Compound Chinese Herbal Formulations enhance Growth and Reduce Hepatic Lipid Deposition in Hybrid Grouper (Epinephelus lanceolatus ♂× E. fuscoguttatus ♀)

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Research Article

Keywords: Grouper, Marine aquaculture, Chinese herbal medicine, Oil Red O staining, Hepatic lipid deposition

Posted Date: July 15th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9834919/v1

Additional Declarations: No competing interests reported.

Compound Chinese Herbal Formulations enhance Growth and Reduce Hepatic Lipid Deposition in Hybrid Grouper (*Epinephelus lanceolatus* ♂× *E. fuscoguttatus* ♀)

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Abstract

This study evaluated the effects of compound Chinese herbal extracts, including *Eucommia ulmoides*, *Paeoniae Radix Alba*, *Glycyrrhiza uralensis*, and *Cornus officinalis*, on growth performance, lipid metabolism, and intestinal morphology in hybrid groupers. Dietary supplementation significantly enhanced specific growth rate in D2 and D3 ($P < 0.05$). Oil Red O staining combined with automated image analysis, revealed reduced hepatic lipid deposition in all supplemented groups, with the lowest lipid area ratio observed in D1. Notably, D1 significantly decreased serum triglycerides and increased intestinal goblet cell numbers, while D2 enhanced high-density lipoprotein cholesterol (HDL) levels. These findings suggest that compound herbal formulations can promote growth and alleviate hepatic lipid accumulation in grouper, providing a promising strategy for sustainable aquaculture.

Key words: Grouper; Marine aquaculture; Chinese herbal medicine; Oil Red O staining; Hepatic lipid deposition

Introduction

Chinese herbal additives have gained increasing attention in aquaculture due to their natural origin, low toxicity, broad-spectrum efficacy, and minimal risk of drug resistance (Abdallah et al., 2019; Li et al., 2024; Arshad et al., 2025). These additives function through various mechanisms, including improving feed palatability, digestion, and nutrient metabolism to promote growth (Cai et al., 2023; Arshad et al., 2025), as well as mobilizing disease-resistant factors, enhancing tissue and organ functions, and exerting direct antibacterial and antioxidant effects (Yin et al., 2009; Abdallah et al., 2019; Li et al., 2022; Zhao et al., 2025a). For example, components of *E. ulmoides*, such as geniposidic acid, pinoresinol diglucoside, and quercetin, have demonstrated antioxidant and anti-inflammatory activities, with potential synergistic effects in regulating lipid metabolism (Shao et al., 2021; Wu, 2021; Ren et al., 2023). The extract of *E. ulmoides* leaves has been shown to enhance growth performance and non-specific immunity in *Mylopharyngodon piceus* (Xu et al., 2015) and increase antioxidant capacity while protecting *Micropterus salmoides* from liver damage and immune dysfunction induced by a high-starch diet (Liu et al., 2023a).

Groupers (*Epinephelus* spp.), belonging to the order Perciformes, are warm-water reef fish commonly found in tropical and subtropical waters of the Indian and Pacific Oceans (Wang et al., 2025). Known for their tender meat, pleasant taste, high-quality protein, and rich content of unsaturated fatty acids, groupers are highly valued as premium seafood with significant economic potential (Ding et al., 2018). In 2024, China's grouper culture reached 267,000 tons, with major production areas in Guangdong (113,000 tons), Hainan (102,000 tons), and Fujian (45,000 tons) (Fisheries Bureau of the Ministry of Agriculture and Rural Affairs, 2025). The primary species farmed include *Epinephelus coioides*, *Epinephelus akaara*, and the hybrid *Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀ (Zhang et al., 2023).

As market demand for groupers increases, the scale and intensity of grouper culture continue to grow. However, high-density farming systems are prone to infections caused by viral, bacterial, and parasitic agents (Xu et al., 2014; Juniar et al., 2018; Natnan et al., 2021), leading to higher morbidity and increased reliance on antibiotics and chemicals. Long-term dependence on such treatments can lead to various risks, including the emergence of drug-resistant pathogens, metabolic disruptions in fish, deterioration of the aquatic environment, accumulation of drug residues in products, and potential hazards to human health (Elgendy et al., 2024). These issues contradict China's sustainable development goals, such as "green, high-quality fishery development" and "antibiotic-free, healthy culture." Additionally, unbalanced feeding and overfeeding in farmed fish have been shown to cause widespread metabolic health problems, particularly fatty liver disease in groupers (Du, 2014; Zhao et al., 2025b). Excessive hepatic lipid accumulation not only increases metabolic burdens but also weakens immune response and stress resistance. Fish suffering from fatty liver disease exhibit significantly higher mortality rates following pathogen infection than healthy individuals (Li et al., 2016; Zhao et al., 2025b), leading to reduced meat quality and economic returns (Ma et al., 2020). Consequently, there is a pressing need to explore natural alternatives that promote growth, protect liver health, and enhance immunity in order to establish sustainable, antibiotic-free grouper culture systems.

Most studies on Chinese herbal medicines in aquaculture have primarily focused on the effects of single herbs. However, compound herbal preparations are more commonly used in practice. Research on the application of compound Chinese herbal formulations for marine fish remains limited, particularly regarding their effects on regulating lipid metabolism and protecting liver health. There is a lack of systematic experimental evidence and practical application guidelines for compound herbal treatments in this area. Therefore, it is essential to investigate the combined effects of multiple Chinese herbal medicines with similar functions. Given the significant role of *E. ulmoides* in regulating hepatic lipid metabolism, this study uses liver lipid accumulation characteristics as a key parameter for evaluation. Moreover, Oil Red O, an azo fat-soluble dye that specifically stains neutral fats such as triacylglycerols, is employed to visualize lipid droplet distribution and accumulation (Shihana et al., 2023; Li et al., 2024). This technique has been successfully applied in the pathological evaluation of fatty liver in economic fish (Chen et al., 2023). However, studies using imaging systems to calculate lipid-positive area ratios remain scarce.

In this study, the hybrid grouper (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀) was selected as the experimental model to evaluate the effects of different herbal combinations and dosages on growth, serum biochemistry, tissue structure, and immune parameters. The primary goal of this study is to provide both theoretical and practical insights for the scientific application of Chinese herbal additives in antibiotic-free marine fish culture and to propose new strategies for modernizing aquaculture practices and promoting sustainable, healthy fish farming systems.

Materials and Methods

Experimental Materials

A 0.33-hectare pond with 12 cages (1.5 m diameter, 1.5 m height) was used for the experiment at the Yangxi Marine Fish Breeding Farm. A batch of healthy, active groupers, with uniform size and no visible diseases, were selected for the study. The average body weight and total length of the groupers were 20.90 ± 1.61 g and 10.23 ± 0.25 cm, respectively.

Feed Preparation

The compound herbal extract, which included *E. ulmoides* leaves, *Paeoniae Radix Alba*, *G. uralensis*, and *C. officinalis*, was provided by Beijing Center Biology Co. Ltd. The feed ingredients were thoroughly mixed to prepare a powdered feed according to the formulation provided in Table 1. The compound herbal extract was blended into the powder at the designated dosage, then formed into pellets. The pelleted feed was air-dried for approximately 2–3 days, packaged separately, and stored at a low temperature for daily feeding during the grouper culture experiment.

The formulated pelleted feed contained 42.05% crude protein, 9.39% crude lipid, 7.91% moisture, and 10.83% crude ash. Four treatment groups were established, based on different supplementation levels of the compound Chinese herbal preparation (Table 2). Three replicates (cages) were used in each group, with a stocking density of 80 fish per cage.

Daily Management

During the 60-day feeding period, water temperature, salinity, and dissolved oxygen (DO) levels in the pond were continuously monitored. The water temperature ranged from 12 to 29 °C, salinity was maintained at 15–18‰, and the DO concentration remained above 6.0 mg/L. The daily feeding amount was calculated as 2–3% of the body weight of the groupers in each treatment group. The feeding amount was adjusted based on feeding behavior, and the actual feed intake was recorded throughout the trial.

Sample Collection and Processing

After 60 days of rearing, 4 to 5 fish were sampled from each cage. Blood was collected from the caudal vein of each fish, allowed to stand, and then centrifuged to obtain serum, which was stored at -80 °C and tested within 48 hours. The sampled fish were dissected on ice, and liver and foregut tissues were collected and fixed in 4% paraformaldehyde for histological section analysis. Additional liver tissue samples were stored at -80 °C for enzyme activity determination. The remaining samples were stored at -20 °C for subsequent testing.

Growth Performance Indicators

Body weight, length, visceral weight, and liver weight were measured on days 0 and 60.

The following growth performance indicators were calculated: survival rate (SR), feed intake rate (FR), specific growth rate (SGR), weight gain rate (WR), condition factor (CF), hepatosomatic index (HSI), viscerosomatic index (VSI), and intestinal fat ratio (IPF). These indicators were computed using the formulas provided below:

$$\mathrm{SR} = \frac{N_f}{N_i} \times 100\% \tag{1}$$

$$\mathrm{FR} = \frac{I_0}{(W_t + W_0)/2} \times 100\% \tag{2}$$

$$\mathrm{SGR} = \frac{\ln W_t - \ln W_0}{t} \times 100\% \tag{3}$$

$$\mathrm{WR} = \frac{W_t - W_0}{W_0} \times 100\% \tag{4}$$

$$\mathrm{CF} = \frac{W_t}{L^3} \times 100\% \tag{5}$$

$$\mathrm{HSI} = \frac{W_h}{W} \times 100\% \tag{6}$$

$$\mathrm{VSI} = \frac{W_v}{W} \times 100\% \tag{7}$$

$$\mathrm{IPF} = \frac{W_i}{W} \times 100\% \tag{8}$$

Where, N_f is final fish number; N_i is initial fish number; W_0 is initial body weight (g); W_t is final body weight (g); I_0 is total feed intake (g); t is culture cycle (d); L is average body length (cm); W_h is liver weight (g); W_v is visceral weight (g); W_i is intestinal fat weight (g).

Serum Lipid Indicators

Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were measured using a Hitachi 7180 automatic biochemical analyzer.

Determination of Total Fat Content in Whole Fish

Total fat content was determined using the Soxhlet extraction method, with petroleum ether as the extractant. Whole fish samples were collected, dried at 105 °C, ground into powder, and then analyzed using a Soxtec AVanti 2050 automatic analyzer (Foss Tecator AB, Switzerland).

Liver and Intestinal Tissue Sections

Liver sections were stained with Oil Red O for analysis. After drying, the sections were fixed in a fixative for 15 minutes, rinsed with water, and dried. The sections were then immersed in Oil Red O staining solution for 8–10 minutes (protected from light). Following this, the sections were sequentially immersed in two dishes of 60% isopropanol for 3s and 5s, respectively, for differentiation, and then in two dishes of pure water for 10s each. The sections were subsequently stained with hematoxylin, coverslipped, and examined microscopically. Three fields of view were photographed for each sample. An optical microscope (ZEISS Axio Scope. A1) was used to observe and record the images, and Aipathwell® analysis software was employed for automatic image analysis. The process is as follows:

a) **Trace**: The area of interest is automatically located and traced based on tissue characteristics, with manual positioning available if needed.

b) **Color Selection**: Automatic positive judgment based on HSI, with manual correction possible if necessary.

c) **Calculation**: The software automatically calculates parameters such as the positive area and tissue area within the region of interest.

d) **Analysis**: The area of interest is analyzed at high magnification, and the software calculates the positive area, tissue area, and the percentage of positive area in the measured area using the original data and algorithm. The calculation formula is as follows:

$$\text{Lipid - Positive Area Ratio} = \frac{S_o}{S_t} \times 100\% \qquad (9)$$

Where, S_o is Oil Red O positive area; S_t is total tissue area.

Foregut sections were prepared and observed using hematoxylin-eosin (H&E) staining. The sections underwent sequential ethanol dehydration, xylene clearing, paraffin embedding, microtome sectioning, H&E staining, and neutral resin mounting. Observations and image acquisition were conducted using an optical microscope (ZEISS Axio Scope A1). Subsequently, using the SlideViewer analysis system, five intact intestinal villi were randomly selected from each section. For each selected villus, morphological parameters such as length, width, and muscular layer thickness were measured, and the number of goblet cells was counted. These data were further analyzed to characterize structural changes in the foregut.

Data Analysis

SPSS 22 software was used for one-way ANOVA and Duncan's multiple comparison. Results are presented as "mean ± standard deviation", with $P < 0.05$ indicating significant differences.

Results and Analysis

Effects on Feed Intake and Growth Performance

During the culture period, no noticeable diseases or mortality were observed in any of the grouper groups, and the SR remained above 95% (Table 3). Groupers in all supplemented groups exhibited normal feeding behavior, with visible feeding competition, indicating good feed palatability. The SGR of D2 and D3 was significantly higher than that of Dc ($P < 0.05$). CF of D1 was significantly higher than that of Dc ($P < 0.05$), while no significant differences were observed among the other supplemented groups ($P > 0.05$). Additionally, no significant differences in HSI, VSI, and IPF were found among all treatment groups ($P > 0.05$).

Effects of Compound Chinese Herbal Preparations on the Intestinal Structure

The intestinal tissue structure of groupers in all treatment groups was well-defined, with distinct muscularis and serosa layers. The average villus length ranged from 312.03 to 391.43 μm, villus width ranged from 70.09 to 86.72 μm, and muscularis thickness ranged from 59.09 to 75.35 μm (Table 4, Fig. 1). No significant differences were found in the number or length of villi among all of the treatment groups ($P > 0.05$). However, a significant difference was observed in the number of goblet cells on intestinal villi ($P < 0.05$). D1 exhibited the highest number of goblet cells on intestinal villi, with an average of 10–13 cells per villus, significantly higher than those in Dc, D2, and D3.

Hepatic Lipid-Positive Characteristics and Body Fat Percentage

The results of Oil Red O staining of liver tissue showed that, at the beginning of the experiment (D0), the degree of positive fat staining in the liver was low (Fig. 2), with the positive fat area ratio ranging from 4.04% to 9.62% (Fig. 3). After 60 days of feeding, the positive fat area ratio in the liver of all treatment groups increased to varying degrees. The positive fat area ratios in D1, D2, and D3 were significantly lower than that in Dc ($P < 0.05$), with D1 showing the lowest ratio (18.8%) (Fig. 3).

Compared to Dc, all of the supplemented groups exhibited fewer, smaller, and lighter-colored lipid accumulations (red-stained regions) in hepatocytes, with less than 25% of the hepatocytes showing steatosis. In contrast, Dc displayed numerous, large, and dark-colored lipid accumulations in hepatocytes, which compressed the hepatocyte nuclei (blue-stained regions). The proportion of hepatocytes with steatosis in Dc was nearly 30% of the entire liver tissue, indicating that lipid deposition in the hepatocytes of Dc was more severe than in the other groups.

The average body fat percentage varied between 26.20% and 27.49%. No significant differences in body fat content were detected in groupers across the different treatments ($P > 0.05$), although values exhibited an ascending order of D1 < D2 < D3 < Dc.

Effects on Serum Lipids

Chinese herbal medicine additives had significant effects on the blood lipid profiles of groupers (Table 5). The serum levels of TC and HDL in D2 were significantly higher than those in Dc, D1, and D3 ($P < 0.05$), while no significant differences were observed among these three groups ($P > 0.05$). The levels of LDL in the herbal supplemented groups (D1, D2, and D3) were significantly higher than that in Dc ($P < 0.05$). Additionally, the TG content in D1 was significantly lower than that in Dc and D2 ($P < 0.05$), while no significant differences in TG levels were found among D2, D3, and Dc ($P > 0.05$).

Discussion

The present study demonstrated that dietary supplementation with compound Chinese herbal medicines significantly improved growth performance and reduced hepatic lipid deposition in hybrid grouper. These effects are likely attributed to the synergistic actions of multiple bioactive compounds involved in growth regulation, antioxidant defense, and lipid metabolism. Chinese herbal medicines contain a variety of bioactive substances that effectively enhance growth, immunity, and overall health in aquatic animals (Pu et al. 2017; Tadese et al. 2022; Peng et al. 2023). The results showed that the compound extract of *E. ulmoides*, *G. uralensis*, and *Paeoniae Radix Alba* significantly increased the SGR of grouper, with the D2 (4% addition level) exhibiting the optimal effect. This suggests that appropriate supplementation effectively promotes growth. The growth-promoting effect is likely related to the synergistic regulation of growth pathways by multiple bioactive components: *E. ulmoides* is rich in lignans, chlorogenic acid, and polysaccharides (Leng et al. 2008), which can promote cell metabolism and protein synthesis by upregulating the *igf1/tor* pathway (Yang et al. 2022). Previous studies have confirmed that *E. ulmoides* extract can significantly enhance the growth performance and antioxidant capacity of grass carp (*Ctenopharyngodon idellus*) (Meng et al. 2007; Leng et al. 2008), turbot (*Scophthalmus maximus*) (Zhang et al. 2019), rainbow trout (*Oncorhynchus mykiss*) (Yao et al. 2020), and Pacific white shrimp (*Litopenaeus vannamei*) (Liu et al. 2013). *G. uralensis* extract enhances the immune function and alleviates inflammatory responses of fishes by increasing serum lysozyme activity, immunoglobulin content, and upregulating the expression of C-reactive protein, mTOR, and heat shock protein-90-related genes (Abdel-Tawwab et al. 2021; Yang et al. 2020). Anthraquinones in *Paeoniae Radix Alba* extract enhance the physiological adaptation and stress resistance of fish and shrimp to high-temperature environments by increasing antioxidant enzyme activity and the expression of iNOS-NO immune-related proteins (Liu et al. 2010; Song et al. 2020). The synergistic effects of the three Chinese herbal medicines not only promote growth through the regulation of growth pathways by *E. ulmoides* extract but also provide a stable internal environment for growth through the immune enhancement of *G. uralensis* and stress resistance protection of *Paeoniae Radix Alba*, overcoming the limitations of single Chinese herbal extracts in aquatic animals and highlighting the unique advantages of compound herbal formulations.

Fatty liver is a prevalent metabolic disorder afflicting farmed groupers, and is commonly associated with the administration of high-energy and high-sugar diets (Yu et al. 2025). The blood and liver are central organs governing lipid metabolism in fish (Nguyen et al. 2008), and their lipid content and distribution constitute reliable biomarkers for evaluating physiological health status. In the present study, the hepatic lipid-positive rate was significantly lower in every supplemented group relative to the control group ($P < 0.05$), with D1 displaying the most prominent hypolipidemic effect, indicative of effective lipid-lowering and liver-protecting functions, whereas analysis of body fat percentage revealed that Chinese herbal medicine additives exerted no significant effects on whole-body lipid accumulation and metabolism in groupers. The serum HDL in the D2 was significantly increased, which contributes to improving liver lipid transport and metabolic homeostasis. Meanwhile, TG in D1 significantly reduced , TC, HDL and LDL in D2 significantly increased, while the blood lipid indices in D3, supplemented with *C. officinalis*, demonstrated little change. These results suggest that different Chinese herbal medicines have complementary effects in lipid metabolism regulation. Previous studies have confirmed that *E. ulmoides* extract can promote lipid breakdown by upregulating LPL and inhibiting fat synthesis (Meng et al. 2007). *G. uralensis* can enhance hepatopancreatic function in shrimp and reduce alanine aminotransferase levels (Panita et al. 2019). *Paeoniae Radix Alba*'s paeoniflorin increases antioxidant enzyme activities (SOD, GSH-Px) and reduces liver fibrosis by decreasing collagen deposition (Zhang et al. 2025).

Furthermore, Oil Red O staining results confirmed that the compound Chinese herbal medicines significantly reduce liver lipid deposition and protect hepatocyte structure. In this study, the liver fat-positive rate of groupers in all supplemented groups was significantly lower than that in the non-supplemented group (Dc) ($P < 0.05$). Although the HSI of all supplemented groups was higher than the reference threshold of the grass carp (*C. idellus*) fatty liver model (Meng et al. 2007), the HSI values were all lower than those of Dc, indicating that the critical level of hepatic lipid accumulation in grouper may differ from that in freshwater fish. The results of this study provide a reference for establishing diagnostic criteria and prevention strategies for grouper fatty liver. Notably, the blood lipid indices in this study did not follow the typical pattern of increased HDL, decreased LDL, and decreased TG. This is consistent with the phenomenon observed in healthy tilapia (*Oreochromis niloticus*) fed tea polyphenols, which temporarily increase LDL, suggesting enhanced metabolism (Daniel et al. 2024). In fish, moderate increases in LDL and TG may contribute to energy supply, growth support, and immune response rather than inducing atherosclerosis.

As a key organ for nutrient absorption and immune barrier function, there were no significant differences in the intestinal villus morphology of groupers across all of the treatment groups. However, the number of goblet cells in D1 was significantly higher ($P < 0.05$), indicating that an appropriate amount of Chinese herbal extract helps strengthen the intestinal mucosal barrier, reduce pathogen adhesion, and enhance intestinal health. Glycyrrhizic acid and its derivatives have been shown to inhibit intestinal inflammation and promote the proliferation of intestinal goblet cells (Liu et al. 2023b). Due to the low addition level of *C. officinalis* extract (approximately 0.04%) in the D3, it did not demonstrate significant advantages in blood lipid regulation. This contrasts with the effects of 6.5% *C. officinalis* extract (COV) in a mouse model, which reduces serum TG and LDL cholesterol, increases HDL cholesterol levels, and significantly improves non-alcoholic fatty liver (Cao et al. 2022). This suggests that the optimal addition level of *C. officinalis* in feed requires further optimization.

Overall, the compound Chinese herbal extract in this study has multiple effects, including promoting growth, regulating fat metabolism, and alleviating fatty liver. Different ratios show functional specificity: the D1 focuses more on lipid-lowering, liver protection, and intestinal health, while the D2 excels in growth promotion. This difference is likely related to the addition level and synergistic effects of the components: a higher addition level (D2) more effectively activates growth regulatory pathways such as *igf1/tor*, while a lower addition level (D1) preferentially regulates lipid breakdown-related genes, reduces liver fat deposition, and enhances intestinal barrier function. The feed used in this experiment had a low fat content and a short culture cycle, which did not induce a high-fat model but may instead stimulate liver lipid synthesis and export pathways to enhance metabolic efficiency, supporting rapid growth and stress resistance needs

Conclusion

In conclusion, dietary supplementation with compound Chinese herbal extracts effectively enhances growth performance and alleviates hepatic lipid accumulation in hybrid grouper. The combined application of Oil Red O staining and automated image analysis provides a reliable and quantitative method for assessing lipid deposition. These findings support the potential application of compound herbal additives in sustainable aquaculture.

Author contribution: **Zhang Bo:** Experimental Setup; Investigation; Methodology; Writing-original draft **Lv Huiyuan:** Ration formulation; Supervision **Li Junwei:**

Conceptualization; Data curation; Investigation; Material preparation; Writing-review & editing **Li Ting:** Data collection; Statistical analysis; Writing-original draft **Lan Kunpeng:** Fish rearing; Investigation; Data collection **Jiang Liangzheng:** Fish rearing; Investigation; Validation. All authors read and approved the final manuscript.

Funding: This work was supported by the Basal Research Fund, Chinese Academy of Fishery Sciences (2023TD15).

Declarations

Competing interests: All the authors declare no relevant financial or non-financial interests.
Data availability statement: The datasets generated during and/or analysed during the current study are not publicly available.

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Tables

Table 1 Experimental Feed Raw Materials and Addition Ratios

Raw Materials	**Addition Ratio (%)**
Imported Fish Meal	32.83
Peanut Meal	5.63
Soybean Meal	18.39
Distillers Dried Grains with Solubles (DDGS)	2.81
Wheat Flour	16.89
Squid Meal	9.38
Fish Oil	4.69
$Ca(H_2PO_4)_2$	1.50
Phospholipid Oil	1.88
NaCl	0.28
Premix	1.88
Fermented Product	3.84

Table 2 Dosage of herbal additive formulations in different treatment groups

Groups	**Chinese herbal ingredient (mass ratio)**	**Additive content(‰)**
Dc	——	——
D1	*E. ulmoides*, *Paeoniae Radix Alba*, *G. uralensis* extracts (3:1:1)	2
D2	*E. ulmoides*, *Paeoniae Radix Alba*, *G. uralensis* extracts (3:1:1)	4
D3	*E. ulmoides*, *Paeoniae Radix Alba*, *G. uralensis*, *C. officinalis* extracts (3:1:1:1)	3

Table 3 Feeding and growth performance of grouper in different treatments

Index	Dc	D1	D2	D3
Weight / g	50.74±10.52	52.96±7.75	54.68±6.90	54.88±6.85
SR/%	98.00±1.90	100.00±0.00	96.67±0.72	95.00±5.72
FR/%	1.80±0.30	1.70±0.17	1.73±0.15	1.74±0.16
SGR/%·d^{-1}	1.47±0.40^{b}	1.56±0.24^{ab}	1.62±0.20^{a}	1.63±0.21^{a}
WR /%	142.80±50.33	153.39±37.10	161.62±33.03	162.58±32.78
CF /g·cm^{-3}	2.89±0.34^{b}	3.26±0.33^{a}	3.14±0.27^{ab}	3.22±0.50^{ab}
VSI /%	11.82±0.64	11.71±1.08	12.02±0.76	11.97±1.42
HSI /%	4.25±0.52	3.76±0.69	4.23±0.60	3.77±0.56
IPF	0.15±0.06	0.15±0.07	0.15±0.07	0.17±0.06

Note: Values with different superscript letters within the same index are significant ($P < 0.05$).

Table 4 Intestinal Villus Characteristics and Goblet Cell Abundance in Grouper under Different Treatments

Index	Dc	D1	D2	D3
Villus length/μm	332.70±32.42	391.43±20.69	389.68±10.59	322.26±57.95

Villus width/μm	82.73±8.59	86.72±4.09	77.91±9.31	77.13±11.25
Muscular thickness/μm	72.22±4.93	75.35±4.36	74.04±9.43	63.99±15.89
Number of goblet cells /per Villus	10.27±1.21^{b}	11.93±1.10^{a}	9.13±1.55^{b}	5.67±1.22^{c}

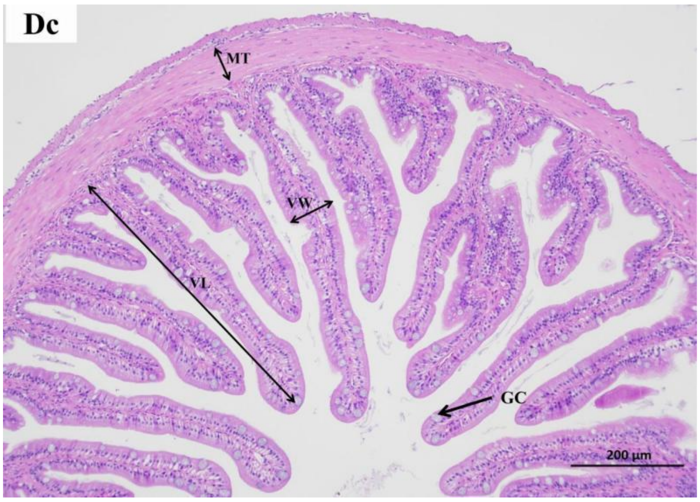
Note: Different superscript letters in the same row indicate significant differences ($P < 0.05$).

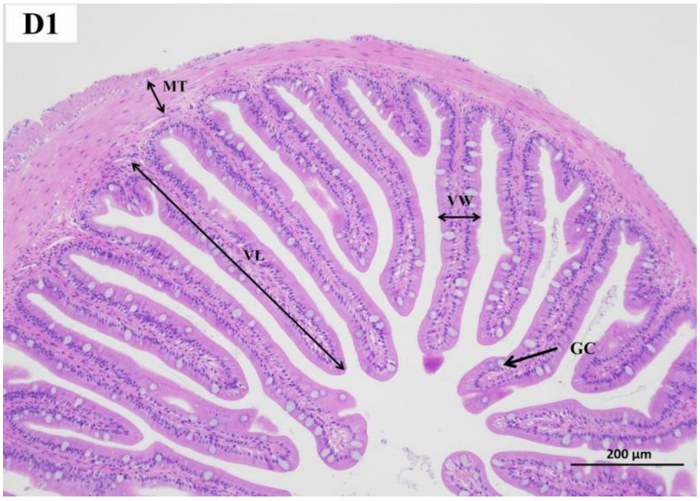
Table 5 Serum Lipid Indicators of Groupers in Different Groups

Index	Dc	D1	D2	D3
TC /mmol·L^{-1}	1.94±0.24^{b}	2.01±0.14^{b}	2.46±0.34^{a}	2.14±0.06^{ab}
TG /mmol·L^{-1}	1.21±0.06^{a}	0.92±0.05^{b}	1.28±0.24^{a}	1.11±0.15^{ab}
HDL /mmol·L^{-1}	1.26±0.25^{b}	1.20±0.06^{b}	1.66±0.20^{a}	1.34±0.07^{b}
LDL /mmol·L^{-1}	0.26±0.04^{b}	0.36±0.06^{a}	0.35±0.06^{a}	0.34±0.02^{a}

Note: Different superscript letters in the same row indicate significant differences ($P < 0.05$).

Figure captions





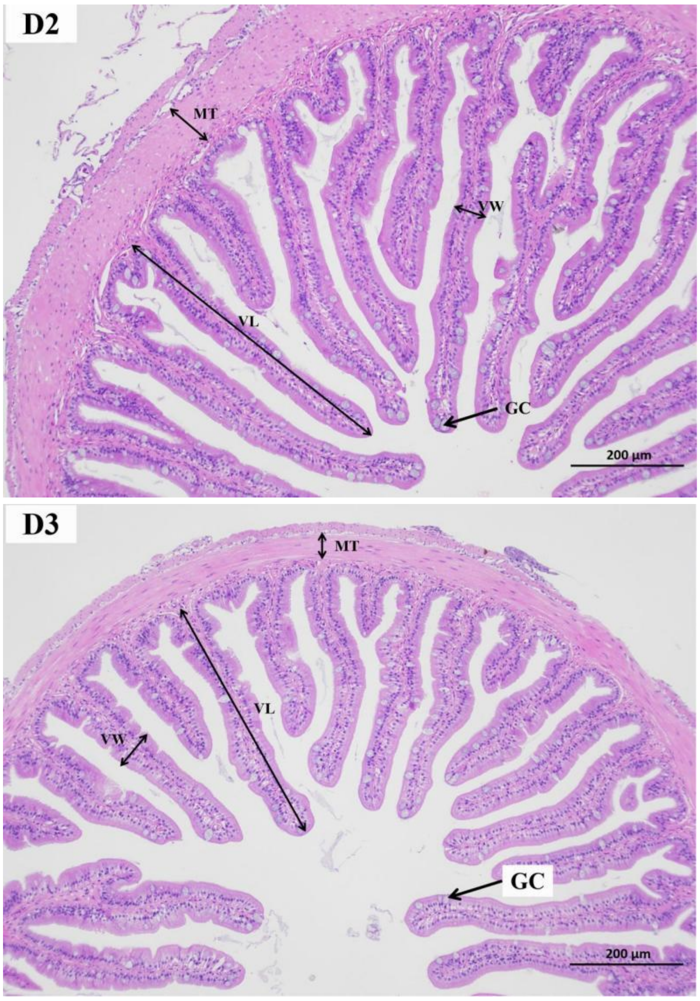
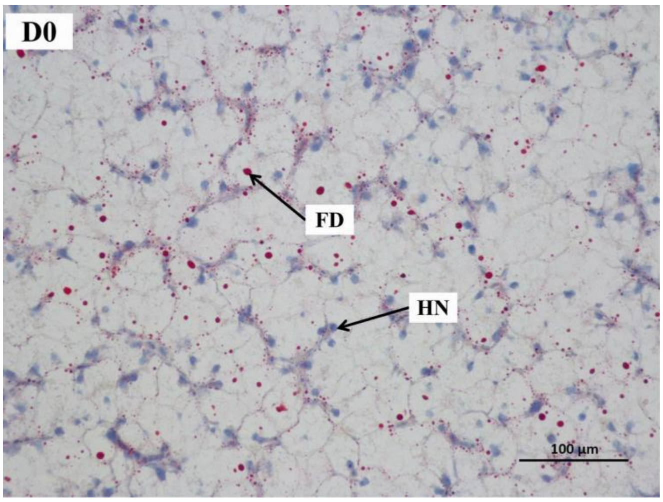
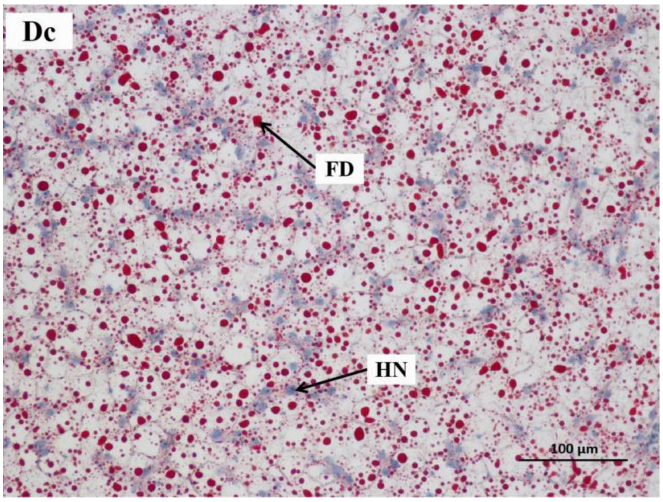


Fig.1 Intestinal Tissue Structure of Groupers in Different Groups

Dc, control group; D1, group 1; D2, group 2; D3, group 3.

GC， goblet cell; VL, villus length; VW, villus width; MT, muscularis thickness.

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FD
HN
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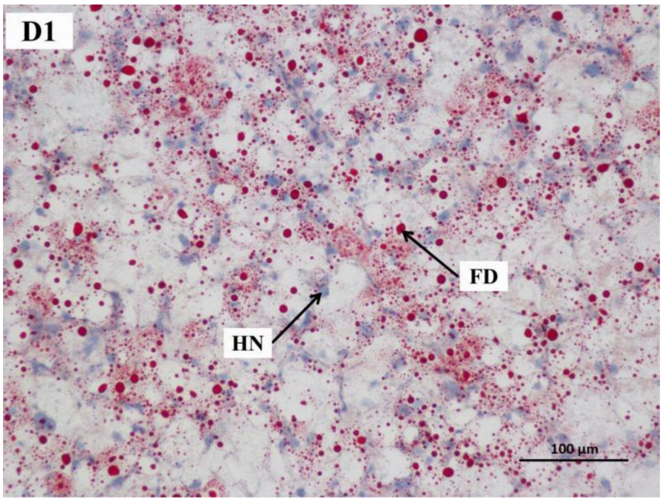
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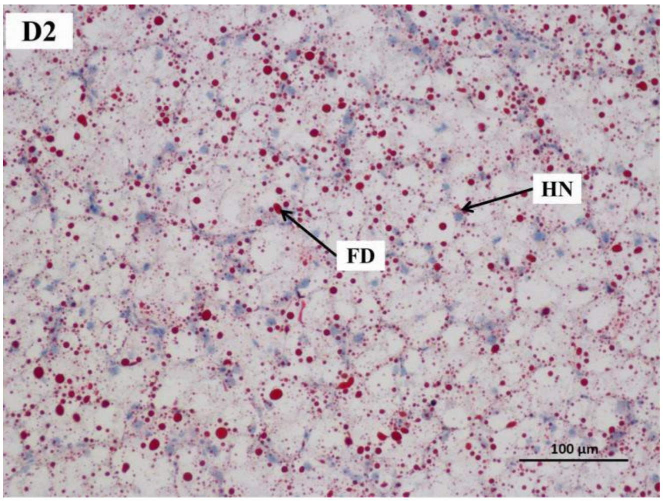
D2
HN
FD
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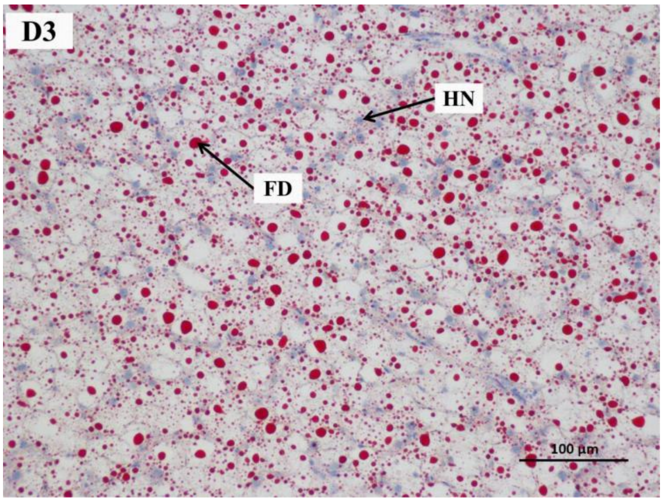

Fig.2 Hepatic Lipid-Positive Characteristics of Groupers in Different Groups

D0, The beginning of the experiment; Dc, Control group; D1, Group D1; D2, Group D2; D3, Group D3.

FD, fat deposition in the liver; HN, hepatocyte nuclei.

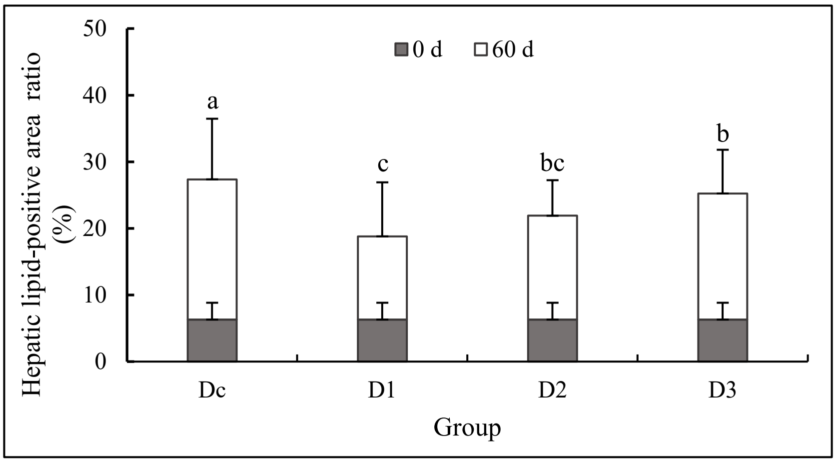

Fig. 3 Hepatic Lipid-Positive Area Ratio of Groupers in Different Groups