Impacts of dietary konjac glucomannan supplementation on growth, antioxidant capacity, hepatic lipid metabolism and inflammatory response in golden pompano (*Trachinotus ovatus*) fed a high fat diet

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Abstract

This study examined the effect of konjac glucomannan (KGM) supplementation in diets with different lipid levels on growth, antioxidant capacity, hepatic lipid metabolism and inflammatory response in juvenile pompano (*Trachinotus ovatus*). Triplicate groups of fish (12.07 ± 0.21g) were fed eight experimental diets containing two lipid levels of 9 % (basal-fat diet, BFD) and 18 % (high-fat diet, HFD), and four levels of KGM (0, 0.5, 1 and 2 %) for 60 days. Pompano fed HFD exhibited markedly lower growth and feed efficiency than fish fed BFD, and KGM supplementation, particularly at 1 % inclusion, significantly improved growth performance and feed efficiency in fish fed both BFD and HFD. In addition, Pompano fed HFD showed higher whole-body lipid and lower protein contents than fish fed BFD. Irrespective of dietary lipid level, whole body, dorsal and ventral muscle were reduced by dietary KGM supplementation up to 1 % while the opposite was found for whole body protein content. Dietary KGM also significantly reduced the HSI, liver lipid contents, as well as lipid droplets in the liver. Supplementation of KGM to both BFD and HFD significantly reduced serum total cholesterol, triglyceride, low-density lipoprotein cholesterol and free fatty acids levels. Furthermore, irrespective of dietary lipid level, increasing dietary KGM supplementation enhanced serum IgM level, lysozyme and alkaline phosphatase activities, and reduced serum alanine aminotransferase and aspartate aminotransferase activities. Dietary KGM also increased liver total antioxidant capacity, superoxide dismutase and catalase activities, and decreased malondialdehyde concentration in fish fed both HFD and BFD. Dietary KGM significantly increased serum glutathione peroxidase, superoxide dismutase and catalase activities and reduced malondialdehyde concentrations in fish fed BFD, and enhanced serum total antioxidant capacity and glutathione peroxidase activity in fish fed HFD. Supplementation of KGM increased the expression of genes associated with lipid catabolism (*pparα*, *cpt1*, *hsl*) and lipid transport (*fabp1*, *apoB100* and *cd36*), and decreased the expression of lipid anabolism related genes (*srebp1*, *pparγ*, *fas* and *acc*). Moreover, the expression of pro-inflammatory cytokines including tumor necrosis factor α and interleukin-8 were down-regulated, while expression of the anti-inflammatory cytokine transforming growth factor β1 was increased by KGM supplementation. In conclusion, supplementation of KGM at 1 % of diet improved growth, feed efficiency, antioxidant capacity, hepatic lipid metabolism and inflammatory responses in juvenile pompano, and can mitigate excessive deposition of lipid in tissues of farmed fish.

Keywords: Konjac glucomannan, High-fat diets, Antioxidant capacity, Hepatic lipid metabolism, Inflammatory response

1. Introduction

The aquaculture industry has developed rapidly to become an important source of food and aquatic products in China, and now accounts for over 50 % of the world's seafood production for human consumption (Gao et al., 2018; Sprague et al., 2017). In order to shorten the production cycle, high-energy/fat diets have been widely employed in sustainable aquaculture practices (Asaoka et al., 2013). As well as lower price, dietary lipid aids the absorption and transportation of fat-soluble vitamins, and also provides essential fatty acids and phospholipids for the maintenance of cell normal structure and biological function in fish (NRC, 2011; Sargent et al., 1999). Furthermore, the protein-sparing effect of dietary lipid improves feed efficiency and growth in many fish species (Cho et al., 2015; Du et al., 2005; Li et al., 2012; Shi et al., 2018) and this, in particular, has encouraged the widespread use of high-fat diets (HFD) in intensive aquaculture (Li et al., 2017a). However, the use of HFD can often be accompanied by increased lipid deposition in tissues, particularly liver, of farmed fish, leading to increased incidence and scale of fatty liver disease, especially in marine species (Asaoka et al., 2013). Excessive lipid accumulation in the abdominal cavity and liver of cultured fish induces lipid metabolic disorders, liver damage and immune dysfunction leading to deteriorated growth and health and eventually huge economic losses (Huang et al., 2018; Li et al., 2016; Yan et al., 2015; Zhang et al., 2017a). As well as content, lipid composition of key components of aquafeeds also affects lipid deposition in fish and so has been the subject of considerable research in fish nutrition. There have been many studies to determine the optimal dietary lipid level to minimize excess fat accumulation and promote healthy growth of various aquatic species, including *Larimichthys crocea* (Yan et al., 2015), *Clarias magur* (Mir et al., 2019), *Micropterus salmoides* (Guo et al., 2019) and *Scylla paramamosain* (Xu et al., 2020), among others.

Recently, the potential of dietary additives which help to reduce the risk of hepatic lipid metabolic disorders and/or alleviate the development of fatty liver in farmed fish is gaining increasing interest. Konjac glucomannan (KGM) is a water-soluble polysaccharide consisting of β-D-glucose and β-D-mannose mainly linked by β-1,4-glycosidic bonds extracted from the edible corm (tuber) of *Amorphophallus konjac* or "Devil's tongue" belonging to the Araceae family (Shah et al., 2015; Zhang et al., 2005). KGM has been recognized as a food additive by the U.S. Food and Drug Administration since 1994 (Li et al., 2017b) and, recently, has been applied widely in food, pharmaceutical and cosmetic industries owing to its good thickening, stability and biocompatibility properties (Devaraj et al., 2019; Yang et al., 2017). KGM cannot be decomposed by digestive enzymes in the upper gastrointestinal tract of human, but it could be enzymatically digested by the extracellular enzyme produced by gut microbiota (Ouyang et al., 2020). Previous studies indicated that KGM polysaccharides, after being subjected to enzyme hydrolysis, have exerted a variety of beneficial physiological effects such as immunoregulation (Chang et al., 2013; Onishi et al., 2005), antioxidation (Jian et al., 2017), anti-obesity (Keithley et al., 2005) and gut microbial metabolism. It has also been demonstrated that KGM can prevent some metabolic disorders, reducing serum cholesterol, and alleviating hyperlipidemia and hyperglycemia (Chen et al., 2003; Sood et al., 2007). The potential roles of KGM in the prevention of HFD-induced nonalcoholic fatty liver

syndrome (NAFLS) have been explored in mammals and it was shown that KGM modulated HFD-induced NAFLS through activating the PI3K pathway and regulating expression of key genes associated with lipid metabolism (Shang et al., 2019). Given the functional properties of KGM in mammals, increasing attention has been paid to the application of KGM and its derivatives in aquafeeds. Studies have shown that oxidized KGM and acidolysis-oxidized KGM could promote growth performance, improve immune function and up-regulate expression of the immune-related genes of the cyprinid *Schizothorax prenanti* (Chen et al., 2016; Zhang et al., 2013; Zheng et al., 2015). Zhang et al. (2017b) showed that dietary supplementation of oxidized KGM beneficially affected serum lipid metabolism and reduced flesh lipid content in *S. prenanti.* However, the specific role(s) of KGM in lipid metabolism and tissue fat deposition in mitigating HFD-induced hepatic lipid dysfunction are still unclear in fish.

Pompanos, which constitute around 21 species of the genus *Trachinotus* in the Carangidae or jack family, are carnivorous marine fish that mainly feed on zooplankton, shellfish and small fish (Zhang et al., 2019a). Most *Trachinotus* species have desirable taste characteristics and are thus valued as food fish with limited commercial fisheries, and some species that exhibit rapid growth, high disease resistance and suitability for cage culture have made pompano species, such as *T. ovatus* and *T. blochii*, promising candidates for intensive aquaculture in Southern China with annual production of around 120,000 tons (Li et al., 2020; Tan et al., 2017). Dietary protein requirement of pompano at around 50 % is relatively high (Wang et al., 2020; Zhang et al., 2019a), so that improving utilization of dietary lipid as an economical source of energy to spare protein for somatic growth is crucial for viable farming of pompano. The optimum dietary lipid level for juvenile pompano has been suggested to be 5-12 % of diet (Tan et al., 2013), and commercial feed produced for this species in China contains 9 % lipid. Our recent studies showed that dietary lipid level for juvenile pompano could be increased to 12.5 % in experimental conditions (Guo et al., 2020; Zhang et al., 2019c), but increasing lipid content in feeds to 18 % led to growth depression and abnormal fat deposition in tissues (unpublished). The objectives of the present study were to evaluate the effects of dietary KGM supplementation on growth performance, lipid metabolism, antioxidant capacity, and non-specific immune response in pompano fed diets with two lipid levels, 9 % (basal-fat diet, BFD) and 18 % (high-fat diet, HFD). The overall aim was to elucidate the role(s) of KGM in lipid metabolism in pompano, and the possible effects of KGM in mitigating oxidative damage and inflammation induced by HFD in marine fish.

2. Materials and methods

2.1. Experimental diets

Eight experiment diets were prepared to contain two dietary lipid and four KGM levels. Four diets were formulated to contain 9 % lipid and 45 % protein (basal-fat diets, BFD) and supplemented with 0, 0.5, 1 and 2 % KGM, and four diets contained 18 % lipid and 39 % protein (high-fat diets, HFD) with the same four KGM levels. Fishmeal, fermented soybean meal and soy protein concentrate were used as the major protein sources, and a mixture of palm oil, rapeseed

oil and perilla oil (3:1:1, by volume) was used as the lipid source in the experiment diets. The feeds were produced as described in detail previously (Zhang et al., 2019a). All dry ingredients were ground into fine powder, sieved through size 40 mesh (425 micron), and mixed thoroughly before adding the vegetable oil mixtures and the appropriate amount of water. Uniform pellets (4 mm and 5 mm diameter) were wet-extruded using a laboratory feed-pelletizer (SLC-45, Fishery Machinery and Instrument Research Institute, China), air-dried to approximately 10 % moisture, sealed in plastic bags and stored in a freezer at -20 °C until used. Formulations and proximate compositions of the experimental diets are presented in Table 1.

2.2. Experimental fish and feeding trial

Golden pompano (*T. ovatus*) juveniles were obtained from a local commercial hatchery in Dongshan (Fujian province, China) and species confirmed by cytochrome oxidase sequencing (COI 5p). All procedures were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No.8023, revised 1978) and approved by the Institutional Animal Care and Use Committee of Shantou University. The culture experiment was carried out at Nan Ao Marine Biology Station (NAMBS) of Shantou University.

Prior to the start of the feeding trial, all fish were maintained in two floating sea cages (2 m × 2 m × 2 m) for two weeks during which they were all fed a composite feed comprised of a thorough mix of equal proportion of all eight diets to acclimate them to the culture conditions and feeding regime. At the end of the acclimation period, 720 healthy fish of similar size (average weight 12.07 ± 0.21g) were selected and distributed into 24 floating sea cages (1 m × 1 m × 1.5 m) at a density of 30 fish per cage. Triplicate groups of fish were hand fed to apparent satiation with the experimental diets twice a day (6:00 and 17:00) for 60 days. During the feeding trial, water temperature ranged between 26.0 °C and 29.6 °C, salinity was 30–32 ‰, NH_4^+ nitrogen was kept at < 0.5 mg l^{-1} and dissolved oxygen level was > 5.0 mg l^{-1}.

2.3. Sample collection

At the end of feeding trial, following a 24 h fast, all the fish in each cage were anesthetized with 0.01 % 2-phenoxyethanol (Sigma-Aldrich, St. Louis. MO, USA), and counted and weighed to determine weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR). Fish for the provision of experimental samples were selected randomly and killed by a blow to the head. All fish and tissue samples collected were flash frozen in liquid nitrogen and stored at −80 °C prior to subsequent analysis. Three fish per cage were immediately frozen for whole-body composition analysis. Blood samples were collected from the caudal vein of four fish per cage (12 fish per treatment) using non-heparinized syringes and centrifuged at 3000 g for 10 min at 4 °C, and serum collected for analysis of biochemical parameters. The same four fish per cage were used for determination of organosomatic indices including condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI). Muscle (dorsal and abdominal)

samples were collected from six fish per cage for the analysis of proximate composition. Liver samples were collected from the same six fish for analysis of antioxidant enzymes activity and RNA extraction, and another set of liver sample was collected from a further six fish for determination of lipid content. Intestine samples were pooled per tank into 1.5 ml centrifuge tubes for assessment of digestive enzymes activity and RNA extraction. Two fish per cage were selected for the assessment of liver histology analysis with oil red O staining.

2.4. Proximate composition analysis

Dry matter, crude protein, crude lipid and ash contents of the experimental diets, muscle and whole-body samples were quantified following the methods of the Association of Official Analytical Chemists (AOAC, 2006). Dry matter was determined by drying to a constant weight in an oven at 105 °C. Crude protein was determined by measuring nitrogen (N * 6.25) using the Kjeldahl System (Kjeltec™8400; FOSS). Crude lipid was measured by ether petroleum (B.P. 40–60 °C for 4 h) extraction using the Soxlet method (SZF-06A; Xinjia Yiqi). Ash was measured by combusting the samples in a muffle furnace (CWF1100; Carbolite) at 550 °C for 6 h.

2.5. Serum non-specific immune and biochemical parameters

Serum immunoglobulin M (IgM), total cholesterol (TC), triglyceride (TG), free fatty acid (FFA), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels, and the activities of lysozyme (LZM), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AKP) were determined by the colorimetric enzymatic method using commercial kits (Nanjing Jiancheng Bioengineering Co. Ltd.) as described in detail previously (Li et al., 2020). The contents of TC and TG were measured spectrophotometrically at 510nm, while FFA, HDL-C and LDL-C contents were measured at 546nm. One unit of LZM activity was defined as the amount of serum lysozyme needed to cause a 0.001 decrease of absorbance per min at 530nm. The amount of enzyme that generates 1 mmol of pyruvate per min at 37 °C defined as one ALT activity unit. One unit of AST activity was defined as the amount of enzyme that generates 1 mmol of glutamate per min at 37 °C measured at 510nm. One unit of AKP activity was defined as the amount of enzyme activity required to produce 1 mg phenol per 100 ml serum in 15 min at 37°C measured at 520nm. All the enzymatic activities and nonenzymatic factor contents were calculated according to the manufacturer's instructions

2.6. Serum and liver antioxidant parameters

Serum superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities, total antioxygenic capacity (T-AOC), and malondialdehyde (MDA) concentration were analyzed using commercial assay kits (Nanjing Jiancheng Bioengineering Co. Ltd.) as described previously (Zhang et al., 2019a). Malondialdehyde was determined according to the thibabituric acid method (TAB) introduced by commercial kits. One unit of SOD was defined as the amount of enzyme essential to produce a 50% inhibition in colour formation measured

spectrophotometrically at 550nm. One unit of GSH-Px activity was defined as the amount of enzyme that the GSH concentration in the reaction system at 1 μ mol l^{-1} per min. One unit of CAT activity was defined as the amount of enzyme that catalyzed the decomposition of 1μmol of H_2O_2 per min. One unit of T-AOC activity was defined as a 0.01 increment of absorbance of the reaction system caused by serum per milliliter reacting at 37 °C for 1 min. Liver samples (approximately 0.1 g) were homogenized in normal saline (9 volumes, 0.86 %) and supernatant was separated after centrifugation at 1800 *g* for 15 min at 4 °C. The analyses of SOD, CAT and GSH-PX activities, T-AOC and MDA concentration in the liver supernatants were determined using the same kits used for serum antioxidant parameters. The protein contents of liver supernatants were determined using a Bradford protein assay kit (Beyotime Biotechnology). All the antioxidant parameters were calculated according to the manufacturer's instructions.

2.7. Digestive enzyme activities

Pooled intestine samples were homogenized in an ice bath with 10 volumes (v/w) of chilled saline in a tissue homogenizer and centrifuged at 3000 *g* for 30 min at 4 °C. The resultant supernatant was stored at -80°C for subsequent analysis. Protein concentrations of supernatants were measured using bovine serum albumin (Sigma) as standard to enable the calculation of enzyme specific activities. Trypsin, lipase and amylase activities were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Co. Ltd.), which were calculated according to the manufacturer's instructions. Trypsin activity was monitored at 37 °C with the absorbance of 253nm, and one unit of trypsin activity was defined as a 0.003 change in absorbance per minute of trypsin per milligram of protein. Amylase activity was measured at 405nm by the rate of 2-chloro-4-nitrophenol formation at 37 °C. Lipase activity was determined the rate of methylresorufin formation at 37°C with the absorbance of 580nm, which was proportional to the concentration of catalytic lipase present in the sample homogenate.

2.8. Total RNA extraction, cDNA synthesis and quantitative real-time PCR (qRT-PCR)

Expression of lipid metabolism-related genes including *srebp1*, *pparγ*, *fas*, *acc*, *fabp1*, *apob100*, *cd36*, *pparα*, *cpt1* and *hsl* in liver, and inflammatory genes such as *tnf-α*, *il-8*, *tgf- β1* and *il-10* in intestine were measured by quantitative real time PCR (qRT-PCR). The sequences of primers are provided in Table 2. Total RNA was extracted using Trizol reagent according to manufacturer's instructions (Takara, Japan), and high-quality RNA samples with A260/A280 ratios between 1.8 and 2.0 were used for further analysis. The cDNA samples were synthesized using FastQuant® RT kit (Tiangen, China) with a genomic DNA elimination reaction, according to the manufacturer's specifications. The qRT-PCR were run in a Lightcycler 480 system (Roche, Switzerland) with the reaction mixture consisting of 5 μL SYBR Green Supermix (Roche, Switzerland), 0.5 μL of each primer (10 μM), 2 μL ddH_2O, and 2 μL diluted cDNA (10 ng μL^{-1}), with a total reaction volume made up to 10 μL. The qRT-PCR conditions were as follows: an initial DNA denaturation step at 95 °C for 5 min, 40 cycles of 95 °C for 10 s, the annealing temperature for 20 s, and 72°C for 20 s. After the amplification phase, a melting curve of 0.1 °C increments from 65 °C to 95 °C was performed. The expression

levels of target genes were calculated using the optimized comparative C_t ($2^{-\Delta\Delta Ct}$) value method as described previously (Livak and Schmittgen, 2001), and *actb* (*β-actin)* of *T. ovatus* was used as a reference gene to normalize the C_t value in each sample.

2.9. Histological assessment of lipid droplets content in liver

The liver tissues were fixed in 4% paraformaldehyde solution at 4°C for 24h for the histological analysis. Preparation of paraffin-embedded tissue sections and oil red O staining was performed by Wuhan Servicebio Technology Co. Ltd. The relative area stained with oil red O solution was quantified using Image-Pro Plus 6.0 software. Six visual fields of the same size were randomly selected and the average relative area stained with oil red O were calculated to determine the relative content of lipid droplets.

2.10. Statistical analysis

All data were subjected to one-way and two-way Analysis of Variance (ANOVA) to evaluate the effects of dietary lipid and KGM levels, and their interaction. Difference between means were determined by a Duncan's Multiple Range test at $P < 0.05$ level of significance using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Data are presented as means ± standard error of the mean (SEM) with n values as indicated.

3. Results

3.1. Growth, feed utilization and organosomatic indices

Growth performance and feed utilization were significantly affected by both dietary lipid and KGM levels (two-way ANOVA, $P < 0.05$) without interaction (two-way ANOVA, $P > 0.05$) (Table 3). The groups fed BFD exhibited higher growth rates and lower FCR values than the corresponding HFD groups, while FBW, WG and SGR were significantly enhanced and FCR decreased by increasing KGM level up to 1% at both dietary lipid levels. HSI was also affected by both dietary lipid and KGM levels and there was interaction, with increasing KGM level at the lower lipid level increasing HSI while inclusion of 1% KGM in fish fed HFD significantly decreased HSI. VSI was only affected by dietary lipid level with the groups of pompano fed HFD having higher values than groups fed BFD. Fish survival and CF were unaffected by diet ($P > 0.05$).

3.2. Whole-body and muscle composition

Protein and lipid contents of whole body were affected by both dietary lipid and KGM levels (two-way ANOVA, $P < 0.05$) (Table 4). The HFD groups showed higher whole-body lipid and lower protein content than the BFD groups, while increasing KGM level up to 1 % at both dietary lipid levels decreased whole-body lipid content while protein content showed the opposite tendency (one-way ANOVA, $P < 0.05$). Whole-body ash content was influenced by the interaction of dietary lipid and KGM levels with highest value recorded in fish fed HFD supplemented with 1 % KGM.

Proximate compositions of dorsal and ventral muscles of fish fed the experimental diets is shown in Table 5. Lipid

contents of both dorsal and ventral muscles were influenced by dietary lipid and KGM levels (two-way ANOVA, $P < 0.05$). In addition, protein content of dorsal muscle was affected by dietary levels of lipid and KGM and their interaction, while protein content of ventral muscle was only affected by dietary lipid level. Addition of 1 % KGM in HFD significantly reduced lipid content and increased protein content of dorsal muscle (one-way ANOVA, $P < 0.05$), while supplementation of 1 % KGM in both BFD and HFD reduced lipid content of ventral muscle.

3.3. Liver lipid content, lipid droplets content, and serum immune and biochemical parameters

Liver lipid content of pompano fed HFD was considerably higher than in fish fed BFD and was significantly decreased by increasing KGM level to 1% at both dietary lipid levels (Fig. 1). As shown in Fig. 2A, the proportion of lipid droplets with Oil Red O stain in HFD groups were significantly increased compared with that in the BFD groups and 1 % KGM supplementation in fish fed both BFD and HFD significantly decreased the size of lipid droplets. The relative contents of lipid droplets were significant reduced with KGM added (Fig. 2B). Serum IgM level, and activities of AKP, LYZ, ALT and AST were influenced by dietary lipid and KGM levels and their interaction (Table 6). IgM level, and AKP and LYZ activities were significantly increased by increasing KGM supplementation level fed in fish both BFD and HFD, whereas ALT and AST activities were decreased by dietary supplementation of KGM at both dietary lipid levels. Serum lipid parameters including TC, TG, HDL-C, LDL-C and FFA are shown in Table 7. Serum TC, TG and FFA concentrations were significantly reduced by increasing KGM inclusion level up to 1% in fish fed both BFD and HFD. In contrast, serum HDL-C and LDL-C levels showed increasing trends with increased KGM supplementation level.

3.4. Serum and liver antioxidant parameters

Serum GSH-PX, SOD and CAT activities and MDA concentration were affected by dietary level of KGM and showed interaction between dietary lipid and KGM levels, while T-AOC was influenced by dietary lipid level and also showed interaction between the two factors (Table 8). Increasing KGM supplementation level significantly increased GSH-PX, SOD and CAT activities and decreased MDA level in fish fed BFD, while supplementation of 0.5-1 % KGM increased AOC and GSH-PX activity in fish fed HFD. Liver T-AOC, MDA concentration, SOD and CAT activities were all affected by both dietary lipid and KGM levels, but only CAT activity showed any interaction (Table 9). Increasing KGM level increased T-AOC and SOD activity in fish fed both BFD and HFD whereas MDA concentration showed a decreasing trend. Highest liver CAT activities were found with supplementation of 1 and 2 % KGM in fish fed BFD and HFD, respectively.

3.5. Digestive enzyme activity

Intestinal lipase activity was influenced by both dietary lipid and KGM levels with increased activity observed at higher lipid and KGM levels (Table 10). Amylase activity was affected by both dietary factors and their interaction, and was increased by increasing KGM level up to 1 % in fish fed both BFD and HFD. Trypsin activity was also influenced

by both dietary lipid and KGM levels, and was increased in fish fed BFD supplemented with 1% KGM.

3.6. Expression of genes related to lipid metabolism in liver

Fig. 3 shows the expression of lipid metabolism-related genes in the liver of pompano including those associated with anabolism (Fig. 3A), transport (Fig. 3B) and catabolism (Fig. 3C). Expression of genes involved in hepatic lipid anabolism including *srebp1*, *pparγ*, *fas* and *acc* were significantly up-regulated in pompano fed HFD compared to fish fed BFD, and KGM supplementation induced decreasing trends at both dietary lipid levels. In contrast, while the expression of lipid transport-related genes such as *fabp1*, *apob100* and *cd36* and lipid catabolism-related genes such as *pparα*, *cpt1* and *hsl* were also up-regulated in fish fed HFD compared to pompano fed BFD, supplementation of KGM (particularly at 1 %) enhanced expression of the abovementioned genes at both dietary lipid levels.

3.7. Expression of inflammatory genes in intestine

Expression levels of pro-inflammatory genes such as *tnf-α* and *il-8*, and anti-inflammatory cytokine *tgf-β1* in intestine were significantly affected by dietary lipid and KGM levels and their interaction (Fig. 4). Expression levels of *tnf-α* and *il-8* were markedly up-regulated by HFD compared to BFD, and KGM supplementation decreased their expression levels. In contrast, the expression level of *tgf-β1* was reduced in HFD compared to BFD and KGM application significantly enhanced its expression in fish fed HFD. Few significant effects of dietary lipid and KGM levels were found on *il-10* gene expression.

4. **Discussion**

With the increasing demand for aquafeeds of high efficiency and low-cost, HFD have been widely applied in intensive aquaculture due to their protein-sparing and growth-promoting effects (Li et al., 2012; Shi et al., 2018). However, ingesting HFD may lead to undesirable levels of fat deposition in fish, resulting in increased tissue lipid peroxidation and associated inflammatory responses, which consequently affect the growth performance and health of farmed fish. In the present study, pompano fed HFD exhibited reduced WG and SGR, which was in agreement with previous studies on blunt snout bream (*Megalobrama amblycephala*) (Dong et al., 2020; Zhou et al., 2019) and juvenile cobia (*Rachycentron canadum*) (Wang et al., 2005). This could be due to excessive lipid accumulation in fish resulting in metabolic disorders and suppressed immune function, ultimately resulting in impaired growth performance (Guo et al., 2019; Yan et al., 2015; Zhang et al., 2017a). It has also been suggested that depressed growth of fish fed HFD could also be due to impaired nutrient balance due to excessively high, dietary fat-driven, energy intake resulting in reduced feed intake and uptake of other non-lipid nutrients (Ding et al., 2020; Wang et al., 2005). Perhaps linked to this latter point, the pompano fed HFD showed reduced activities of intestinal digestive enzymes including trypsin and amylase compared to those fed BFD, which was also reflected in the higher FCR in fish fed HFD.

Recently, plant polysaccharides have gained increasing attention in fish nutrition due to their growth promoting

properties. Astragalus polysaccharides (APS), the major active component in the herb *Astragalus membranaceus*, a traditional Chinese medicine, increased digestive enzyme activities and facilitated the absorption of nutrients in large yellow croaker (*Larimichthys crocea*) larvae and thus improved growth performance (Liu et al., 2020). In the present study, increasing supplementation level of KGM up to 1 % of diet at both dietary lipid levels significantly reduced FCR accompanied with increased FBW, WG and SGR. Consistent with this, dietary KGM enhanced the activities of intestinal digestive enzymes including lipase, amylase and trypsin. Similarly, Zheng et al. (2015) showed that dietary oxidized KGM supplementation promoted feed utilization, beneficially affected gut morphology and modulated gut microbiota in *S. prenanti*, with the optimum dose suggested to be 1.6 % of diet. Zhang et al. (2013) had previously reported that supplementation of 0.8 % oxidized KGM in diets for *S. prenanti* enhanced growth performance, and improved antioxidant status and immune function. Serum AKP and LZM activities, and IgM level are considered as key components of innate immunity in fish playing crucial roles in protecting cells against bacterial incursion and clearance of pathogens (Chen et al., 2016; Chen et al., 2017; Saurabh & Sahoo, 2008; Zhou et al., 2012). In the present study, the activities of AKP and LZM, and IgM level in serum were significantly increased by KGM supplementation of juvenile pompano indicating beneficial effects on health status. It has been suggested that growth and immune enhancing effects of KGM and derivatives in fish may be due to the similarity of its structure to β-glucan that could be recognized and bound by β-glucan receptor thereby evoking the recognition and phagocytosis of microbes *in vivo* (Chen et al., 2016; Zhang et al., 2013; Zheng et al., 2015).

Dietary lipid level is closely correlated with fish whole body lipid content and feeding diets with excessive lipid is generally accompanied with increased fat deposition in muscle, viscera and liver (Ghanawi et al., 2011; Regost et al., 2001; Song et al., 2009; Wang et al., 2005). In the present study, increasing dietary lipid level to 18 % significantly increased HSI and VSI, and lipid contents of whole body, liver, dorsal and abdominal muscles, which were in agreement with previous studies on *Rachycentron canadum* (Wang et al., 2005), *Odontesthes bonariensis* (Gómez-Requeni et al., 2013) and *Megalobrama amblycephala* (Lu et al., 2013; Zhang et al., 2017a). Moreover, in the present study KGM supplementation led to significant reductions in HSI, whole body and muscle lipid contents suggesting that KGM can reduce lipid accumulation in fish tissues. It has been showed that KGM can be fermented by gut bacteria to produce SCFAs, which upregulated the expression of PPARα and increased fatty acids β-oxidation, resulting in decreasing lipids in fish meat (Zhang et al., 2017b). In addition, there are several reports indicating that KGM can reduce hepatic lipid deposition though activation of AMPK and PPAR pathways and down-regulation of key lipid metabolism-related genes in mice fed HFD (Zhai et al., 2018; Shang et al., 2019).

Serum TG, TC, FFA, HDL-C and LDL-C levels are closely correlated with lipid metabolism, with TG and TC generally secreted from liver (Zhao et al., 2019) whereas FFA in serum could be transported to the liver for *de novo* lipogenesis (Kawano & Cohen, 2013). In the present study, serum TG, TC and FFA concentrations were significantly

higher in pompano fed HFD compared to fish fed BFD indicating increased endogenous lipid transport, which was consistent with studies in largemouth bass (Guo et al., 2019; Yin et al., 2020). Regardless of dietary lipid level, serum TG, TC and FFA concentrations were significantly decreased by supplementation of diets with KGM, clearly indicating its impact in regulating lipid metabolism. This was consistent with a study on rats that reported dietary KGM supplementation significantly reduced concentrations of TC, TG and FFA in both serum and liver (Shang et al., 2019). Oil red O staining and the relative contents of lipid droplets further support this finding. In addition, dietary KGM increased the serum concentration of HDL-C in pompano fed HFD possibly reflecting increased metabolism of lipoproteins (VLDL and LDL) by peripheral tissues with surplus cholesterol being transported back to the liver (Li et al., 2016; Shin et al., 2010). However, detailed information on the precise roles of KGM in regulating lipid metabolism in fish is scare and further studies are required to elucidate the underlying mechanisms.

In order to explore the influence of KGM in hepatic lipid metabolism, mRNA expression of several transcription factors and genes associated with the regulation of lipid metabolism were examined. Pparγ and Srebp1 are the important transcription factors that regulate lipogenesis by inducing the expression of adipogenic genes such as *fas* and *acc* (Meng et al., 2018; Oku et al., 2008). The present study showed that the expression levels of *pparγ*, *srebp1*, *fas* and *acc* genes were significantly up-regulated in pompano fed HFD compared to fish fed BFD indicating increased fatty acid synthesis in liver, which was consistent with previous studies on rainbow trout (Rollin et al., 2003) and grass carp (Li et al., 2016; Zhao et al., 2019). Meanwhile, the expression levels of these genes were reduced by dietary KGM supplementation suggesting that KGM decreased the rate of lipid synthesis. Previously, it was suggested that a possible mechanism could be activation of AMPK phosphorylation by KGM, which can result in suppressed liver expression of downstream lipid metabolism targets of AMPK such as *srebp1*, *fas* and *acc* (Shang et al., 2019).

It is well known that lipolysis and fatty acid oxidation are two important pathways that can reduce lipid accumulation (Zhang et al., 2019b). HSL is a rate-limiting lipolytic enzyme that initiates the hydrolysis of intracellular TG producing FFA. In the present study, the expression of *hsl* in liver was significantly increased by KGM supplementation to HFD suggesting that KGM enhanced lipolysis, which may also be reflected in the higher FFA concentration in serum. Furthermore, it has been shown that Pparα plays an important role in mediating lipid catabolism through regulating the β-oxidation pathway and related genes such as Cpt1 (import of fatty acids into mitochondria, directly affecting β-oxidation), Cd36 (transmembrane transport of fatty acids) and Fabp1 (intracellular transport of fatty acids from cell membrane to mitochondria) (Bai et al., 2013; Carvalho et al., 2008; Lee et al., 2003; Pravenec et al., 2002). In the present study in pompano, the up-regulated expression of *pparα*, *cpt1*, *cd36* and *fabp1* by dietary supplementation with KGM, particularly at an inclusion level of 1 % of diet, indicated that an appropriate dose of KGM can likely increase lipid catabolism in fish liver. Moreover, the interaction between dietary lipid and KGM on hepatic lipid catabolism showed that KGM can at least partly mitigate the increased lipid deposition in liver of fish fed HFD. In addition, the increased expression of *apob100* in pompano by KGM supplementation to HFD showed that KGM enhanced the transport of TG

in the form of VLDL/LDL from liver, thereby reducing hepatic lipid level (Hussain et al., 2008). Therefore, the possible mechanism of action of KGM on hepatic lipid metabolism in pompano could be through a combination of metabolic mechanisms including: 1) decreasing lipid synthesis via activating AMPK phosphorylation and down-regulating the expression of downstream lipid metabolism targets of AMPK in the liver and, 2) reducing lipid deposition in fish though simultaneous enhancement of lipid transport and lipid catabolism.

Generally, increased fat deposition in fish tissues, particularly liver, can induce the generation of reactive oxygen species (ROS) causing oxidative stress and cell damage (Lu et al., 2017; Jia et al., 2017; Zhao et al., 2019), with antioxidant enzymes such as GSH-Px, SOD and CAT operating to degrade ROS and thus protect cells against this stress (Wang et al., 2009). The concentration of MDA is a direct measure of toxic processes caused by lipid peroxides and, therefore, increased MDA production is considered as an indicator of lipid peroxidation (Koruk et al., 2004; Valko et al., 2007), while T-AOC is as an important marker of the antioxidant capacity of fish (Tan et al., 2017). In the present study, significantly lower T-AOC, SOD and CAT activities and higher MDA concentrations were found in liver of pompano fed HFD indicating reduced hepatic antioxidant capacity compared to fish fed BFD, and supplementation of HFD with KGM increased hepatic T-AOC, SOD and CAT activities and decreased MDA concentrations. Serum antioxidant parameters showed similar trends to those of the hepatic antioxidant indices. In agreement with these data, dietary KGM enhanced the antioxidant defense capability of rats by increasing the enzymatic and non-enzymatic antioxidants thereby reducing the liver oxidative damage (Shang et al., 2019; Zhao et al., 2020). The activities of AST and ALT in serum are considered as important indicators of liver damage as these enzymes leak into the blood stream through the damaged membranes of hepatic cells (Ashouri et al., 2015; Meng et al., 2018; Rao et al., 2006; Sayed et al., 2017). Previously, Zhai et al. (2018) showed that dietary KGM supplementation decreased the serum AST and ALT activities in mice fed HFD. Similarly, the present study showed that KGM inclusion particularly in HFD reduced serum AST and ALT activities in pompano indicating that KGM can play an important role in preventing / alleviating liver damage.

It has been reported by several studies that the oxidative stress induced by increased and/or excessive lipid accumulation in tissues of fish fed HFD induces inflammatory responses (Dai et al., 2019; Jin et al., 2019; Xie et al., 2020). Expression levels of pro-inflammatory cytokines such as Tnf-α and Il-8 are considered as markers of an inflammatory response, whereas increased expression of anti-inflammatory cytokines such as Tgf-β and Il-10 inhibit inflammation in fish (Shi et al., 2015). In the present study, increasing dietary lipid level from 9 % to 18 % led to up-regulated expression of *tnf-α* and *il-8* and reduced expression of *tgf-β* in intestine of pompano indicating the occurrence of an inflammatory response in fish fed HFD. Dietary supplementation with KGM significantly reduced the expression of *tnf-α* and *il-8* and increased the *tgf-β* expression in intestine of pompano. Similarly, Zhai et al. (2018) reported that KGM down-regulated the expression of *Tnf-α* and *Il-6* in C57/6J mice fed HFD, displaying the mitigating effect of dietary KGM on inflammatory response. Additionally, Zhao et al. (2020) demonstrated that dietary KGM reduced

expression levels of the genes and proteins of pro-inflammatory cytokines such as p-NF-κB, TNF-α, IL-β and IL-6 in T2DM rats through regulation of the NF-κB pathway, thereby inhibiting the inflammatory response. Although the potential molecular mechanisms through which dietary KGM regulated anti-inflammatory responses have been investigated in rodents, detailed molecular studies in fish are scare and need to be elucidated further in future studies.

5. Conclusion

In summary, the present study showed that dietary KGM supplementation improved growth performance, antioxidant activity, immune response, and lipid metabolism in juvenile pompano, and suggested that the optimum level is around 1 % of diet. Additionally, dietary supplementation with KGM could prevent excessive lipid accumulation through beneficially influencing pathways of lipid synthesis, transport and catabolism.

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FIGURES

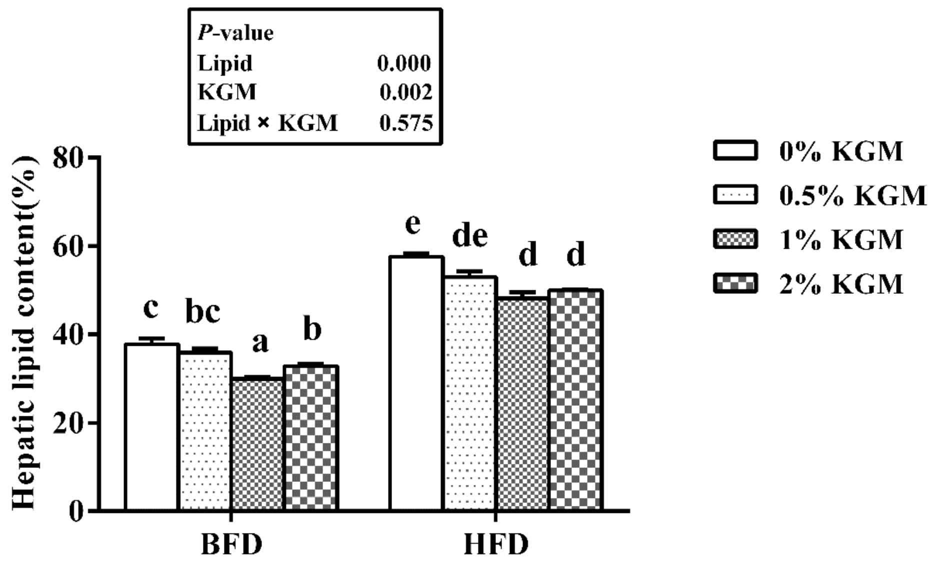
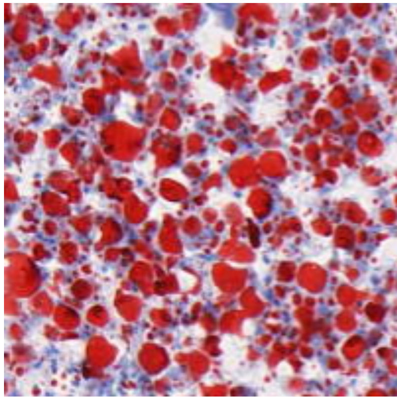


Fig.1. Hepatic lipid content of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days (means ± SEM, n = 6). Bars within a dietary fat group (BFD, 9 % basal fat diet; HFD, 18 % high-fat diet) with different letters are significantly different ($P < 0.05$) as determined by one-way ANOVA. The results of the two-way ANOVA analysis are shown in the black box; values within the same row indicate significantly different at $P < 0.05$.

（A）



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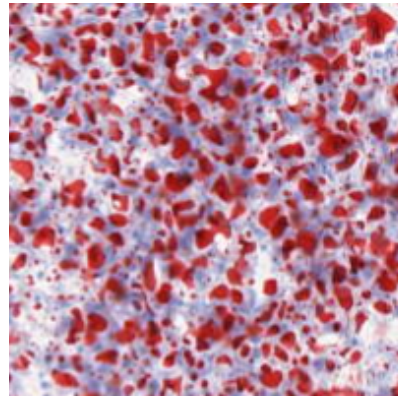

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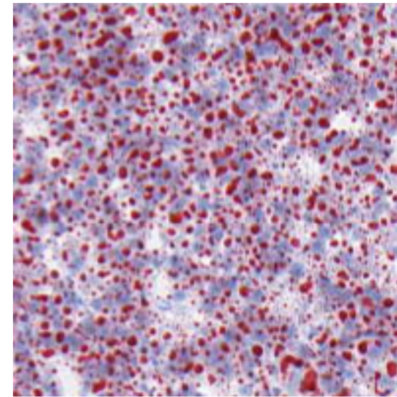
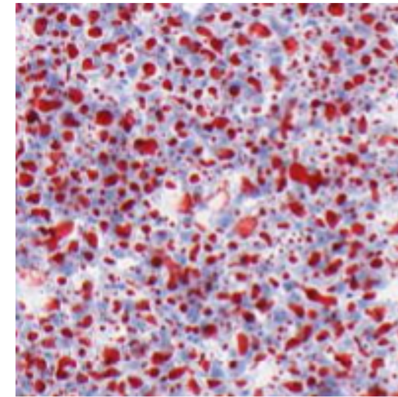
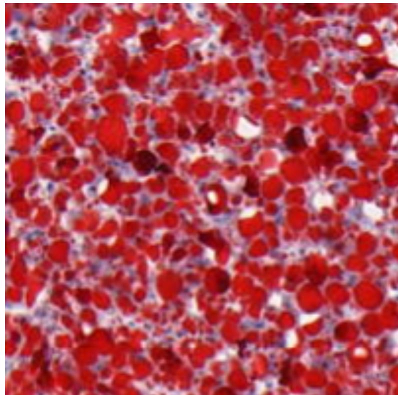


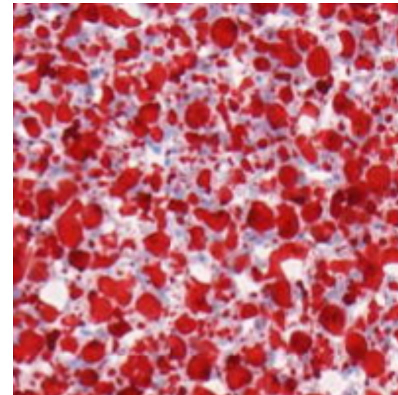
BFD (1% KGM)



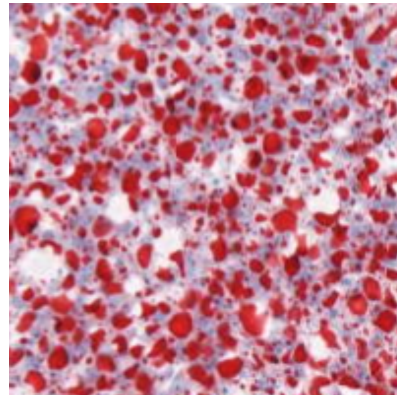
BFD (2% KGM)



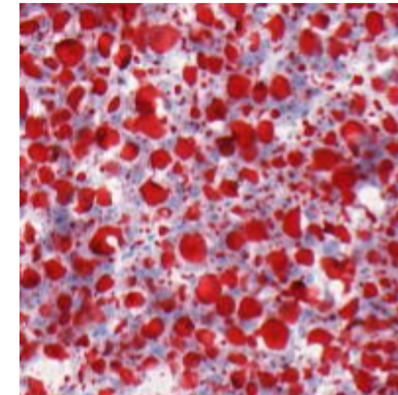
HFD (0% KGM)



HFD (0.5% KGM)



HFD (1% KGM)



HFD (2% KGM)

（B）

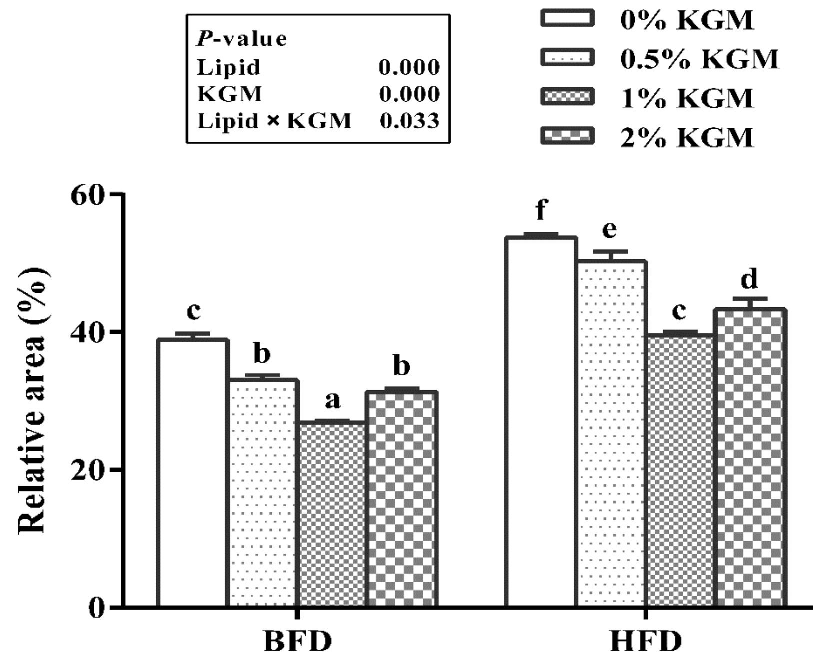
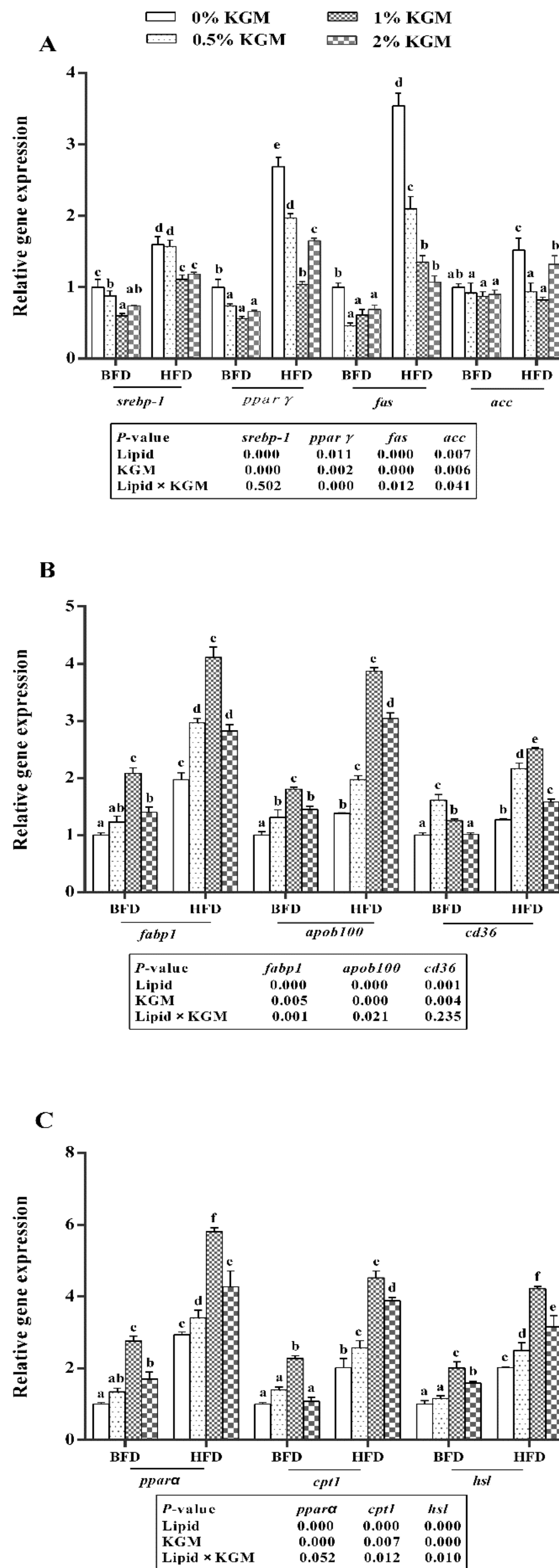


Fig.2. Hepatic histology of golden pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days (mean ± SEM, n = 6). A: Hepatic histology with oil red O staining (Magnification × 10); B: The relative area of red lipid droplets in the hepatic section area (%). Bars within a dietary fat group (BFD, 9 % basal fat diet; HFD, 18 % high-fat diet) with different letters are significantly different ($P < 0.05$) as determined by one-way ANOVA. The results of the two-way ANOVA analysis are shown in the black box; values within the same row indicate significantly different at $P < 0.05$.



P-value	srebp-1	ppar γ	fas	acc
Lipid	0.000	0.011	0.000	0.007
KGM	0.000	0.002	0.000	0.006
Lipid × KGM	0.502	0.000	0.012	0.041

P-value	fabp1	apob100	cd36
Lipid	0.000	0.000	0.001
KGM	0.005	0.000	0.004
Lipid × KGM	0.001	0.021	0.235

P-value	pparα	cpt1	hsl
Lipid	0.000	0.000	0.000
KGM	0.000	0.007	0.000
Lipid × KGM	0.052	0.012	0.010

Fig.3. Relative expression of lipid metabolism-related genes including anabolism (A), transport (B), and catabolism (C) associated genes in the liver of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days (means ± SEM, n = 6). Bars within a dietary fat group (BFD, 9 % basal fat diet; HFD, 18 % high-fat diet) with different letters are significantly different ($P < 0.05$) as determined by one-way ANONA. The results of the two-way ANOVA analysis are shown in the black box; values within the same row indicate significantly different at $P < 0.05$.

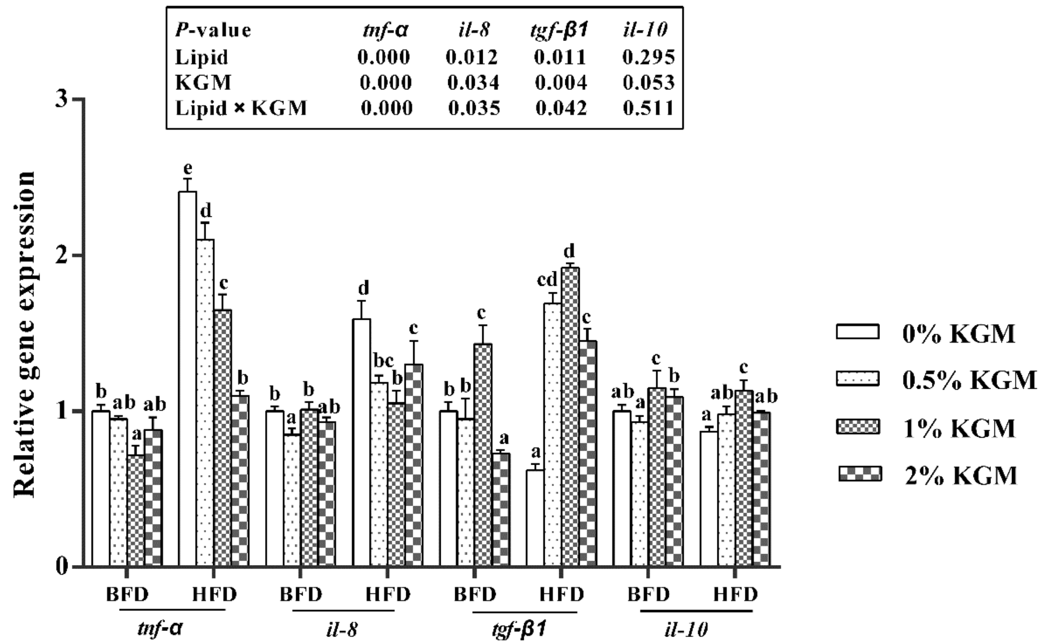


Fig.4. Relative expression of inflammation related genes in intestine of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days (means ± SEM, n = 6). Bars within a dietary fat group (BFD, 9 % basal fat diet; HFD, 18 % high-fat diet) with different letters are significantly different ($P < 0.05$) as determined by one-way ANONA. The results of the two-way ANOVA analysis are shown in the black box; values within the same row indicate significantly different at $P < 0.05$.

TABLES

Table 1. Formulations and proximate compositions of the experimental diets (% dry weight).

Diets	Lipid level (%)							
	9				18			
	KGM level (%)							
	0	0.5	1	2	0	0.5	1	2
Fishmeal	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Fermented soybean meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soy protein concentrate	29.00	29.00	29.00	29.00	20.00	20.00	20.00	20.00
Bran meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Konjac glucomannan (KGM)[1]	0	0.50	1.00	2.00	0	0.50	1.00	2.00
Soybean lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mixed vegetable oil[2]	5.00	5.00	5.00	5.00	14.00	14.00	14.00	14.00
α-starch	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Extruded corn	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Carboxymethyl cellulose	4.00	3.50	3.00	2.00	4.00	3.50	3.00	2.00
Vitamin premix[3]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[4]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lutein	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calcium hydrogen phosphate	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Proximate composition (%)								
Dry matter	91.15	91.05	90.99	91.24	91.36	91.26	91.26	90.98
Ash	10.16	10.11	10.51	10.44	9.90	9.89	10.04	9.78
Crude protein	45.11	45.03	45.13	45.04	39.54	39.46	39.56	39.39
Crude lipid	9.04	9.00	9.09	8.97	18.01	18.16	17.97	18.05

[1]Konjac glucomannan (purity, 99 %) was purchased from Qiangsen Konjac Technology Company (Hubei Province, China).

[2] Mixed vegetable oil, palm oil / rapeseed oil / perilla oil (3:1:1, by volume).

[3]Vitamin premix (mg kg^{-1}), including 1100000 IU vitamin A, 320000 IU vitamin D3, 80 mg vitamin E, 8 mg vitamin B_{12}, 120 mg vitamin C, 1000 mg vitamin K3, 1500 mg vitamin B_1, 2800 mg vitamin B_2, 2000 mg calcium pantothenate, 7800 mg nicotinamide, 400 mg folic acid, 12800 mg inositol, and 1000 mg vitamin B_6.

[4]Mineral premix (mg kg^{-1}), 2 mg NaF, 0.8 mg KI, 50 mg $CoCl_2$ (1 %), 10 mg $CuSO_4$, 50 mg $ZnSO_4$, 60 mg $MnSO_4$, 1200 mg $MgSO_4$, 100 mg NaCl.

Table 2. Sequences of primers used for species identification and real-time PCR.

Target gene	Forward primer (5' -3')	Reverse primer (5' -3')	Reference
actb	CGCCCGAGTGTGTATGAGAAATG	GTCATGTGGATCAGCAAGCAGGA	Tan et al. (2017)
srebp1	GAGCCAAGACAGAGGAGTGT	GTCCTCTTGTCTCCCAGCTT	Li et al. (2020)
pparγ	TCAGGGTTTCACTATGGCGT	CTGGAAGCGACAGTATTGGC	XM_022748373
fas	GAAGGAGAGGGGGTGGAGTC	GTGTGAAGGTGGAGGGTGTG	Liu et al. (2018)
acc	GTTGTCAATCCCAGCCGATC	ATCCACAATGTAGGCCCCAA	Li et al. (2020)
fabp1	AGTCATTGTCTGGGGAGGG	GTCAAGGCGGTGGTTCA	Liu et al. (2018)
apob100	AAAAGCCACAAGACGAAAGCA	GAAGCAGCAAAAGGCAGAGC	Liu et al. (2018)
cd36	CTCTTCATGGAAAGCAGGCC	CAGCTCCGAATACAACAGCC	Li et al. (2020)
pparα	AATCTCAGCGTGTCGTCTT	GGAAATGCTTCGGATACTTG	You et al. (2019)
cpt1	CTTTAGCCAAGCCCTTCATC	CACGGTTACCTGTTCCCTCT	Liu et al. (2018)
hsl	TCATACCTCCACACCAACCC	GTCTCGCAGTTTCTTGGCAA	Li et al. (2020)
tnf-α	GCTCCTCACCCACACCATCA	CCAAAGTAGACCTGCCCAGACT	You et al. (2019)
il-8	TGCATCACCACGGTGAAAAA	GCATCAGGGTCCAGACAAATC	You et al. (2019)
tgf-β1	GAGATACGGAAAAGAGTGGGG	TGACAAAGCGGGAAGCAAG	Tan et al. (2017)
il-10	CTCCAGACAGAAGACTCCAGCA	GGAATCCCTCCACAAAACGAC	Tan et al. (2017)

acc, acetyl-CoA carboxylase; *actb*, β-actin; *apob100*, apolipoprotein b100; *cd36*, fatty acid translocase 36; *cpt1*, carnitine palmitoyl transferase 1; *fabp1*, fatty acid binding protein 1; *fas*, fatty acid synthase; *hsl*, hormone-sensitive lipase; *il-8*, interleukin 8; *il-10*, interleukin 10; *pparα*, peroxisome proliferator-activated receptor alpha; *pparγ*, peroxisome proliferator-activated receptor gamma; *srebp1*, sterol-regulatory element binding protein-1; *tgf-β1*, transforming growth factor β1; *tnf-α*, tumor necrosis factor α.

Table 3. Growth, feed utilization and organosomatic indices of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days.

Diets		IBW[1] (g)	FBW[2] (g)	WG[3] (%)	SGR[4] (% day^{-1})	Feed Intake (g)	FCR[5]	SUR[6] (%)	HSI[7] (%)	VSI[8] (%)	CF[9] (g cm^{-3})
Lipid (%)	KGM (%)										
9	0	12.27±0.05	65.19±0.43^{bc}	438.72±8.62^{ab}	2.69±0.03^{a}	2315.51±11.88^{b}	1.71±0.01^{d}	92.22±1.11^{ab}	1.38±0.04^{a}	7.01±0.08^{a}	3.48±0.01
	0.5	12.27±0.05	67.56±0.59^{cd}	450.36±7.33^{b}	2.74±0.04^{ab}	2355.31±1.60^{b}	1.49±0.04^{b}	93.02±0.82^{ab}	1.39±0.02^{a}	7.46±0.20^{abc}	3.61±0.01
	1	12.22±0.11	72.03±0.58^{e}	489.55±9.03^{c}	2.89±0.02^{bc}	2376.19±7.89^{b}	1.27±0.01^{a}	93.26±0.07^{ab}	1.48±0.03^{ab}	7.19±0.17^{a}	3.54±0.06
	2	12.16±0.96	68.63±1.05^{d}	481.81±5.15^{c}	2.84±0.02^{bc}	2339.28±10.23^{b}	1.50±0.20^{b}	91.03±1.03^{a}	1.54±0.01^{ab}	7.39±0.21^{ab}	3.43±0.02
18	0	11.72±0.05	60.57±1.38^{a}	422.89±8.80^{a}	2.67±0.05^{a}	2170.97±14.50^{a}	1.83±0.02^{e}	90.00±1.92^{a}	1.86±0.08^{c}	8.13±0.25^{d}	3.42±0.13
	0.5	11.92±0.15	64.15±0.21^{b}	447.32±2.63^{b}	2.70±0.03^{a}	2297.48±34.07^{b}	1.54±0.06^{bc}	91.11±1.11^{a}	1.82±0.07^{c}	8.33±0.12^{d}	3.56±0.02
	1	12.25±0.25	67.51±0.82^{cd}	479.91±6.97^{c}	2.83±0.03^{bc}	2329.42±3.54^{b}	1.45±0.13^{b}	95.56±2.22^{b}	1.55±0.09^{ab}	7.96±0.02^{cd}	3.59±0.04
	2	11.77±0.06	65.22±0.44^{bc}	453.79±3.77^{b}	2.76±0.02^{ab}	2324.07±13.01^{b}	1.64±0.05^{cd}	92.07±1.03^{ab}	1.62±0.09^{ab}	7.83±0.07^{bcd}	3.44±0.05
Means of main effect											
9		12.23±0.21	68.36±0.80^{Z}	465.11±7.17^{Z}	2.79±0.02^{Z}	2346.57±26.54^{Z}	1.50±0.05^{Y}	92.38±0.42	1.45±0.02^{Y}	7.27±0.09^{Y}	3.54±0.01
18		11.92±0.15	64.37±0.83^{Y}	450.98±6.64^{Y}	2.74±0.03^{Y}	2297.15±46.46^{Y}	1.62±0.04^{Z}	92.18±0.94	1.73±0.05^{Z}	8.07±0.08^{Z}	3.50±0.03
	0	11.99±0.05	62.88±1.22^{A}	430.81±4.63^{A}	2.68±0.03^{A}	2243.24±41.79^{A}	1.77±0.01^{C}	91.11±1.02^{A}	1.62±0.04^{B}	7.57±0.11	3.45±0.07
	0.5	12.10±0.10	65.86±0.81^{B}	448.84±3.55^{B}	2.72±0.02^{A}	2302.81±29.65^{B}	1.51±0.07^{B}	92.06±0.36^{AB}	1.62±0.02^{B}	7.90±0.06	3.58±0.02
	1	12.23±0.06	69.78±1.06^{C}	484.73±5.54^{D}	2.86±0.02^{B}	2352.80±25.96^{B}	1.36±0.08^{A}	94.40±0.87^{B}	1.52±0.01^{A}	7.58±0.08	3.56±0.02
	2	11.96±0.08	66.92±0.91^{B}	467.80±6.88^{C}	2.80±0.02^{B}	2331.67±19.95^{B}	1.57±0.09^{B}	91.55±1.03^{AB}	1.58±0.05^{AB}	7.61±0.14	3.49±0.04

Two-way ANOVA										
Lipid	0.057	0.005	0.011	0.029	0.002	0.012	0.829	0.021	0.000	0.056
KGM	0.957	0.021	0.000	0.000	0.000	0.002	0.088	0.045	0.178	0.086
Lipid × KGM	0.615	0.769	0.349	0.865	0.055	0.306	0.256	0.013	0.248	0.253

Values are presented as means ± SEM (n = 3). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Superscript capital letters indicate significant differences between main effect means (Lipid, Y < Z; KGM, A < B < C < D) as determined by two-way ANOVA. Superscript lower-case letters indicate significant differences between all diets as determined by one-way ANOVA. The lack of superscript letter indicates no significant different among treatments ($P > 0.05$).

[1]Initial body weight; [2]Final body weight; [3]Weight gain = [(final body weight – initial body weight) / initial body weight] × 100;

[4]Specific growth rate = [ln (final body weight – initial body weight) / days] × 100; [5]Feed conversion ratio = feed intake / (final body weight – initial body weight).

[6]Survival = (survived fish number / total fish number) × 100; [7]Hepatosomatic index = (liver weight / body weight) × 100.

[8]Viscerosomatic index = (viscera weight / whole body weight) × 100; [9]Condition factor.

Table 4. Whole body composition of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days (% dry weight).

Diets		Dry matter	Protein	Lipid	Ash
Lipid (%)	KGM (%)				
9	0	30.44±0.41	$53.95±0.30^{ab}$	$33.94±0.44^{d}$	$11.57±0.22^{ab}$
	0.5	32.92±2.32	$55.65±1.81^{c}$	$31.75±0.46^{bc}$	$11.96±0.07^{b}$
	1	33.35±1.77	$57.30±1.66^{d}$	$28.51±0.23^{a}$	$11.54±0.25^{ab}$
	2	34.47±1.57	$54.30±1.04^{bc}$	$31.35±0.32^{b}$	$11.97±0.16^{b}$
18	0	33.99±0.61	$49.07±0.13^{a}$	$39.74±0.26^{e}$	$10.79±0.42^{a}$
	0.5	34.25±0.69	$51.07±0.62^{a}$	$34.36±0.17^{d}$	$11.36±0.23^{ab}$
	1	30.64±1.23	$53.39±0.45^{b}$	$32.53±0.33^{c}$	$12.51±0.26^{c}$
	2	32.59±0.66	$50.71±0.84^{a}$	$34.17±0.60^{d}$	$10.93±0.31^{a}$
Means of main effect					
9		32.79±0.71	$55.30±0.70^{Z}$	$31.39±0.52^{Y}$	11.76±0.15
18		32.87±0.63	$51.06±0.52^{Y}$	$35.20±0.72^{Z}$	11.40±0.30
	0	32.22±0.75	$51.51±0.41^{A}$	$36.84±1.12^{C}$	$11.18±0.12^{A}$
	0.5	33.58±0.67	$53.37±1.01^{AB}$	$33.05±0.54^{B}$	$11.66±0.25^{AB}$
	1	31.99±1.33	$55.35±0.87^{B}$	$30.52±0.78^{A}$	$12.02±0.21^{B}$
	2	33.53±0.86	$52.51±0.74^{A}$	$32.76±0.62^{B}$	$11.45±0.28^{AB}$
Two-way ANOVA					
Lipid		0.936	0.000	0.005	0.826
KGM		0.446	0.013	0.000	0.063
Lipid × KGM		0.067	0.919	0.129	0.045

Values are presented as means ± SEM (n = 6). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Superscript capital letters indicate significant differences between main effect means (Lipid, Y < Z; KGM, A < B < C) as determined by two-way ANOVA. Superscript lower-case letters indicate significant differences between all diets as determined by one-way ANOVA. The lack of superscript letter indicates no significant different among treatments ($P > 0.05$).

Table 5. Dorsal and ventral muscle compositions of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days (% dry weight).

Diets		dorsal muscle				ventral muscle			
Lipid (%)	KGM (%)	Dry matter	Protein	Lipid	Ash	Dry matter	Protein	Lipid	Ash
9	0	25.65±0.69	78.74±0.29^{d}	13.73±0.53^{b}	5.49±0.20	33.90±2.18	70.46±0.97^{b}	18.54±0.71^{a}	5.47±0.33
	0.5	27.58±0.99	77.39±0.60^{cd}	14.34±0.74^{bc}	5.33±0.08	32.32±2.17	71.39±0.42^{b}	18.70±0.24^{a}	4.77±0.26
	1	27.64±0.25	79.18±0.84^{d}	11.64±0.15^{a}	5.56±0.25	33.43±0.65	72.14±1.00^{b}	16.74±0.74^{a}	5.00±0.31
	2	26.58±0.26	77.61±0.36^{cd}	12.86±0.78^{ab}	5.21±0.14	34.61±2.86	70.59±0.57^{b}	17.29±0.43^{a}	4.55±0.10
18	0	25.78±0.72	70.22±0.92^{a}	19.53±0.85^{f}	5.22±0.07	35.29±0.63	63.93±0.30^{a}	28.98±0.84^{d}	5.02±0.52
	0.5	26.54±0.61	72.03±0.41^{a}	18.03±0.43^{ef}	5.34±0.23	34.49±1.08	63.38±0.77^{a}	27.73±1.53^{cd}	5.20±0.41
	1	26.29±0.64	75.68±0.22^{b}	15.75±0.51^{cd}	5.04±0.07	36.84±1.10	65.16±0.36^{a}	23.02±0.18^{b}	4.92±0.25
	2	27.18±0.91	74.31±0.84^{b}	16.31±0.18^{de}	5.29±0.19	32.04±2.98	64.21±0.71^{a}	25.31±0.99^{bc}	6.12±0.05
Means of main effect									
9		26.86±0.15	78.23±0.33^{Z}	13.15±0.25^{Y}	5.40±0.07	33.57±1.06	71.15±0.24^{Z}	17.82±0.73^{Y}	4.95±0.35
18		26.45±0.79	73.06±0.69^{Y}	17.41±0.49^{Z}	5.23±0.24	34.67±1.40	64.17±0.54^{Y}	26.26±0.28^{Z}	5.32±0.12
	0	25.72±0.20	74.48±1.95^{A}	16.63±0.09^{B}	5.36±0.03	34.60±1.01	67.19±0.53	23.76±0.37^{B}	5.25±0.22
	0.5	27.06±0.12	74.72±1.24^{AB}	16.19±0.52^{B}	5.34±0.25	33.41±1.12	67.38±0.23	23.22±0.59^{B}	4.98±0.38
	1	26.97±0.44	77.43±0.87^{C}	13.69±0.43^{A}	5.30±0.12	35.14±0.82	68.65±0.58	19.88±0.41^{A}	4.96±0.48
	2	26.88±0.98	75.96±0.84^{B}	14.59±0.58^{A}	5.25±0.07	33.33±1.92	67.41±0.44	21.29±0.51^{A}	5.34±0.08
Two-way ANOVA									
Lipid		0.726	0.022	0.001	0.169	0.433	0.000	0.000	0.121
KGM		0.827	0.001	0.022	0.934	0.736	0.173	0.001	0.558
Lipid × KGM		0.921	0.012	0.214	0.287	0.468	0.643	0.119	0.055

Values are presented as mean ± SEM (n = 6). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Superscript capital letters indicate significant differences between main effect means (Lipid, Y < Z; KGM, A < B < C) as determined by two-way ANOVA. Superscript lower-case letters indicate significant differences between all diets as determined by one-way ANOVA. The lack of superscript letter indicates no significant different among treatments ($P > 0.05$).

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Table 6. Serum biochemical and innate immune parameters of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days.

Diets		AKP[1]	LYZ[2]	IgM[3]	ALT[4]	AST[5]
Lipid (%)	KGM (%)	U l^{-1}	U ml^{-1}	mg ml^{-1}	U l^{-1}	U l^{-1}
9	0	1.59±0.04^{a}	138.02±2.34^{c}	2.42±0.08^{a}	5.96±0.13^{b}	14.76±0.98^{b}
	0.5	1.72±0.02^{a}	161.03±5.63^{d}	4.45±0.09^{b}	5.18±0.13^{ab}	17.32±0.43^{c}
	1	3.35±0.06^{d}	246.01±11.04^{f}	8.29±0.44^{d}	4.43±0.12^{a}	11.29±0.57^{a}
	2	2.04±0.05^{b}	245.07±3.38^{f}	7.21±0.44^{c}	5.45±0.58^{b}	13.04±0.51^{ab}
18	0	2.11±0.04^{b}	77.46±3.67^{a}	1.66±0.05^{a}	16.05±0.42^{d}	34.23±0.76^{f}
	0.5	2.12±0.07^{b}	84.97±3.72^{a}	2.53±0.03^{a}	12.19±0.26^{c}	25.64±0.58^{e}
	1	2.43±0.05^{c}	115.96±5.69^{b}	4.45±0.17^{b}	5.06±0.22^{ab}	20.05±0.25^{d}
	2	2.70±0.05^{c}	185.45±4.62^{e}	3.87±0.45^{b}	5.83±0.23^{b}	14.96±0.30^{b}
Means of main effect						
9		2.17±0.04^{Y}	197.53±14.94^{Z}	5.62±0.71^{Z}	5.25±0.21^{Y}	14.11±0.74^{Y}
18		2.34±0.02^{Z}	115.96±12.99^{Y}	3.15±0.35^{Y}	9.78±1.38^{Z}	23.72±2.17^{Z}
	0	1.85±0.04^{A}	107.74±13.68^{A}	2.04±0.17^{A}	11.01±2.26^{D}	24.49±4.39^{D}
	0.5	1.92±0.02^{A}	123.00±17.27^{B}	3.53±0.45^{B}	8.68±1.57^{C}	21.48±1.89^{C}
	1	2.89±0.04^{C}	180.98±29.60^{C}	6.42±0.86^{D}	4.74±0.18^{A}	15.67±1.98^{B}
	2	2.37±0.05^{B}	215.25±13.57^{D}	5.54±0.80^{C}	5.64±0.29^{B}	14.00±0.50^{A}
Two-way ANOVA						
Lipid		0.012	0.018	0.012	0.011	0.000
KGM		0.003	0.012	0.002	0.024	0.021
Lipid × KGM		0.041	0.033	0.032	0.042	0.020

Values are presented as means ± SEM (n = 6). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Superscript capital letters indicate significant differences between main effect means (Lipid, Y < Z; KGM, A < B < C < D) as determined by two-way ANOVA. Superscript lower-case letters indicate significant differences between all diets as determined by one-way ANOVA. The lack of superscript letter indicates no significant different among treatments ($P > 0.05$).

[1]Alkaline phosphatase activity; [2]Lysozyme activity; [3]Immunoglobulin M; [4]Alanine aminotransferase; [5]Aspartate aminotransferase.

Table 7. Serum lipid metabolism markers of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days.

Diets		TC[1]	TG[2]	HDL-C[3]	LDL-C[4]	FFA[5]
Lipid (%)	KGM (%)	mmol l^{-1}	mmol l^{-1}	mmol l^{-1}	mmol l^{-1}	mmol l^{-1}
9	0	4.92 ± 0.02^{bc}	2.06 ± 0.00^{c}	2.58 ± 0.17^{a}	1.81 ± 0.02^{b}	0.34 ± 0.01^{b}
	0.5	4.43 ± 0.04^{b}	2.07 ± 0.01^{c}	2.66 ± 0.03^{a}	1.76 ± 0.03^{ab}	0.31 ± 0.01^{ab}
	1	3.06 ± 0.04^{a}	1.85 ± 0.02^{b}	3.56 ± 0.16^{cd}	2.01±0.05c	0.27 ± 0.01^{a}
	2	4.65 ± 0.06^{b}	1.82 ± 0.07^{b}	3.25 ± 0.12^{c}	2.26 ± 0.02^{d}	0.30 ± 0.01^{ab}
18	0	7.42 ± 0.12^{d}	4.18 ± 0.04^{d}	2.86 ± 0.02^{b}	2.28 ± 0.03^{d}	0.79 ± 0.03^{e}
	0.5	5.58 ± 0.07^{c}	2.07 ± 0.02^{c}	3.14 ± 0.02^{c}	1.86 ± 0.08^{b}	0.67 ± 0.02^{d}
	1	4.74 ± 0.04^{bc}	1.44 ± 0.04^{a}	3.75 ± 0.09^{d}	1.62 ± 0.08^{a}	0.57 ± 0.01^{c}
	2	5.36 ± 0.13^{c}	1.70 ± 0.02^{b}	3.49 ± 0.07^{c}	1.77 ± 0.10^{ab}	0.64 ± 0.03^{d}
Means of main effect						
9		4.27 ± 0.22^{Y}	1.86 ± 0.04^{Y}	2.97 ± 0.11^{Y}	1.96±0.06	0.30 ± 0.01^{Y}
18		5.84 ± 0.30^{Z}	2.34 ± 0.32^{Z}	3.31 ± 0.10^{Z}	1.88±0.08	0.67 ± 0.03^{Z}
	0	6.17 ± 0.56^{C}	3.12 ± 0.47^{C}	2.71 ± 0.08^{A}	2.05 ± 0.11^{B}	0.56 ± 0.10^{C}
	0.5	5.14 ± 0.32^{B}	1.96 ± 0.06^{B}	2.89 ± 0.11^{B}	1.81 ± 0.04^{A}	0.48 ± 0.08^{B}
	1	3.90 ± 0.37^{A}	1.63 ± 0.09^{A}	3.57 ± 0.09^{D}	1.82 ± 0.10^{A}	0.42 ± 0.07^{A}
	2	5.01 ± 0.17^{B}	1.70 ± 0.03^{A}	3.36 ± 0.06^{C}	2.01 ± 0.12^{B}	0.47 ± 0.08^{B}
Two-way ANOVA						
Lipid		0.000	0.035	0.028	0.077	0.000
KGM		0.002	0.000	0.024	0.001	0.000
Lipid × KGM		0.023	0.016	0.255	0.007	0.006

Values are presented as means ± SEM (n = 6). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Superscript capital letters indicate significant differences between main effect means (Lipid, Y < Z; KGM, A < B < C < D) as determined by two-way ANOVA. Superscript lower-case letters indicate significant differences between all diets as determined by one-way ANOVA. The lack of superscript letter indicates no significant different among treatments ($P > 0.05$).

[1]Total cholesterol; [2]Triglyceride; [3]High density lipoprotein-cholesterol; [4]Low density lipoprotein-cholesterol; [5]Free fatty acids.

Table 8. Serum antioxidant parameters of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days.

Diets		T-AOC[1]	GSH-Px[2]	SOD[3]	MDA[4]	CAT[5]
Lipid (%)	KGM (%)	mmol l^{-1}	U ml^{-1}	U ml^{-1}	nmol ml^{-1}	U ml^{-1}
9	0	$0.54±0.02^{a}$	$168.07±5.53^{a}$	$11.78±0.21^{a}$	$5.75±0.52^{c}$	$3.19±0.20^{a}$
	0.5	$0.52±0.01^{a}$	$182.18±9.13^{ab}$	$11.86±0.49^{a}$	$5.16±0.16^{bc}$	$4.15±0.53^{ab}$
	1	$0.61±0.04^{ab}$	$174.45±8.79^{a}$	$12.97±0.21^{a}$	$3.56±0.68^{a}$	$4.62±0.42^{ab}$
	2	$0.59±0.04^{ab}$	$220.17±5.22^{c}$	$15.01±0.28^{c}$	$3.28±0.55^{a}$	$6.75±0.42^{c}$
18	0	$0.64±0.02^{b}$	$165.38±4.76^{a}$	$12.54±0.45^{a}$	$5.08±0.76^{b}$	$4.50±0.46^{ab}$
	0.5	$0.73±0.00^{c}$	$205.04±3.96^{b}$	$13.36±0.13^{b}$	$4.52±0.44^{ab}$	$4.83±0.78^{b}$
	1	$0.63±0.02^{b}$	$197.98±2.87^{b}$	$13.23±0.52^{b}$	$3.97±0.16^{b}$	$4.71±0.25^{b}$
	2	$0.66±0.01^{b}$	$169.75±1.21^{a}$	$13.63±0.53^{b}$	$4.43±0.29^{ab}$	$5.23±0.68^{b}$
Means of main effect						
9		$0.56±0.02^{Y}$	186.22±6.86	12.91±0.41	4.44±0.38	4.68±0.43
18		$0.66±0.01^{Z}$	184.54±5.40	13.19±0.22	4.49±0.23	4.82±0.22
	0	0.59±0.02	$166.72±3.32^{A}$	$12.53±0.28^{AB}$	$5.41±0.44^{B}$	$3.85±0.37^{A}$
	0.5	0.63±0.04	$193.61±6.78^{B}$	$12.61±0.41^{A}$	$4.84±0.25^{AB}$	$4.49±0.35^{A}$
	1	0.62±0.02	$186.22±6.69^{B}$	$13.10±0.26^{B}$	$3.76±0.33^{A}$	$4.67±0.22^{A}$
	2	0.62±0.03	$194.96±11.52^{B}$	$14.32±0.41^{C}$	$3.86±0.38^{A}$	$6.00±0.49^{B}$
Two-way ANOVA						
Lipid		0.000	0.086	0.319	0.873	0.670
KGM		0.458	0.001	0.000	0.011	0.002
Lipid × KGM		0.026	0.000	0.013	0.031	0.038

Values are presented as means ± SEM (n = 6). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Superscript capital letters indicate significant differences between main effect means (Lipid, Y < Z; KGM, A < B < C) as determined by two-way ANOVA. Superscript lower-case letters indicate significant differences between all diets as determined by one-way ANOVA. The lack of superscript letter indicates no significant different among treatments ($P > 0.05$).

[1]Total antioxidant capacity; [2]Glutathione peroxidase activity; [3]Superoxide dismutase activity; [4]Malondialdehyde concentration; [5]Catalase activity.

Table 9. Liver antioxidant parameters of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days.

Diets		T-AOC[1]	GSH-Px[2]	SOD[3]	MDA[4]	CAT[5]
Lipid (%)	KGM (%)	mmol g prot^{-1}	mmol g prot^{-1}	U mg prot^{-1}	nmol mg prot^{-1}	U mg prot^{-1}
9	0	16.30±0.08^{b}	18.41±0.17	24.45±0.32^{b}	1.02±0.07^{d}	69.39±4.17^{b}
	0.5	17.98±0.68^{b}	19.17±0.82	28.80±0.29^{bc}	0.43±0.04^{a}	76.01±1.39^{b}
	1	22.02±1.45^{d}	20.29±0.56	30.39±1.26^{c}	0.63±0.08^{ab}	84.49±0.69^{c}
	2	25.75±0.75^{e}	21.31±1.59	33.99±1.23^{d}	0.50±0.05^{ab}	73.45±3.03^{b}
18	0	12.39±0.49^{a}	19.74±0.55	20.91±0.62^{a}	1.52±0.13^{e}	57.82±0.98^{a}
	0.5	18.65±0.32^{bc}	20.09±1.38	26.68±0.16^{ab}	0.82±0.05^{cd}	72.11±0.76^{b}
	1	20.74±0.72^{cd}	20.94±1.48	25.42±0.93^{b}	0.67±0.04^{bc}	66.91±3.31^{b}
	2	22.98±0.99^{d}	22.58±1.05	27.81±0.32^{b}	0.55±0.05^{ab}	75.36±4.65^{b}
Means of main effect						
9		20.52±1.17^{Z}	19.77±0.52	29.41±1.10^{Z}	0.64±0.07^{Y}	75.84±2.02^{Z}
18		18.70±1.22^{Y}	20.84±0.60	25.22±0.83^{Y}	0.89±0.11^{Z}	68.05±2.35^{Y}
	0	14.35±0.90^{A}	19.07±0.39	22.68±0.85^{A}	1.27±0.13^{B}	63.60±3.22^{A}
	0.5	18.32±0.37^{B}	19.58±0.75	27.75±0.50^{B}	0.62±0.09^{A}	74.06±1.12^{B}
	1	21.38±0.78^{C}	20.61±0.72	27.91±1.31^{B}	0.65±0.04^{A}	75.69±4.21^{B}
	2	24.37±0.83^{D}	21.95±0.90	31.93±1.48^{C}	0.53±0.04^{A}	74.17±2.52^{B}
Two-way ANOVA						
Lipid		0.015	0.178	0.015	0.020	0.001
KGM		0.000	0.072	0.011	0.000	0.002
Lipid × KGM		0.056	0.988	0.088	0.053	0.016

Values are presented as means ± SEM (n = 6). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Superscript capital letters indicate significant differences between main effect means (Lipid, Y < Z; KGM, A < B < C < D) as determined by two-way ANOVA. Superscript lower-case letters indicate significant differences between all diets as determined by one-way ANOVA. The lack of superscript letter indicates no significant different among treatments ($P > 0.05$).

[1]Total antioxidant capacity; [2]Glutathione peroxidase activity; [3]Superoxide dismutase activity; [4]Malondialdehyde concentration; [5]Catalase activity.

Table 10. Intestinal digestive enzyme activities of pompano fed diets with different lipid and konjac glucomannan (KGM) levels for 60 days.

Diets		Lipase	Amylase	Trypsin
Lipid (%)	KGM (%)	U g $prot^{-1}$	U mg $prot^{-1}$	U mg $prot^{-1}$
9	0	$0.46±0.01^{a}$	$0.36±0.01^{d}$	$2898.78±130.03^{b}$
	0.5	$0.42±0.00^{a}$	$0.39±0.01^{de}$	$3055.76±115.25^{bc}$
	1	$0.60±0.01^{a}$	$0.65±0.01^{f}$	$3691.30±36.98^{d}$
	2	$0.62±0.12^{a}$	$0.41±0.03^{e}$	$3238.88±127.78^{c}$
18	0	$1.08±0.02^{b}$	$0.20±0.16^{a}$	$1975.94±58.61^{a}$
	0.5	$1.07±0.15^{b}$	$0.27±0.01^{b}$	$2130.86±18.14^{a}$
	1	$1.51±0.22^{c}$	$0.31±0.00^{c}$	$2283.56±74.08^{a}$
	2	$1.33±0.15^{bc}$	$0.29±0.01^{bc}$	$2145.09±169.77^{a}$
Means of main effect				
9		$0.53±0.03^{Y}$	$0.45±0.03^{Z}$	$3221.18±100.98^{Z}$
18		$1.24±0.08^{Z}$	$0.27±0.01^{Y}$	$2133.86±53.01^{Y}$
	0	$0.77±0.13^{A}$	$0.28±0.04^{A}$	$2437.36±216.04^{A}$
	0.5	$0.74±0.16^{A}$	$0.33±0.03^{B}$	$2593.31±213.29^{AB}$
	1	$1.05±0.22^{B}$	$0.48±0.07^{C}$	$2987.43±316.95^{C}$
	2	$0.98±0.18^{AB}$	$0.35±0.03^{B}$	$2691.99±118.07^{B}$
Two-way ANOVA				
Lipid		0.000	0.023	0.000
KGM		0.042	0.031	0.001
Lipid × KGM		0.063	0.044	0.104

Values are presented as means ± SEM (n = 6). Values in the same column with different superscript letters are significantly different ($P < 0.05$). Superscript capital letters indicate significant differences between main effect means (Lipid, Y < Z; KGM, A < B < C) as determined by two-way ANOVA. Superscript lower-case letters indicate significant differences between all diets as determined by one-way ANOVA. The lack of superscript letter indicates no significant different among treatments ($P > 0.05$).

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