, specific growth rate; FI, feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio; HSI, hepatosomatic index; VSI, viscera index; IFI, intraperitoneal fat index; CF, condition factor.

Values are means ± standard deviations (n=3).

Table 5 Effect of DBSFLM on proximate composition in tissues of fish[1] (% wet weight)

Proximate composition	Dietary groups				
	FM	DBSFLM25	DBSFLM50	DBSFLM75	DBSFLM100
Hepatopancreas					
Crude protein	20.53±1.18	20.61±1.02	21.42±1.06	20.71±0.88	21.17±0.29
lipid	32.24±4.18[a]	25.56±0.77[b]	22.64±2.50[b]	25.94±2.48[b]	24.10±4.05[b]
Moisture	69.25±2.59	71.38±2.19	72.13±5.53	72.26±1.00	68.63±2.55
Ash	1.64±0.09	1.63±0.18	1.50±0.03	1.54±0.11	1.62±0.04
Muscle					
Crude protein	22.99±1.95	23.30±0.59	23.15±0.57	23.50±0.62	23.51±1.19
lipid	2.57±0.54	2.22±0.24	2.18±0.79	2.17±0.19	2.14±0.50
Moisture	79.17±0.63	79.19±0.79	79.13±0.75	79.26±0.25	79.46±0.96
Ash	1.30±0.04	1.31±0.01	1.27±0.01	1.37±0.05	1.31±0.05

Whole body					
Crude protein	19.59±0.52	19.88±0.69	19.62±0.84	20.09±0.19	19.06±0.42
lipid	16.32±1.09	16.71±1.50	16.11±0.65	16.77±1.68	16.12±0.95
Moisture	75.37±0.68	75.02±0.98	74.45±0.61	74.35±0.52	74.97±0.69
Ash	3.91±0.27	3.92±0.13	3.88±0.19	3.91±0.10	3.99±0.45

[1] Values are means and standard deviations with different superscripts in the same row are significantly different ($P < 0.05$) from each other. (n=3)

Table 6 Serum biochemical indices of the experimental fish[1]

Index	Dietary groups				
	FM	DBSFLM25	DBSFLM50	DBSFLM75	DBSFLM100
ALT	16.05±3.05	12.20±5.68	13.65±2.17	13.05±3.77	12.47±2.96
AST	178.00±71.76	120.43±51.96	126.90±45.69	108.67±18.38	145.50±66.74
TP	23.28±2.29	18.88±2.96	21.38±2.67	19.80±3.24	20.97±3.10
ALB	11.18±1.29	8.90±1.36	10.00±1.10	9.05±1.32	9.80±1.24
GLO	12.10±1.11	9.98±1.63	11.38±1.73	10.75±1.94	11.17±1.92
A/G	0.92±0.06	0.89±0.04	0.89±0.08	0.85±0.04	0.89±0.07
GLc	5.81±1.33	5.35±1.63	6.17±2.85	4.82±1.42	5.92±1.45
Chol	3.11±0.35^{a}	2.52±0.40^{b}	2.52±0.29^{b}	2.13±0.23^{b}	2.47±0.32^{b}
TG	2.52±0.35	1.99±0.38	2.20±0.26	1.89±0.34	2.18±0.41

[1] Values are means and standard deviations with different superscripts in the same row are significantly different ($P < 0.05$, one-way ANOVA) from each other (n=3).

ALT (U ml^{-1}), alanine aminotransferase; AST (U ml^{-1}), aspartate aminotransferase; TP (g L^{-1}), total protein; ALB (g L^{-1}), albumin; GLO (g L^{-1}), globulin; A/G, albumin/ globulin; Glc (mmol L^{-1}), glucose; Chol (mmol L^{-1}), cholesterol; TG (mmol L^{-1}), triglyceride.

Figure legends

Fig. 1. Alpha-amylase, lipase and trypsin in the intestine of Jian carp fed diet substituting fishmeal with defatted black soldier fly larvae meal for 8 weeks.

Fig. 2. Serum oxidation indices: superoxide dismutase (SOD) activity, malondialdehyde (MDA) content, catalase (CAT) activity. Values are means (±SD) of three replications containing three fish per replication. [a,b]Means with different letters are significantly different (P<0.05) from each other.

Fig. 3. The midgut construction.

Fig. 4. Hepatocyte construction.

Fig. 5. Expression of hepatic HSP70 gene. Values are means (±SD) of three replications containing three fish per replication. [a,b]Means with different letters are significantly different (P<0.05) from each other.

Fig. 1

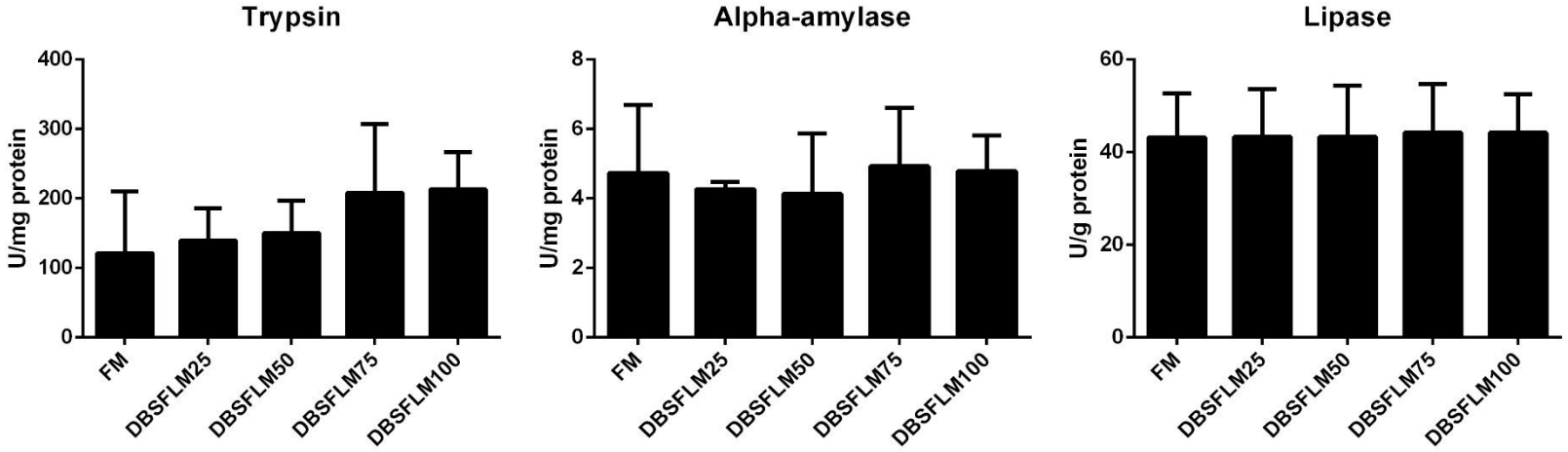


Fig. 2

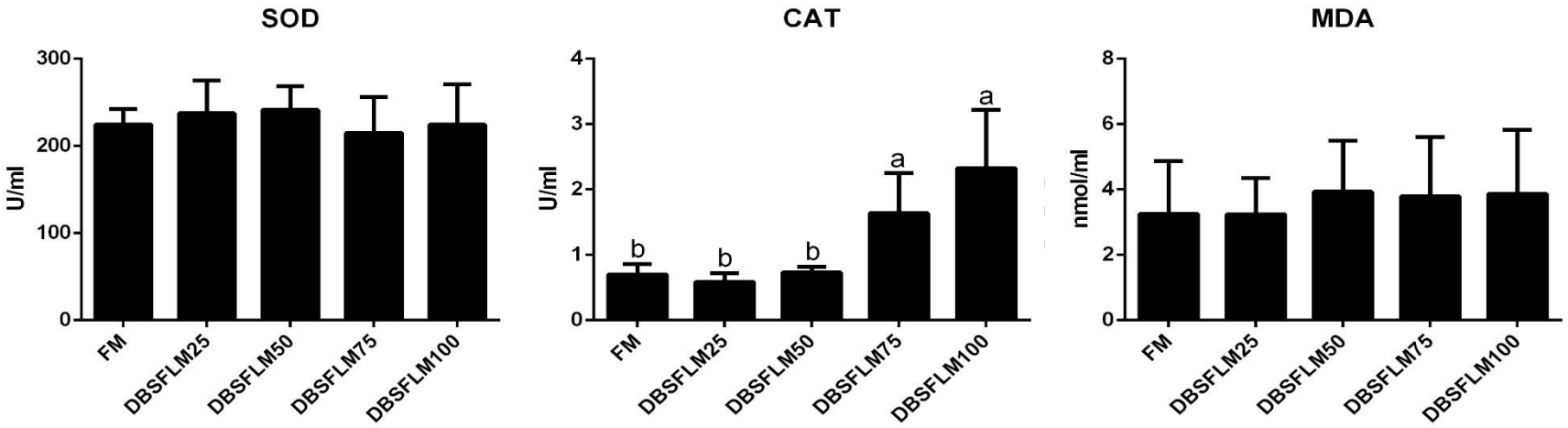
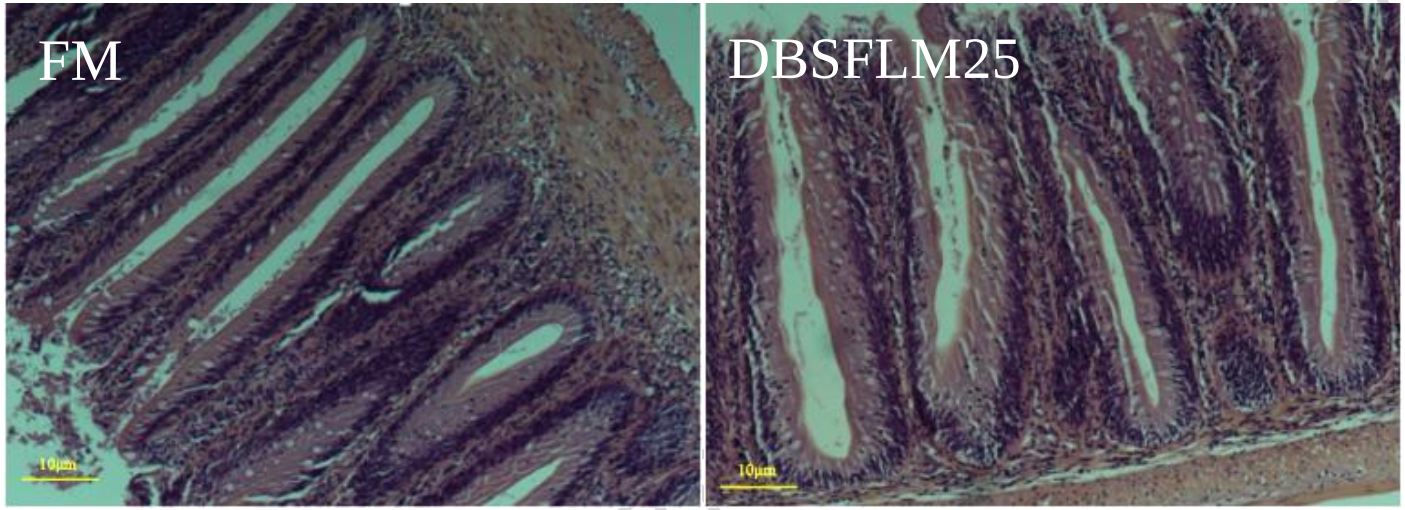


Fig. 3



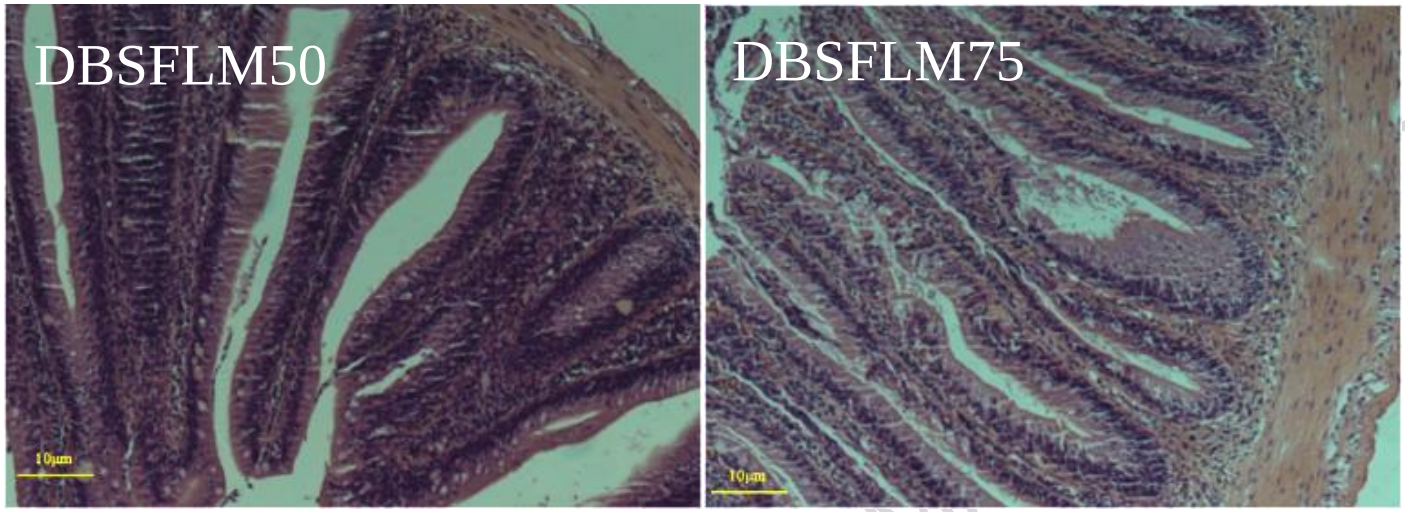
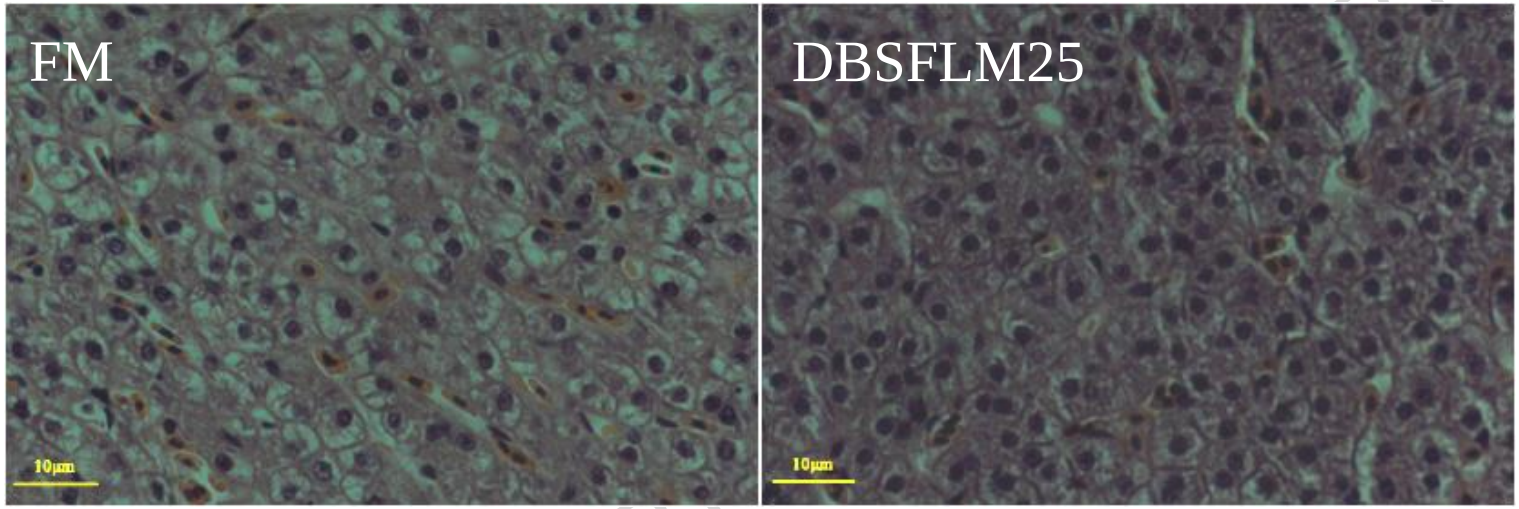
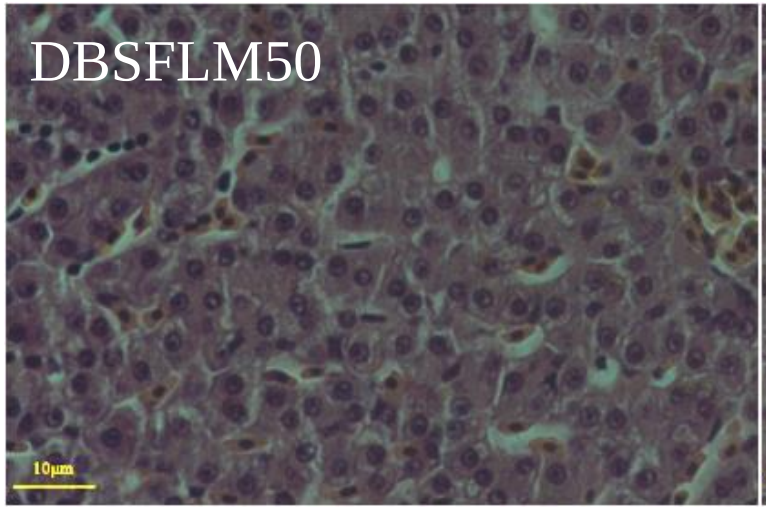
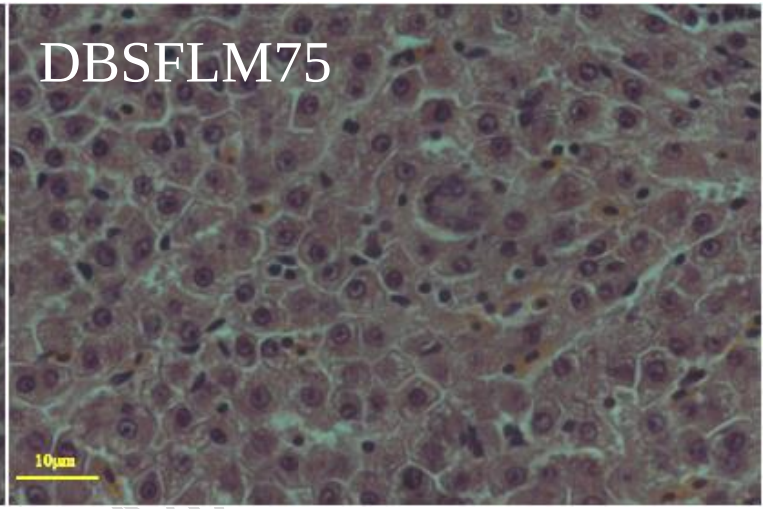


Fig. 4







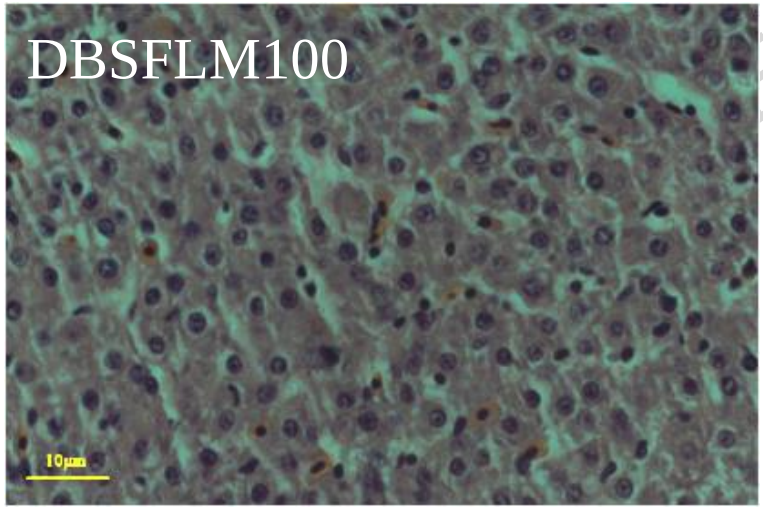
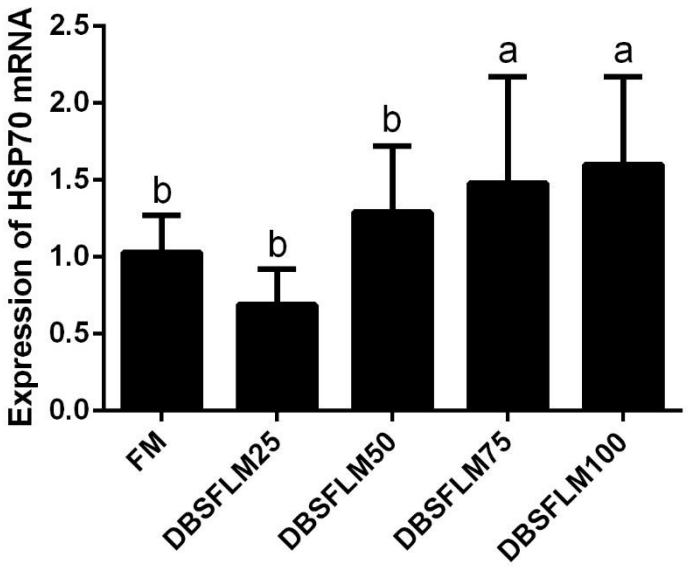


Fig. 5



Highlights

Defatted black soldier fly larvae meal boosted antioxidant status of fish by higher CAT activity.

Defatted black soldier fly larvae meal significantly decreased the hepatopancreas lipid and serum cholesterol content of fish.

It is practical to replace 50% of the Jian carp dietary fishmeal protein with defatted black soldier fly larvae meal.

High levels of substitution of fishmeal with defatted black soldier fly larvae meal resulted in stress and intestinal histopathological damage of fish.