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Yeast cultures alleviate gossypol induced inflammatory response in liver tissue of Ussuri catfish (*Pseudobagrus ussuriensis*)

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ABSTRACT

The present study explored the mechanisms of gossypol induced inflammatory response in liver tissue of Ussuri catfish (*Pseudobagrus ussuriensis*), and investigated whether the yeast culture could relieve the inflammatory response of fish. For this purpose, Ussuri catfish were fed two diets with or without yeast culture for 8 weeks, and then fish were treated with 300 mg gossypol/kg body weight by intragastric administration for 15 days. Fish pre-fed diet without yeast culture supplementation and treated with gossypol oral administration (Gos group) had higher interleukin-1β (IL-1β) and interleukin 8 (IL-8) mRNA levels compared with fish pre-fed diet without yeast culture supplementation and treated with sodium carboxymethylcellulose solution oral administration (control group) and pre-fed yeast culture diet and treated with gossypol oral administration (Gos/YC group), but had lower interleukin 10 mRNA level than the control group ($P < .05$). Simultaneously, hepatic toll-like receptor 2 (TLR2), myeloid differentiation factor 88 (MyD88) and nuclear transcription factor kappa B p65 (NF-κB p65) mRNA levels of fish in Gos group were also up-regulated. However, dietary yeast culture supplementation remarkably prevented the increase of IL-1β and IL-8 mRNA levels and the decrease of IL-10 mRNA level by inhibiting the excessive activation of TLR2-MyD88-NF-κB signaling pathway through down-regulating TLR2, NF-κB p65 ($P < .05$) and MyD88 ($P > .05$) mRNA levels. Besides, the lowest serum lysozyme and alkaline phosphatase activities were observed in fish of Gos group. There was an increased trend in immunoglobulin M content among the groups. Fish in Gos group had significantly lower hepatic catalase, superoxide dismutase and total antioxidant capacity compared with those fish in the control group ($P < .05$). Higher occurrences of the dilatation in sinusoids, karyolysis, hydropic degenerations and nuclei shifting to the cellular periphery were detected in Gos group compared with those in the control group, coupled with increased serum aspartate aminotransferase activity. Lower occurrences of these histopathological symptoms were observed in the Gos/YC group compared with those of fish in the Gos group. Overall, the results mentioned above indicated that gossypol could result in immune and oxidative damage, liver injury and inflammatory response and dietary yeast culture supplementation could alleviate these negative responses for Ussuri catfish.

1. Introduction

The yeast culture (YC) is a complicated yeast fermentation product composed of the yeast and its growth media. In the process of yeast cultures production, yeast cells are only used for the production of their metabolites. Recently, YC is widely used in animal feed including aquafeed due to the fact that it contains protein, lipid, B-vitamins, which may possibly serve as an alternative protein source to fish meal (Sanderson and Jolly, 1994; Cheng et al., 2004; Barnes et al., 2006). Moreover, YC possessed immunomodulatory constituents such as yeast cell walls (β-glucans and mannan-oligosaccharides), peptides, nucleotides, cell solubles, and oligosaccharides (Jensen et al., 2008). These immunostimulants have been used in aquatic animal culture such as carp (*Cyprinus carpio*) (Selvaraj et al., 2005), European sea bass (*Dicentrarchus labrax*) (Torrecillas et al., 2007) and rohu (*Labeo rohita* L.) (Choudhury et al., 2005), altering the immune response and inducing protection against a wide range of diseases (Soltanian et al., 2007). Based on these observations, it could be hypothesized that YC might be a potential immune modulator for fish. Moreover, previous study has shown that YC also has anti-inflammatory property (Jensen et al.,

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2008).

Ussuri catfish (*Pseudobagrus ussuriensis*) is an omnivorous fish widely distributed in inland rivers in China and East Asia. The farming production of this fish has spread all over China, and it is well popular with Chinese consumers due to its high nutritional value, high resistance against diseases and availability of reproduction technology. It lives at the bottom of freshwater, the most suitable growth temperature of 25 to 28 °C and the pH range of 7.0 to 8.5. Up to now, the study of fish meal replacement with cottonseed meal in diets for Ussuri catfish has been well assessed in our laboratory, and it was noteworthy that dietary too high proportion of cottonseed meal may result in the inhibition of the growth, immune response, disease resistance and liver function of fish, speculating that dietary free gossypol toxicity may be the main reason responsible for the above-mentioned negative effects (Bu et al., 2017). Previous study demonstrated that dietary free gossypol can cause toxic effects on fish. It is reported that dietary 431.3 mg kg^{-1} free gossypol supplementation could damage the growth performance, while dietary 647.0 mg kg^{-1} free gossypol supplementation could impair the liver for common carp (*Cyprinus carpio*) (Wang et al., 2014a, 2014b). Bian et al. (2016) demonstrated that high levels of free gossypol in diets could cause liver fibrosis and stimulate chemokine and proinflammatory cytokine secretions for turbot (*Scophthalmus maximus* L.). Toll-like receptors (TLRs) are considered to mediate the inflammatory reaction, which interact with their corresponding adaptor molecules such as myeloid differentiation factor 88 (MyD88) to elicit downstream signaling events, resulting in nuclear transcription factor kappa B (NF-κB) activation and the induction of several types of cytokines (Thompson and Locarnini, 2007). Several previous studies have suggested that TLR2 activation could initiate a pro-inflammatory response, which depends on the activation of NF-κB (Hajishengallis et al., 2009). It is reported that signaling molecules NF-κB could regulate various cytokines (Jiang et al., 2017), and up-regulation of proinflammatory cytokines can be directly toxic on hepatocytes (Kono et al., 2001). Moreover, fish immunity is generally associated with cytokines, which were regulated by NF-κB signaling pathway. However, up to data, the possible mechanisms of dietary free gossypol toxicity and whether the yeast culture could relieve the inflammatory response of Ussuri catfish (*Pseudobagrus ussuriensis*) have not been well carried out, and deserves deep investigation.

Therefore, we investigate the effects of YC on gossypol induced inflammatory response in liver tissue of Ussuri catfish. And based on these effects, the potential mechanisms of YC-modulated immune and anti-inflammatory responses were evaluated.

2. Materials and methods

2.1. Ethical considerations

All animal care and handing procedures in this study were conducted under the Guidance of the Care and Use of Laboratory Animals in China and were approved by the Animal Care Committee of Northeast Agricultural University of China.

2.2. Experimental diets and chemicals

Two isonitrogenous (crude protein, 450 g kg^{-1}) and isolipidic (crude lipid, 77 g kg^{-1}) experimental diets were formulated, which contained 0 or 10 g kg^{-1} YC (Table 1). Ingredients were finely ground through a 320-μm mesh and fully mixed, then the oil was added to the mixture as needed, next the mixture was dissolved by adding deionized water (100 mL kg^{-1} diet) to produce dough. The dough was then extruded by a laboratory extruder (QL, Henan, China) of diameter 1.5 mm at room temperature. The resultant moist pellets were dried in a convection oven at 45 °C for approximately 6 h, and then allowed to cool overnight at room temperature. Finally, the sinking diets were stored at −20 °C until used.

Table 1
Formulation and proximate composition of the experimental diets (g kg^{-1} dry matter).

Ingredients	Diet	Diet
	Control	YC
Fish meal [a]	280	270
Soybean meal [a]	300	300
Cottonseed meal [a]	110	110
Corn gluten meal [a]	70	70
Wheat meal	164	164
Soybean lecithin	10	10
Soybean oil	33	33
Vitamin premix[b]	5	5
Mineral premix [c]	5	5
Choline	3	3
$Ca(H_2PO_4)_2$	20	20
Yeast culture [d]	0	10
Proximate composition (g kg^{-1} dry matter)		
Dry matter	932.7	926.0
Crude protein	450.3	458.3
Crude lipid	77.7	77.0
Gross energy (kJ g^{-1})	184.9	185.7
Ash	121.3	118.1
Crude fiber	31.5	31.6
Nitrogen free extract	243.9	243

[a] Fish meal: crude protein: 645.0 g kg^{-1}, crude lipid: 85.0 g kg^{-1}; soybean meal: crude protein: 467.9 g kg^{-1}, crude lipid: 31.4 g kg^{-1}; cottonseed meal: crude protein: 482.0 g kg^{-1}, crude lipid: 14.7 g kg^{-1}); corn gluten meal: crude protein: 602.0 g kg^{-1}, crude lipid: 14.7 g kg^{-1}; These ingredients were supplied by Huada feed Co., Ltd. (Harbin, China).

[b] Vitamin premix (IU or mg kg^{-1} dry diet): retinol (V_A) 3000 IU; cholecalciferol (V_D) 1500 IU; tocopherol (V_E) 40 mg; menadione (V_K) 4.5 mg; thiamin (V_{B1}) 8 mg; riboflavin (V_{B2}) 8.5 mg; pyridoxine (V_{B6}) 6.5 mg; cyanocobalamin (V_{B12}) 0.02 mg; nicotinic acid 45 mg; nicotinamide 45 mg; D-Ca pantothenate 17 mg; inositol 40 mg; biotin 0.15 mg; folic acid 1.3 mg; antiscorbic acid 110 mg.

[c] Trace mineral mixture use providing the following concentration (mg kg^{-1} dry diet): copper 6.5 mg; iron 45 mg; selenium 0.35 mg; zinc 70 mg; manganese 8.5 mg; magnesium 100 mg; cobalt 1 mg; iodine 1.2 mg.

[d] Yeast culture: crude protein: 487.0 g kg^{-1}, crude lipid: 42.1 g kg^{-1}, supplied by Beijing Enhalor International Tech Co., Ltd. (Beijing, China).

Gossypol-acetic acid (1 mg = 0.8962 mg gossypol) is the naturally occurring acetic acid form of gossypol and was purchased from Yuanye Biotechnology Co. (purity ≥98%, Shanghai, China). Sodium carboxymethylcellulose (CMC−Na) was purchased from Yongda Reagent Corporation (Tianjin, China).

2.3. Feeding trial procedures and oral gavage with gossypol

Experimental fish were obtained from Fisheries Research Institute of Harbin Academy of Agricultural Sciences (Harbin, China). Prior to starting the feeding trial, all fish were fed the diet without yeast culture for 2 weeks to acclimate to the experimental diet and experimental conditions. After fasting for 24 h, fish of homogeneous size (initial average weight 7.39 ± 0.32 g) were randomly allotted to 6 aquaria (1.0 × 0.5 × 0.8 m, water depth 50–60 cm) with 20 fish to each aquarium. Diet with YC and diet without YC were randomly assigned to triplicate aquaria respectively. Fish were hand-fed to apparent satiation twice daily (08:00 and 16:00). During the experimental period, the flow rate of water was maintained at 2.5 L min^{-1} and each aquarium was provided with continuous aeration to maintain the dissolved oxygen level above 8 mg L^{-1}, the temperature was 25 ± 1 °C, pH 7.5–8.0, ammonia-N was < 0.1 mg L^{-1}, the photoperiod was set at 12-h light and 12-h dark. The feeding experiment lasted for 8 weeks.

At the end of the feeding trial, the experimental fish were anesthetized with eugenol (1: 12000) (Shanghai Reagent Corporation, Shanghai, China). We collected the fish and created three different

groups: i.e., Control, Gos and Gos/YC groups. There were three replicates per group and 10 fish per replicate (For a total of 30 fish in each group). Fish pre-fed diet without YC without gossypol oral gavage or with gossypol oral gavage was referred as the control group and Gos group, respectively. Fish pre-fed diet with YC with gossypol oral gavage was referred as Gos/YC group. In the Gos and Gos/YC groups, fish were given a single dose of 200 μL of 1% CMC-Na solution containing gossypol, namely a final dose of 300 mg gossypol / kg body weight. In the control group, fish fed diet without YC were given a single dose of 200 μL of 1% CMC-Na solution without gossypol addition. Gossypol solution or CMC-Na solution was forced into the stomach of the fish using a 1-mL syringe fitted with an infusion tube. The oral administration experiment was conducted once daily (18:00) and lasted for 15 days.

2.4. Sample collection

At the end of the oral administration experiment, all experimental fish were anesthetized with eugenol (1: 12000) (Shanghai Reagent Corporation, Shanghai, China) before sampling. Serum samples were collected from the fish caudal vein using 1 mL syringe and withdrawn into Eppendorf tubes without anticoagulant. Blood samples in Eppendorf tubes were allowed to clot for 6 h at 4 °C. Following centrifugation (3000 *g* for 10 min), serum was stored at −80 °C for analyses of lysozyme (LZM) and alkaline phosphatase (AKP) activities and immunoglobulin M (IgM) content. After serum samples were collected, the liver tissues were selected on ice pack and were immediately frozen in liquid nitrogen and stored at −80 °C prior to analysis. Liver tissue samples were fixed in Bouin's solution for histological analysis.

2.5. LZM, AKP and AST activities and IgM level measurement

The serum LZM activity was determined by commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, China) as described by Yin et al. (2015). A LZM activity unit was defined as the amount of enzyme producing a decrease in absorbance of 0.001 min^{-1} at 530 nm. AKP activity unit is defined as 15 min per 100 mL plasma at 37 °C and substrate effects, produce 1 mg of phenol by a unit of enzyme activity as described by our previous study (Bu et al., 2017). The serum IgM level was determined by the kit of enzyme linked immunosorbent assay (ELISA) as described by Yu et al. (2014). Serum AST activity was also determined by the commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, China).

2.6. Hepatic antioxidant status analysis

The liver tissues were homogenized in ice-cold 0.86% (w / v) NaCl solution in tissue homogenizer and centrifuged at 3000 *g* 4 °C for 10 min. The supernatants of liver homogenates were used as enzyme source for measuring the hepatic total antioxidant capacity (TAC), activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and hepatic malondialdehyde (MDA) content by using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) as described by our previous study (Bu et al., 2017). The protein content in the liver sample was also determined using a total protein quantification kit (Nanjing Jiancheng Bioengineering Institute, China) with bovine serum albumin as the standard.

2.7. Real-time polymerase chain reaction (PCR) analysis

RNA extraction, reverse transcription and quantitative real-time PCR were conducted according to the previously established methods in our laboratory (Bu et al., 2017). Total RNA was extracted from liver using *TransZol* Up Plus Kit (TransGen Biotech, China) according to the manufacturer's instructions followed by DNAse I treatment. Quantity and quality of the RNA was assessed by spectrophotometric (A260: 280 nm ratio) analysis and electrophoresis in 1.2% agarose gel. Subsequently, the first strand cDNA synthesis was performed using *TransScript®* One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, China). Reaction conditions were recommended by the manufacturer. Quantitative PCR (20 μL) was performed with TransStart® Green qPCR SuperMix kit (TransGen Biotech, China) using Applied Biosystems® 7500 Real-time PCR System (USA) according to the manufacturer's protocol. There were no significant differences in transcript levels of β-actin gene among dietary treatments and was used as internal control. The real-time PCR began with 30 s at 94 °C, followed by 40 cycles of 5 s at 94 °C, 30 s at 60 °C. The melting curve analysis showed only one peak for each PCR product. The gene expression levels were calculated by $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The primers used are shown in Table 2.

Table 2
Primers used in this study.

Primer names	Sequence (5′-3′)	Product size (bp)	Annealing temperature (°C)
TLR2-F	TTGTACAGCTGGATGAGTTG	206	54
TLR2-R	TGTCGTCAGTGAAATGTCTC		
MyD88-F	TCAGACAGCTGGAGCAGACA	93	59
MyD88-R	CGCTGGTGATGGTCCAAACA		
NF-κB p65-F	AAGAACCAGCCATACAAGCCACAC	83	60
NF-κB p65-R	TCAGGCAGGTCCGCTTCGTAG		
IL-1β-F	CCTGAACACCTTCGAGTCGG	102	58
IL-1β-R	AGGTGGCTGGTTTGCTGATT		
IL-8-F	ATCGAAGGAAAAGCAGAGCG	111	57
IL-8-R	CTTTGCACAGGAGCCACTTG		
IL-10-F	TCATACGCCGTCATCCGAGA	161	58
IL-10-R	CTGACTGCACTGGGCAACAC		
β-actin-F	CCTCCGTCTGGATTTGGCTG	141	60
β-actin-R	TCAAGGGCGACGTAGCAGAG		

2.8. Histological analysis

Liver samples were preserved in Bouin's solution fixative for 24 h, dehydrated in a graded ethanol series, embedded in paraffin. The sections (5 μm) were stained with hematoxylin-eosin (HE) and observed with an Axioskop microscope (BX51, Olympus, Tokyo, Japan).

2.9. Statistical analysis

Results are presented with means ± S.E.M (standard error of the means) with superscript letters indicating differences between groups. Data was subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. A significant level of $P < .05$ was employed in all cases. All statistical analyses were performed using SPSS 22.0 for Windows (SPSS, Inc., USA).

3. Results

3.1. Inflammation-related gene expression analysis

As shown in Fig. 1, the mRNA levels of hepatic TLR2, NF-κB p65, IL-1β and IL-8 were significantly up-regulated in Gos group compared with the other groups ($P < .05$), but no significant differences were observed in these parameters between the Gos/YC and the control groups ($P > .05$). The highest hepatic MyD88 mRNA level was also found in the Gos group, but no significant difference was observed between the Gos and the Gos/YC groups ($P > .05$). The lowest hepatic IL-10 mRNA level was found in the Gos group, and fish in the Gos/YC group had significant higher IL-10 mRNA level than fish in the Gos group ($P < .05$).

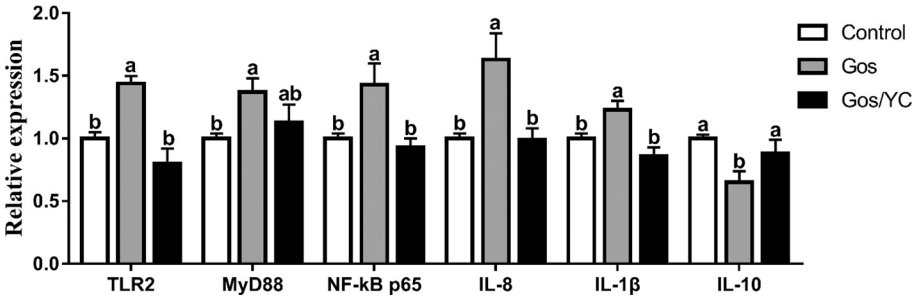

Fig. 1. The effects of dietary pre-supplementation with YC on the hepatic inflammation-related related genes expression in response to gossypol for Ussuri catfish. The immune related genes expression of TLR2, MyD88, NF-κB p65, IL-1β, IL-8 and IL-10 are determined in the liver of Ussuri catfish. Vertical bars represented the means ± S.E.M of three replicates. Different letters in each figure represented significant difference among dietary treatments ($P < .05$). TLR2: toll-like receptor 2; MyD88: myeloid differentiation factor 88; NF-κB p65: nuclear transcription factor kappa B p65; IL-1β: interleukin-1β; IL-8: interleukin 8; IL-10: interleukin 10; S.E.M: standard error of the means.

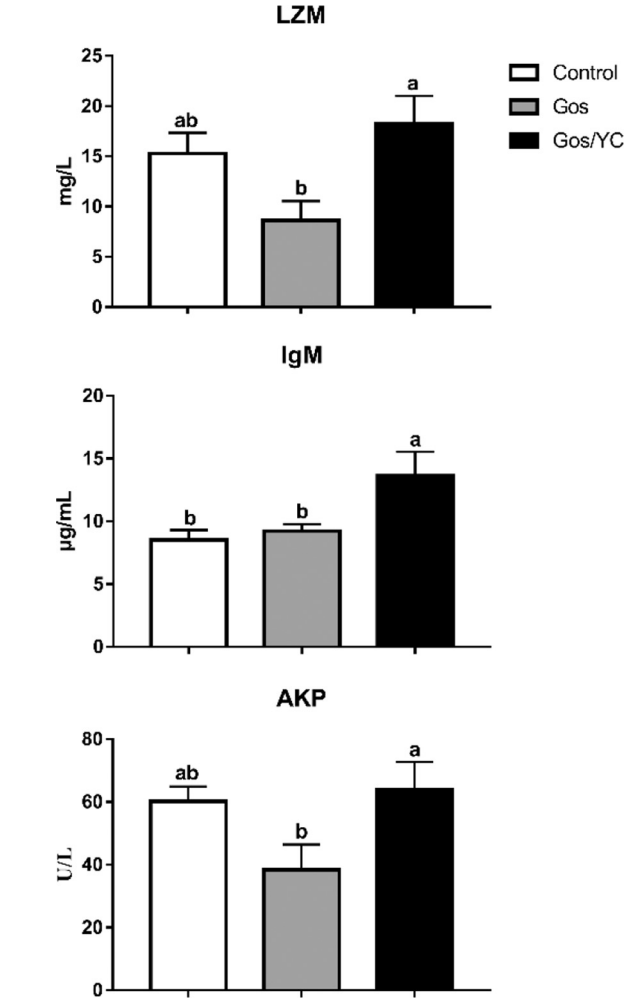

Fig. 2. The effects of dietary pre-supplementation with YC on serum LZM and AKP activities and IgM content in response to gossypol for Ussuri catfish. Vertical bars represented the means ± S.E.M of three replicates. Different letters in each figure represented significant difference among dietary treatments ($P < .05$). LZM: lysozyme; IgM: immunoglobulin M; AKP: alkaline phosphatase; S.E.M: standard error of the means.

3.2. Non-specific immunity parameters

Serum LZM and AKP activities and serum IgM content data are shown in Fig. 2. Fish in Gos/YC group had significantly higher serum LZM and AKP activities than fish in Gos group ($P < .05$). Simultaneously, there was no significant difference in serum IgM content for fish in Gos group compared with fish in control group ($P > .05$), but there was a sharp rising for that of fish in Gos/YC group compared with that of fish in Gos group ($P < .05$).

3.3. Hepatic antioxidant status analysis

As shown in Fig. 3, fish in Gos and Gos/YC groups had significantly lower hepatic CAT activity than the control group ($P < .05$), and there was no significant difference between fish in Gos and Gos/YC groups ($P > .05$). The lowest hepatic SOD and TAC activities were observed in Gos group, and the hepatic TAC in Gos/YC group was significantly higher than that in Gos group. No significant differences were observed in hepatic GPx activity and MDA content among the groups ($P > .05$), but it was noteworthy that the highest MDA content was found in Gos group.

3.4. Liver histology and aspartate aminotransferase activity

No markedly histopathological changes were observed in the liver tissues of fish in the control group (Fig. 4). In the liver tissues of fish pre-fed diet without yeast culture supplementation and treated with gossypol oral administration, higher occurrences of the dilatation in sinusoids, karyolysis, hydropic degenerations and nuclei shifting to the cellular periphery were detected compared with the fish in the control group. However, lower occurrences of these histopathological symptoms were observed in the Gos/YC group compared with those of fish in the Gos group. Meanwhile, fish in Gos group had significantly higher serum AST activity than the other groups ($P < .05$).

4. Discussion

Free gossypol is a yellow polyphenolic compound in the pigment glands of cotton plants *Gossipium* spp., which is known to be toxic to monogastric animals including fish. Herman (1970) reported that gossypol had toxic effects on growth, hematocrit, hemoglobin and total plasma protein of rainbow trout (*Salmo guirdneri* Richardson). Moreover, Bian et al. (2016) found that dietary gossypol could cause liver fibrosis and simulated proinflammatory cytokine secretions. Previous studies suggested that gossypol accumulated mainly in the liver (Jensen et al., 1982; Kim et al., 1996; Lee and Dabrowski, 2002). Thus, at present we evaluated the effect of free gossypol on the gene expression levels of three cytokines (IL-1β, IL-8 and IL-10) in liver tissue of Ussuri catfish. Cytokines, such as IL-1β and IL-8 have a fundamental role in regulation of the pro-inflammatory response in fish (Jung et al., 1995). In the present study, fish in Gos group had significantly higher hepatic IL-1β and IL-8 mRNA expression than fish in control group, however, hepatic IL-1β and IL-8 mRNA expression of fish in Gos/YC group was significantly lower than fish in Gos group, suggesting that free gossypol may have a stimulatory action upon proinflammatory processes and dietary yeast culture supplementation inhibited the inflammatory response. In addition, IL-10 is regarded to have an anti-inflammatory role, potently inhibiting the capacity of cells to secrete inflammatory mediators (Fiorentino et al., 1991). In the present study, dietary yeast culture supplementation upregulated IL-10 mRNA expression level. Taken together, these results suggested that yeast culture could have potentially protective role against gossypol-induced inflammatory response,

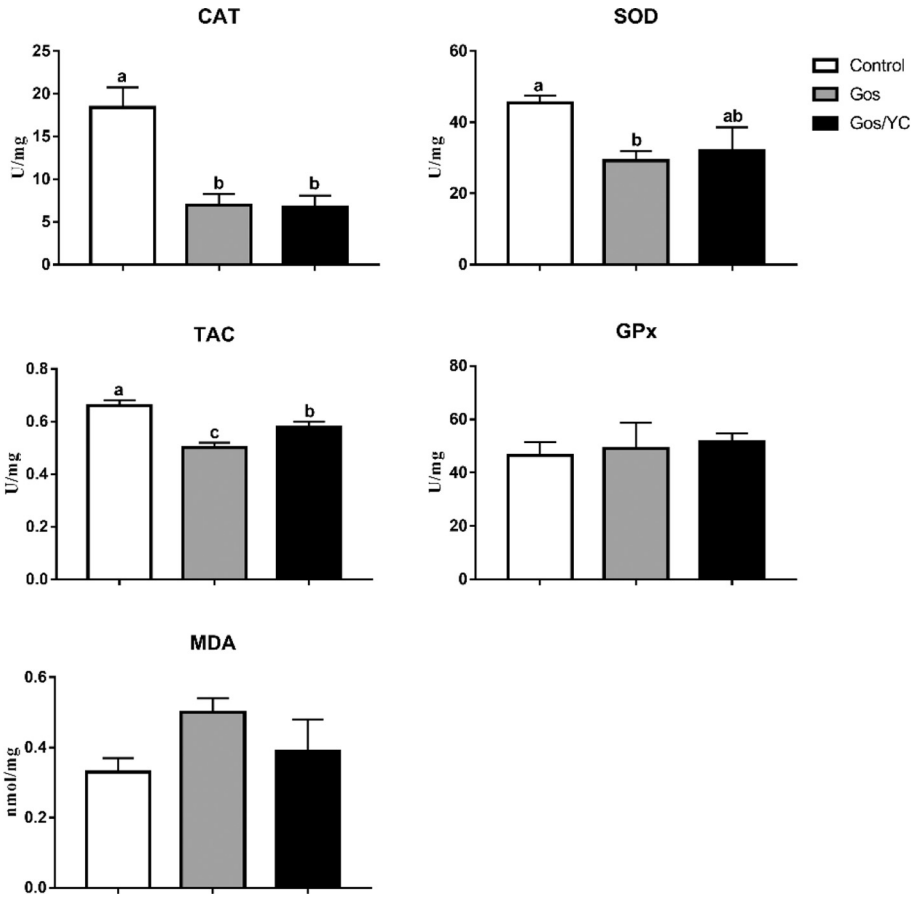

Fig. 3. The effects of dietary pre-supplementation with YC on hepatic antioxidant capacity parameters in response to gossypol of Ussuri catfish. Vertical bars represented the means ± S.E.M of three replicates. Different letters in each figure represented significant difference among dietary treatments ($P < .05$). CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; TAC: total antioxidant capacity; MDA: malondialdehyde; S.E.M: standard error of the means.

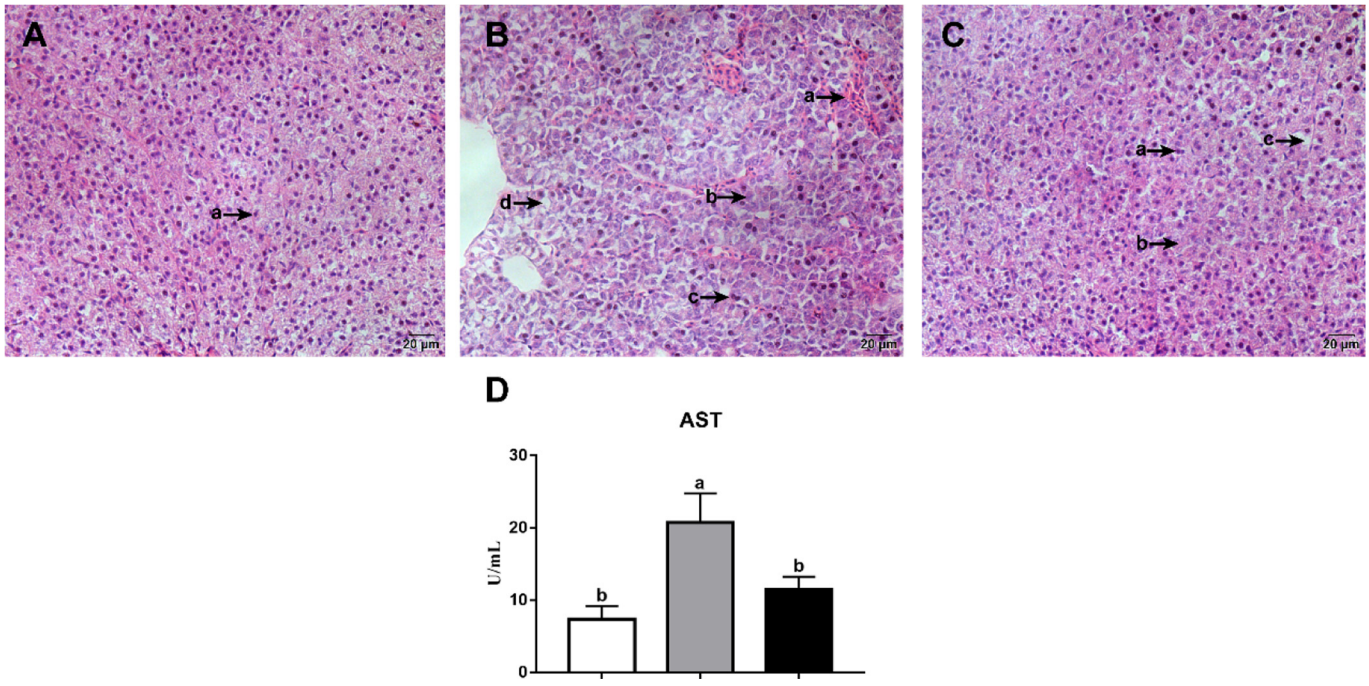

Fig. 4. The effects of dietary pre-supplementation with YC on histological analysis of liver sections and aspartate aminotransferase activity (AST) in response to gossypol for Ussuri catfish. (A) Liver structure of fish in control group: (a) hepatocyte (H & E). (B) Liver structure of fish in Gos group: (a) dilatation in sinusoids, (b) karyolysis, (c) nuclei shifting to the cellular periphery and (d) hydropic degenerations. (C) Liver structure of fish in Gos/YC group: (a) nuclei shifting to the cellular periphery, (b) karyolysis and (c) hydropic degenerations. (D) Aspartate aminotransferase activity. Scale bar = 20 μm.

and this is the first attempt to investigate whether yeast culture could relieve gossypol-induced inflammatory responses in fish.

Bian et al. (2016) also reported that TLRs act an important role in the activation of hepatic immune and inflammatory responses. TLRs are found on immune cells and recognize molecular sequences found on microbes (Kigerl et al., 2007), which serve as major pattern recognition receptors that detect pathogen-associated molecular patterns (PAMPs). The PAMPs could trigger NF-κB signaling pathway and production of pro-inflammatory (Nguyen et al., 2002). TLR2 is one type of pattern recognition receptors, which has been confirmed to have a strongly associated with immunity and inflammatory response of fish (Liu et al., 2016). Moreover, previous study demonstrated that dietary gossypol could induce inflammation of liver (Soares et al., 2012). Similarly, the present results showed that fish pre-fed the control diet and accompanying with gossypol oral gavage had significantly higher hepatic TLR2 mRNA level than that in the control group, suggesting that gossypol exposure may induce the production of inflammatory cytokine through the TLR2 signaling pathway, causing liver inflammation response of Ussuri catfish. However, the hepatic TLR2 mRNA level of fish in Gos/YC group was decreased significantly than that in Gos group. This result again verified that yeast culture may have a mitigative effect on gossypol-induced inflammatory response. To illuminate the regulation mechanisms of inflammation-related cytokine production after the fish was treated with gossypol oral gavage, we evaluated the effects of the main signaling molecules such as TLR2, MyD88 and NF-κB p65 in TLR2-MyD88-NF-κB signaling pathway. It was well known that overactivation of this signaling pathway would aggravate inflammatory reaction and then have negative effects on organism (Jiang et al., 2015). Accordingly, the present results demonstrated that the hepatic MyD88 and NF-κB p65 mRNA levels were up-regulated in response to gossypol, and fish pre-fed yeast culture prevented the increase in the MyD88 and NF-κB p65 mRNA levels. These results suggested that yeast culture may relieve gossypol induced inflammatory response via altering TLR2-MyD88-NF-κB signaling pathway in liver tissue of Ussuri catfish. However, it is necessary to point out that so far it has not been defined in fish how yeast culture regulates the TLR2-MyD88-NF-κB signaling pathway or cytokines gene expression and the mechanisms need to be further investigation.

In fish, the innate immune system is the essential defense mechanism because fish is continuously exposed to numerous opportunistic pathogens and this part of immune response provides the first line of defense for the host (Staykov et al., 2007; Ke and Zhang, 2019). Currently, several approaches were employed to enhance the immunity and control the diseases of fish, such as the using of immunostimulants, probiotics and nucleotides (Selvaraj et al., 2005; Yin et al., 2015; Li et al., 2019). Our previous study also showed that dietary yeast culture supplementation could improve the immunity of Ussuri catfish (Bu et al., 2019). In the present study, three indicators of innate immunity (serum LZM, AKP and IgM) were tested to evaluate the harmful effect of gossypol on immunity of Ussuri catfish and the alleviative effect of yeast culture on gossypol-induced immune damage. Lysozyme, a bactericidal enzyme, is known to act as an important component of the immune defense in fish (Lushchak et al., 2001). It is responsible for breaking down the peptidoglycan layer of the bacterial cell wall, causing lysis and thus stimulating the phagocytosis of bacteria (Hauge et al., 2002). Moreover, alkaline phosphatase is an important lysosomal enzyme with potential protective role in fish (Ghahderijani et al., 2015). In the present study, fish in Gos group had lower serum LZM and AKP activities than the control group though there were no significantly differences between these two groups. These results suggested that the gossypol, to some extent, may cause the disorder of the immune system of fish and reduce the antibacterial ability of Ussuri catfish. Similarly, dietary gossypol could decrease intestinal innate and adaptive immune components of grass carp (*Ctenopharyngodon idella*) (Wang et al., 2019). Furthermore, fish in Gos/YC group had higher serum LZM and AKP activities than fish in Gos group, indicating that fish pre-fed yeast culture could alleviate the gossypol-induced immune damage response for Ussuri catfish. Moreover, IgM is the most widely studied immunoglobulin in fish (Estensoro et al., 2012; Purcell et al., 2012) and it is the earliest produced antibody in the initial humoral immune response, which could be a good biomarker for evaluating the immune status of fish. In the current study, fish pre-fed yeast culture could induce the secretion of IgM. Presumably, the functional components (i.e. β-glucan, mannan-oligosaccharide, peptides) in yeast culture possibly induced B lymphocytes to produce antibodies to activate immune response of fish. But it is unclear why gossypol stress did not cause a decrease in serum IgM level of fish. The mechanisms await further characterization.

The immunity of fish was also related with the antioxidant system. Under normal physiological or stressful conditions, free radicals and reactive oxygen species (ROS) are continuously generated and organisms possess adequate protection systems such as key enzymatic antioxidant defenses to avoid/repair the ROS induced damage (Liu et al., 2018). However, oxidative stress occurs when the balance between the generation and removal of ROS are disrupted. In the current study, fish in Gos group down-regulated the hepatic TAC, CAT and SOD activities significantly, suggesting that gossypol may reduce the antioxidant capacity of Ussuri catfish. In addition, the possible mechanisms of dietary free gossypol toxicity include the generation of oxygen metabolites such as superoxide and hydrogen peroxide radicals (Rinchard et al., 2003). Furthermore, fish in Gos/YC group had significantly higher TAC than the Gos group. Meanwhile, the hepatic SOD activity of fish also had a slightly increased trend in Gos/YC group compared with the Gos group though there was no significantly difference between these two groups. These results demonstrated that fish pre-fed yeast culture, to some extent, had an enhanced effect of antioxidant capacity for Ussuri catfish.

Generally, liver is the vital organ for detoxification, and histological changes in liver are considered an important indicator for evaluating nutritional condition (Van der Oost et al., 2003; Raškovic´ et al., 2011). Thus, the present study investigated the effect of gossypol on liver histology in Ussuri catfish, and found that gossypol oral gavage could cause abnormalities of liver, including dilatation in sinusoids, karyolysis, hydropic degenerations and nuclei shifting to the cellular periphery. Likewise, previous studies reported that these histopathological changes in liver revealed the liver injury or degenerative necrotic condition, which may affect liver function of fish (Zhang et al., 2019; Wang et al., 2014). Moreover, AST, as a sensitive marker of hepatocellular integrity, is generally considered important diagnostic indicators, which is used to estimate the health of fish (Wang et al., 2014). According to the present results, elevated serum AST level of fish in Gos group was also evidence of liver injury. Simultaneously, liver is the preferred organ for evaluating the status of antioxidant defense, and is also highly relevant for biomonitoring researches (Zhang et al., 2019). Therefore, the reduction in antioxidative capacity and up-regulated proinflammatory cytokines may also indirectly verified the liver damage of fish. Moreover, fish pre-fed diet with yeast culture supplementation had lower occurrences of histopathological symptoms in response to gossypol oral gavage, suggesting the mitigative effect of yeast culture on the hepatic lesion induced by gossypol for Ussuri catfish.

In conclusion (Fig. 5), fish treated with gossypol could induce inflammatory response, resulting in up-regulation IL-1β and IL-8 mRNA levels in liver tissue of fish. Dietary yeast culture supplementation could alleviate gossypol-induced inflammatory response via altering TLR2-MyD88-NF-κB signaling pathway and could also relieve immune damage, oxidative stress and liver injury of Ussuri catfish.

Declaration of Competing Interest

No potential conflict of interest.

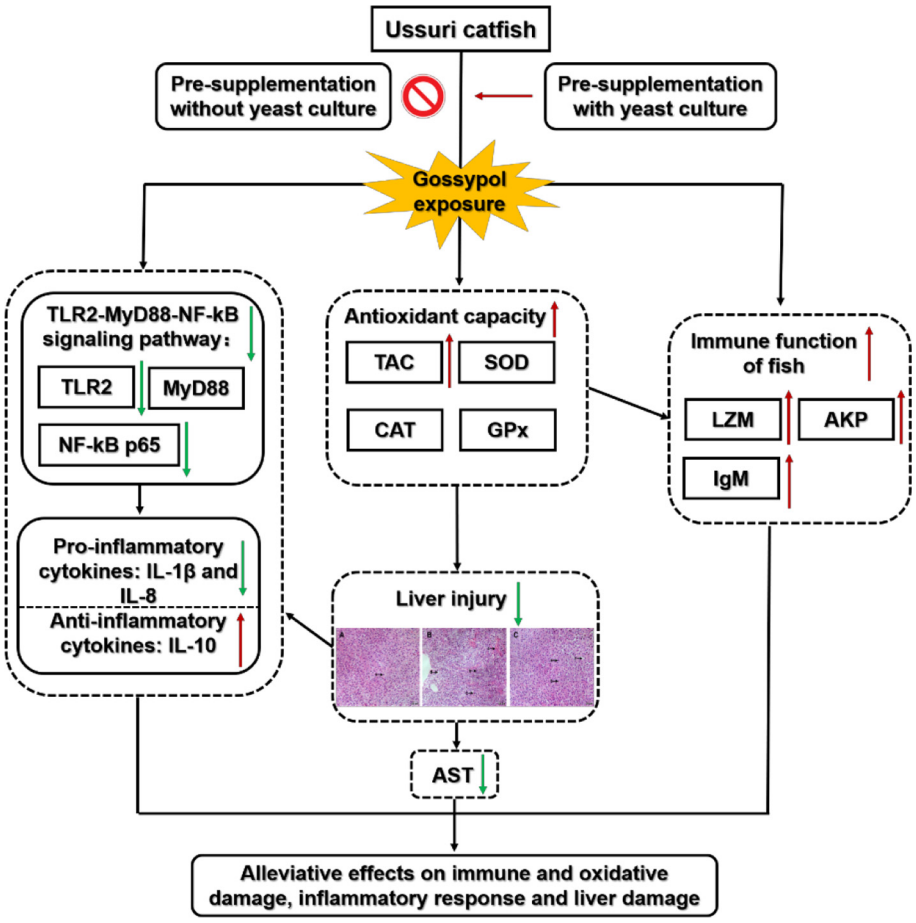

Fig. 5. Potential action pathways of yeast culture relieved gossypol induced immune and oxidative damage and inflammatory response of Ussuri catfish. Fish pre-fed yeast culture could alleviate the immune damage and oxidative stress induced by gossypol oral administration. Meanwhile, yeast culture could alleviate the liver injury though down-regulating TLR2-MyD88-NF-κB signaling pathway. TLR2: toll-like receptor 2; MyD88: myeloid differentiation factor 88; NF-κB p65: nuclear transcription factor kappa B p65; IL-1β: interleukin-1β; IL-8: interleukin 8; IL-10: interleukin 10; CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; TAC: total antioxidant capacity; LZM: lysozyme; IgM: immunoglobulin M; AKP: alkaline phosphatase.

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