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Effects of antibiotics on the growth, antioxidant capacity, activity of digestive enzymes and serum biochemical indices, liver and intestine morphology of *Megalobrama amblycephala* fry

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Abstract
This study evaluated the effects of Oxytetracycline (OTC) and Sulfamethoxazole (SMZ) antibiotics on the growth, antioxidant capacity, activity of digestive enzymes and serum biochemical indices, liver and intestine morphology of *Megalobrama amblycephala* fry. Fish with average initial weights of 3.20 ± 0.09g were fed the control diet, SMZ diet, and OTC diet for 60 days. Compare to the control group, the growth of the Fish fed the antibiotic diet was significantly affected; the immunoglobulin level was significantly lower. Digestive enzymes activity was significantly higher in fish fed SMZ diet than those fed OTC diet. GSH activity was higher in both antibiotic groups, while OTC shows an elevated trend in MDA activity compared to the control group. Histomorphological analysis exhibited changes on liver and intestine tissues of the fish fed the antibiotic diets. These results show that both SMZ and OTC diet impair physiological functions of intestine and Liver and compromise fish immune system.

Keywords: Growth, antioxidant capacity, intestinal enzymes, serum biochemical indices, liver and intestine morphology, *M. amblycephala*

1. Introduction
In recent years, aquaculture has increased rapidly and has contributed to the global food supply. However, intensification of aquaculture has created conditions that favor the development of several numbers of aquaculture related diseases [1]; this has caused heavy losses to fish farmers [2, 3]. Therefore, to overcome these challenges, antibiotics have been used for the treatment and prevention of several of these diseases, and as well as improved growth performance [4, 5]. Generally, the misuse and overuse of antibiotics have been attributed to the development of antibiotic-resistant bacteria, bioaccumulation and environmental pollution in aquaculture production [2, 6]. According to previous studies, antibiotics are no longer considered only beneficial but also potentially harmful agents to fish. For instance, antibiotics were reported to cause microbiota dysfunction [7, 8, 9], bacterial resistance [7], pathogen colonization [8] immunosuppression [10], oxidative stress [9, 10, 11], and body tissues damage [9, 11]. Therefore, the prolonged administration of antibiotics could lead to the presence of residual antibiotics in fish tissue and fish product [12].
Oxytetracycline (OTC) and Sulfamethoxazole (SMZ) are respectively tetracycline and sulfonamide antibiotics mainly used as immune booster and growth promoters in fish farms. These antibiotics are broad-spectrum, cost-effective, and readily available [7, 13]. When administered, OTC and SMZ interfered with bacterial protein production, DNA replication, bacterial cellular metabolism and hindered bacteria from multiplying in the host cell [3]. However, OTC and SMZ antibiotics are commonly used in the treatment against bacterial and fungal diseases in fish [6, 14, 15]. These antibiotics are dose-dependent on fish species and its country-specific legal requirements [3, 9]. Despite these, the two antibiotics are used for long periods in aquaculture production. Previous studies from other literature documented the negative effect of these antibiotics on the immune system of fish [16, 17].

However, few studies elucidate the side effects on long-term administration on fish, which can induce nephrotoxicity, liver damage, and intestinal impairment [18].

Wuchang bream called Blunt snout bream (*Megalobrama amblycephala*) is an essential freshwater fish in China because of its flesh quality, rapid growth, and high larval survival rate [19]. However, this cultural practice has inevitably led to nutritional disease in this species, which correlates directly with a high rate of mortality and reduced growth [20]. The present study was conducted to estimate the impact of a long-term administration of OTC and SMZ on the growth performance, serum biochemical indices, digestive and antioxidant enzymes activities, liver and intestine histomorphology of Wuchang bream fry.

2. Materials and Methods

2.1 Animal ethics

The current study was conducted according to the Guidelines of the Institutional Animal Care and Use Committee (IACUC) of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences (Wuxi, China).

2.2 Experimental diets

The feed was formulated using different ingredients. The feed in the treatment group was formulated using antibiotic concentration, according to Limbu *et al.* [9]. The control group was given a normal feed with no added antibiotics. Diet formulation and proximate composition analysis were shown in Table 1.

Table 1: Formulation of the experimental diets (g kg^{-1})

Ingredient	Control	SMZ	OTC
Casein[a]	300.00	300.00	300.00
Fish meal[b]	150.00	150.00	150.00
Soybean oil[s]	60.00	60.00	60.00
Corn starch[c]	324.75	324.75	324.75
Vitamin premix[1]	20.00	20.00	20.00
Mineral premix[2]	20.00	20.00	20.00
Monocalcium phosphate[d]	20.00	20.00	20.00
Carboxy methyl cellulose [d]	30.00	30.00	30.00
Cellulose[e]	70.00	67.50	68.00
Choline chloride[e]	5.00	5.00	5.00
Butylated hydroxytoluene [e]	0.25	0.25	0.25
Sulfamethoxazole (SMZ) [f]	0.00	2.50	0.00
Oxytetracycline (OTC) [f]	0.00	0.00	2.00
Proximate compostition%			
moisture	8.69	8.61	8.63
Crude protein	32.42	32.40	32.44
Crude fat	6.12	6.22	6.11
Ash	7.55	7.51	7.53

a. Provided by Zhengchang Feed Industry Co., Ltd (Huaian, China).
b. Provided by Hulunbeier Sanyuan Milk Co., Ltd (Inner Mongolia, China).
c. Provided by Coland Feed Industry Co., Ltd., Wuhan, China
d. Purchased from Rousselot Gelatin Co., Ltd (Guangdong, China)
e. Provided by Shanghai Yuanye biotechnology co., Ltd, China.
f. Purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China)
   1. Vitamin premix (mg or IU kg-1): 500,000 I.U. (international units) Vitamin A, 50,000 I.U. Vitamin D3, 2500 mg Vitamin E, 1000 mg Vitamin K3, 5000 mg Vitamin B1, 5000 mg Vitamin B2, 5000 mg Vitamin B6, 5000 mg Vitamin B12, 25,000 mg Inositol, 10,000 mg Pantothenic acid, 100,000 mg Cholin, 25,000 mg Niacin, 1000 mg Folic acid, 250 mg Biotin, 10,000 mg Vitamin C.
   2. Mineral premix (g kg 1): 314.0 g CaCO3; 469.3 KH2PO4; 147.4 g MgSO4.7H2O; 49.8 g NaCl; 10.9 g Fe(II) gluconate; 3.12 g MnSO4.H2O; 4.67 g ZnSO4.7H2O; 0.62 g CuSO4.5H2O; 0.16 g KJ; 0.08 g CoCl2.6H2O; 0.06 g NH4 molybdate; 0.02 g NaSeO3

2.3 Experimental fish and feeding trial

A total of 270 healthy Wuchang breams fry were obtained from the breeding farm of the Freshwater Fisheries Research Center (FFRC) of the Chinese Academy of Fishery Sciences. Before the feeding trial, fish were acclimatized to experimental conditions for 2 weeks. After the acclimation period, fish with an initial weight of 3.20 ± 0.09g were randomly allocated into 9 tanks (filled with a water volume of 250 L /tank), and each tank held 30 fish. Then, fish were randomly assigned to one of three diets. Each treatment has three replicates. Fish were fed three times daily at 8:00, 12:00, and 17:00 h, respectively, for 60 days with feed consumption recorded. Fish were hand-fed to apparent satiation with utmost care to minimize feed waste. During the feeding trial, water parameters such as water temperature, pH, and dissolved oxygen were monitored using a YSI 556MPS multi-probe field meter (Geotech, USA). Water temperature ranged from 26 to 29 ^{0}C, pH fluctuated between 7.3 and 7.8, and dissolved oxygen was maintained approximately at 5.5-6.5 mg L^{-1}

2.4 Sample collection

After the feeding trial, fish were starved for 24 h to empty the content of the alimentary canal before sampling. Then, fish in each tank were counted and weighed, and four fish were randomly collected from each tank and euthanized with MS-222 (100 mg/L; tricaine methanesulfonate, Sigma, USA), and blood samples were immediately obtained from the caudal vein with disposable medical syringes and centrifuged at 3000 rpm, 10 min, and 4℃. The supernatants were collected and stored at -80 ℃ for subsequent analysis. Also, individual viscera, intestine, and liver were dissected to measure the biometric parameters. Also, the mid and distal intestine samples of three fish in each tank were sampled and placed in a 4% formaldehyde solution for the histological evaluation and later stored in -80^{0}C.

2.5 Fish growth performance and feed utilization

In this study, fish growth performances and feed utilization were carried out using the following formulas;

Weight gain (WG, %) = 100 × (final body weight (g) − initial body weight (g)) / initial body weight (g).

Specific growth rate (SGR, % day^{-1}) = 100 × (Ln final body weight (g) − Ln initial body weight (g) / number of days).

Survival rate (SR, %) = 100 × (number of fish at the end/number of fish at the start of the experiment).

Feed conversion ratio (FCR) = feed intake (g) / weight gain (g)

Hepatosomatic index (HSI, %) = 100 × liver weight (g) / bodyweight (g).

Visceralsomatic index (VSI, %) = 100 × viscera weight (g) / body weight (g)

Feed intake (FI, g $fish^{-1}$) = dry feed intake in each tank (g) / Number of fishes in the tank.

2.6 Measures for serum biochemical indices parameters

Serum glucose (GLU), Total protein (TP), Albumin (ALB), Total cholesterol (TC), Triglycerides (TG), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate transaminase (AST) and lactate dehydrogenase (LDH) were analyzed within 24 h of sampling using an automatic biochemical analyzer (Mindray BS-400; Mindray Medical International Ltd., Shenzhen, China) respectively. Immunoglobulin (IgM) content was assayed using the enzyme-linked immunosorbent assay (ELISA) designed specifically for fish according to the methods described by Abasubong *et al*. [21]. Cortisol (COR) in plasma was estimated with a validated radioimmunoassay from Winberg and Lepage [22] as modified by Li *et al*. [23]

2.7 Intestinal enzymes and Liver antioxidant capability assays

The whole intestine and liver were carefully weighed and homogenized in an ice bath with ten volumes (v/w) of chilled saline in a tissue homogenizer. The extract was later centrifuged (3500 rpm, 10 min, 4 °C) with the supernatant stored at -80 ^{0}C for subsequent analysis. The intestinal enzymes such as Amylase (AMS), Protease (PTS) and Lipase (LPS), and the Liver antioxidants including Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH-Px) activities, and Malondialdehyde (MDA) contents were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.8 Histomorphological analysis of the Liver and distal intestine

The histomorphological evaluation was done on the liver and distal intestine samples. The liver and intestine samples were fixed with 10% buffered formaldehyde (pH 7.2) and posteriorly embedded in paraffin. Sections of 5 µm, were obtained in a microtome Leica© RM-2155 (Leyca, Vienna, Austria), and were stained with hematoxylin-eosin for general histological observations for 24 hours. The measurements of the serous layer (SL), the muscular layer (ML), the submucous layer (SML), the villi length (VL), the villi diameter (VD), and the lamina propria (LP) were all performed according to the criteria reported by Martínez-Llorens *et al*. [24].

2.9 Statistical analyses

All data were presented and subjected to one-way analysis of variance (ANOVA) using the software of the SPSS (Ver 13.0; SPSS, Inc., Chicago, IL, USA) for Windows, after testing the homogeneity of variances with the Levene test. Duncan multiple tests were utilized to rank the means. Percentage data were arcsine transformed before the ANOVA and reversed afterwards [25]. All data are presented as the means ± SEM (standard errors of the mean) of three replicates. Mean differences were considered significant at a P-value equal to or less than 0.05.

3. Results

3.1 Growth parameters of Wuchang bream treated by antibiotics

As can be seen from Table 2, VSI, CF, HSI, and SR all showed no statistical difference (P>0.05) among all the treatments. However, FBW, WGR, and SGR were significantly lower (P< 0.05) in fish fed the antibiotics diets than those offered the control diet. FCR was significantly lower (P< 0.05) in fish fed OTC diet compared to those fed SMZ diet, but showed no difference (P>0.05) to those fed the control diet. Similarly, FI was higher (P<0.05) in fish fed SMZ diet compared to the OTC diet, but no statistical difference (P>0.05) was attributed to those fed the control diet.

Table 2: Growth performance, feed utilization and biometric parameters of Wuchang bream (*Megalobrama amblycephala*) fry subjected to different treatments [1]

	Control	SMZ	OTC
Initial weight (g)	3.01 ± 0.01	3.01±0.01	3.02 ± 0.01
Final weight (g)	18.02 ± 0.19^c	13.21 ± 0.58^a	16.09 ± 0.39^b
Specific growth rate(%/day)	2.91 ± 0.03^c	2.33 ± 0.06^a	2.77 ± 0.04^b
Weight gain rate (%)	474.29 ± 11.92^c	319.04 ± 15.97^a	426.21 ± 14.1^b
Feed Conversion ratio	2.10 ± 0.01^b	2.4 ± 0.07^a	2.10 ± 0.01^b
Feed intake (%/ day)	4.93 ± 0.05^b	4.90 ± 0.04^b	4.76 ± 0.02^a
Viscerosomatic index (%)	12.17 ± 0.46	11.27 ± 0.61	11.21 ± 0.50
Hepatosomatic index (%)	1.71 ± 0.07	1.80 ± 0.06	1.78 ± 0.09
Condition factor (%)	2.10 ± 0.06	2.09 ± 0.04	2.07 ± 0.03
Survival rate (%)	96.67± 0.00	96.67 ± 0.00	93.33 ± 0.00

Data are mean values ± SEM of three replicates. Means in the same row with different superscripts are significantly (P < 0.05) different

3.2 Serum biochemical indices

In Table 3, no noticeable difference (P>0.05) was observed in the GLU, ALT, TP, TC, ALP, and COR among all the treatments. IgM activity was significantly lower (P<0.05) in fish fed the antibiotic dietS compared to those fed the control diet. AST activity was significantly higher in fish fed the SMZ diet than those fed the OTC diet, whereas the opposite was true for Albumin activity compares to the control diet. TG was significantly higher (P<0.05) in fish fed OTC diet compare to the control, but no statistical difference (P>0.05) was observed with those fed the SMZ diet. Higher LDH content (P<0.05) was found in the fish fed SMZ diet compare to the control group, but no difference (P>0.05) was attributed to those fed OTC diet.

Table 3: Serum biochemical indices parameters Wuchang bream (*Megalobrama amblycephala*) fry subjected to different treatments [1]

	Control	SMZ	OTC
Alanine aminotransferase (U/L)	3.81 ± 0.87	5.33 ± 0.57	4.58 ± 0.75
Aspartate transaminase (mmol/L)	160.95 ± 18.80^{a}	250.98 ±11.16^{b}	163.93 ±13.16^{a}
Triglyceride (U/L)	2.54 ±0.10^{a}	2.76 ± 0.13^{ab}	3.16 ± 0.04^{b}
Total cholesterol (mmol/L)	8.60±0.11	8.47±0.3	8.33±0.18
Total protein (g/L)	26.05 ± 0.30	24.99 ± 0.67	25.93 ± 0.21
Albumin (g/L)	8.87 ± 0.21^{b}	7.98 ± 0.19^{a}	8.78 ± 0.16^{b}
Immunoglobulin M (gL^{-1})	1.46 ± 0.02^{b}	1.21 ± 0.06^{a}	1.24 ± 0.07^{a}
Alkaline phosphatase (U/L)	47.75 ± 2.41	47.12 ± 2.14	47.33 ± 1.61
Glucose (mmol/L)	25.87 ± 0.87	26.11 ± 0.83	24.20 ± 0.99
Lactate dehydrogenase(U L^{-1})	1121.5 ± 166.1^{a}	1537.33±60.06^{b}	1195.8 ± 81.05^{ab}
Cortisol (ng/mL)	588.48 ±7.41	605.44 ± 3.66	595.18 ±10.65

[1] Data are mean values ± SEM of three replicates. Means in the same row with different superscripts are significantly (P< 0.05) different.

3.3 Antioxidant activity

As shown in Table 4, CAT and SOD activity were not affected (P>0.05) by dietary treatment. However, fish fed the antibiotic diet were higher (P<0.05) in GSH-Px activity compare to the control. In contrast, fish fed OTC diet shows more elevated (P<0.05) trend in MDA activity compared to the lowest in the control group.

Table 4: Antioxidant capacities of Wuchang brea*m (Megalobrama amblycephala)* fry subjected to dietary treatments[1]

	Control	SMZ	OTC
CAT(U/mg)	151.44 ± 0.56	148.41 ± 9.81	151.31 ± 3.67
SOD(U/mg)	20.97 ± 0.37	22.12 ± 0.89	20.51 ± 0.72
GSH(U/mg)	296.4 ± 1.78^{a}	390.51 ± 19.88^{b}	391.93 ± 25.83^{b}
MDA (nmol/mg)	1.4 ± 0.06^{a}	1.85 ± 0.17^{b}	3.87 ± 0.05^{c}

[1] Data are mean values ± SEM of three replicates. Means in the same row with different superscripts are significantly (P < 0.05) different. CAT: Catalase; SOD: Superoxide dismutase; GSH: Glutathione Peroxidase; MAD: Malondialdehyde.

3.4 Digestive enzymes

As shown in Figure 1, AMS, PTS, and LPS activities were significantly higher (P<0.05) in fish fed SMZ diet than those fed OTC diet, but no statistical difference (P>0.05) were attributed to those fed the control diet.

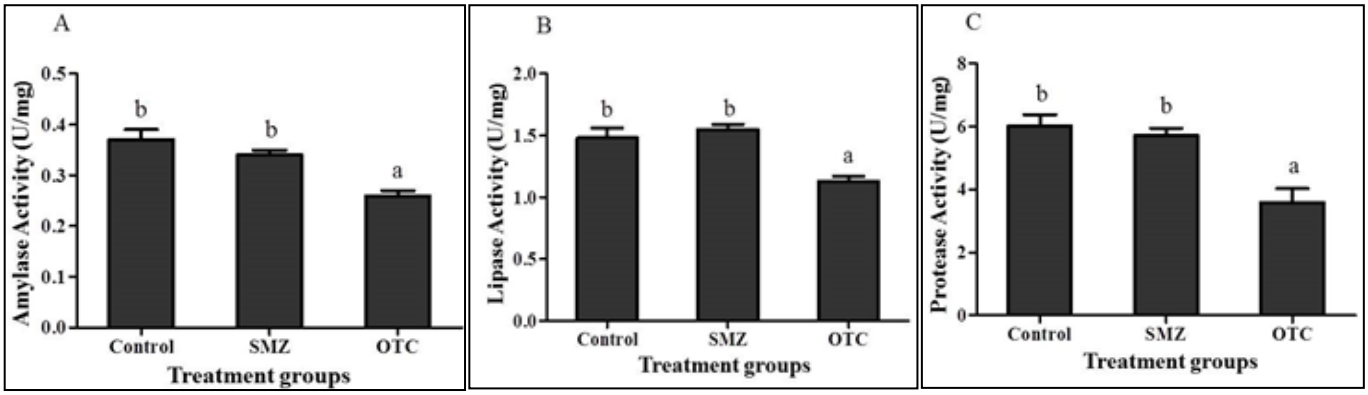

Fig 1: Digestive enzyme activities of blunt snout bream fed with different experimental diets. (A)Amylase activity, (B) Lipase activity, (C) Protease activity. Bars with different superscript letters are significantly different (P< 0.05). Data are mean values ± SEM of three replicates.

3.5 Tissue histomorphology of liver and the distal intestine

As shown in Figure. 2 and Table 5, no significant difference (P > 0.05) was observed in the thickness of SL, ML, SML, V, and LP among all the treatments. However, the distal intestine of fish fed on the control diet presented significantly higher villi length, villi diameter, goblet cells compared to fish fed on the rest of experimental diets. Concerning the liver, the control group shows normal hepatocytes containing a clear spherical nucleus and sinusoidal architectures (Fig 3A). While those fed SMZ and OTC diet had a severe fatty change (FC), cellular swelling (CS) and nuclei aggregation (NA) (Fig 3 B and C).

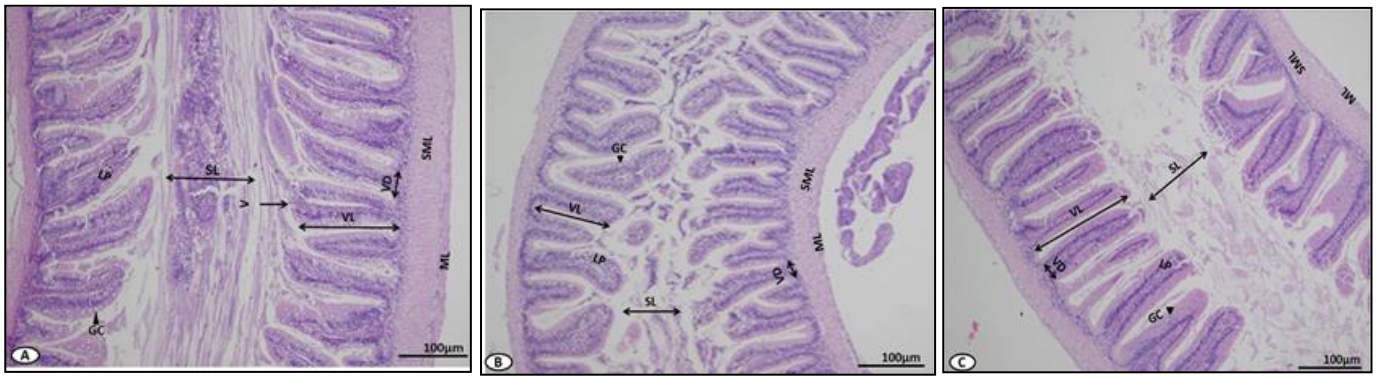

Fig 2: Longitudinal sections of the distal intestine of blunt snout bream fed different diets using hematoxylin-eosin stain (A) Control, (B) SMZ, (C) OTC. Villi (V), Serous layer thickness (SL), Muscular layer (ML), Submucous layer (SML), Villi length (VL), Villi diameter (VD), and Lamina propria (LP) as well as the location of Goblet cells (GC) (black up-pointing triangle). Scale bars = 100 µm

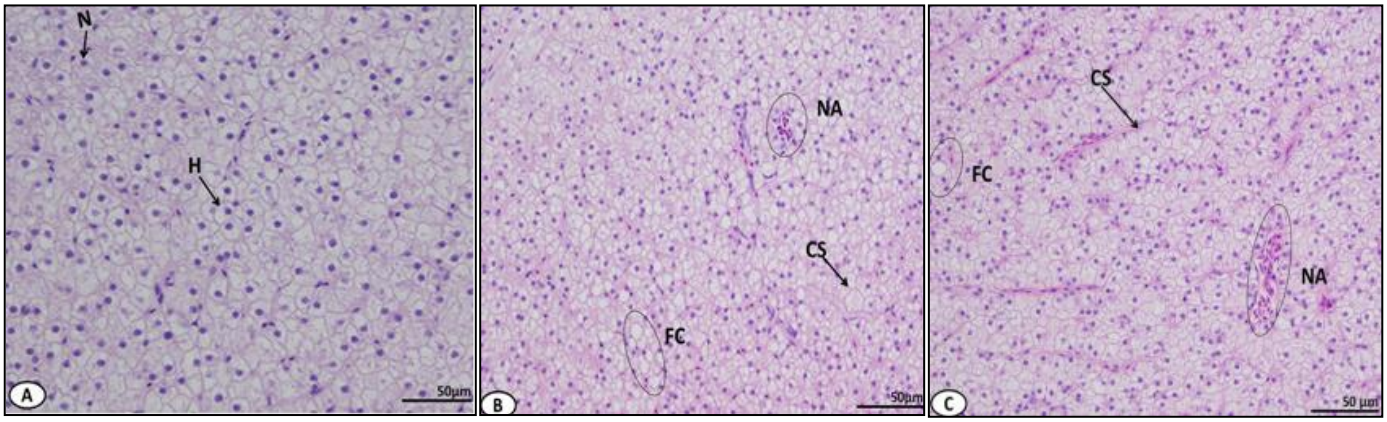

Fig 3: Section of fish liver showing the from Blunt Snout Bream (scale bar = 50 µm). (A) Control (B) SMZ (C) OTC diet. Hepatocyte (H), Nucleus (N) Fatty changes (FC), Cellular swelling (CS), Nuclei aggregate (NA)

Table 5: Measurements of liver and mid intestinal sections of Wuchang bream (*Megalobrama amblycephala*) fry fed various dietary treatments[1]

	Control	SMZ diet	OTC diet
Villi diameter (µm)	1.30 ± 0.04[b]	1.24 ± 0.03[a]	1.26 ± 0.2[a]
Villi length (µm)	0.15 ± 0.12[b]	0.12 ± 0.01[a]	0.13 ± 0.05[a]
Submucous layer thickness(µm)	80. 2 ± 0.21	77. 4 ± 0.33	78. 1 ± 0.23
Muscular layer thickness(µm)	53.6 ± 0.07	51.8 ± 0.11	52.9 ± 0.23
Serous layer thickness(µm)	44.14 ± 3.22	42.35 ± 2.31	41.82 ± 2.23
Goblet cells (Per 100 µm)	5.11 ± 0.20[b]	3.15 ± 011[a]	3.95 ± 0.62[a]
Lamina Propria thickness(µm)	17.76 ± 0.34	15.75 ± 0.21	16.28 ± 0.11

[1]Values are means ± SEM of three replications. Means in the same line with different letters are significantly ($P < 0.05$)

4. Discussion

Growth is the physical aspect that assesses the nutritional status of the fish. In this study, fish fed the antibiotic diets were significantly lower in FBW, SGR, and WGR compared to the control diet. This result suggests that there was as a nutritional impairment in fish fed antibiotic diets. These might be due to toxicological constituent present in antibiotics. Studies have shown that prolong administration of antibiotics could hinder some metabolic activities in animals, leading to poor growth performance. A similar result was also observed in Nile tilapia (*Oreochromis niloticus*) [9]. However, most studies have also reported a positive effect of antibiotics on growth performance. For instance, Koh *et al*. [6] reported that organic acids blend or oxytetracycline improved growth in Nile tilapia (*Oreochromis niloticus)* after 20 weeks of feeding. A similar trend was found by Sanchez-Martinez *et al*. [14] who studied the effect of supplementing channel catfish (*Ictalurus punctatus*) feeds with OTC, they observed that the treated fish exhibited a significant increase in Weight Gain compare to the control. However, the discrepancy in the result might be related to the duration of feeding, feeding strategy, species, feed composition, and fish size. FI was significantly enhanced in fish fed SMZ diet compare to the OTC diet, but no difference was attributed to the control, whereas the opposite was true for FCR. This result suggests that blunt snout bream could efficiently accept SMZ without palatability complications compared to those fed OTC diet. An enhanced feed intake might be due and improved digestive enzyme obtained in these studies. Interestingly, FCR was not improved in the SMZ diet as compared to the OTC diet and the control. These might be due to the disruption of gut microbiota by the action of SMZ antibiotic, which could inhibit the facilitation of nutrients metabolism despite a significant higher FI obtained.

Blood parameters are vital tools for the indication of physiological response as well as the general health condition of fish [26]. In this study, GLU, TC, TP, ALT, and ALP were not affected by dietary treatments. However, fish fed the antibiotic diet were significantly lower in IgM activity compared to the control, indicating that long-term administration of antibiotics could suppress immune functions in fish. This was supported by the fact that IgM is the first

antibody that is produced in the immune system, and it provides a crucial first line of defense [27]. An increase in IgM levels is usually thought to be associated with an enhanced innate immune response in fish [21]. A similar result was also observed in rainbow trout (*Oncorhynchus mykiss*) [28], and sea bream (*Sparus aurata*) [29]. However, the mechanism in which antibiotics suppress immunity remains unknown. ALB activity expectedly has a similar trend with that of IgM except for the OTC diet, indicating that SMZ could as well suppress ALB activity. In these studies, TG significantly increased in OTC diet compare to control, suggesting a disorder in lipoprotein, which might contribute to an explanation of the increased liver fat deposition of fish fed OTC diet [30]. From the results, AST elevates significantly in fish fed SMZ diet compare to the control, possibly indicating damage to membranes of hepatocytes from stress, toxicity, and lipid peroxidation produced by drugs and antibiotics. LDH activity was significantly higher in fish fed SMZ diet compare to the control indicating tissue and heart damage in this fish fed SMZ diet. This was supported by the fact that an elevated LDH in the blood cell is commonly regarded as a marker of injuries and disease such as heart failure [31], it is possible that an increase in LDH activity could lead to higher AST activity in these fish fed SMZ diet.

In vertebrates, the phagocytic process is followed by the production of reactive oxygen species, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-), all of which are highly microbicidal. The primary enzymes which detoxify reactive oxygen species are SOD, CAT, and GSH-Px, all of them abundant in fish tissues [32]. In the present study, CAT and SOD activities were not affected by dietary treatment. However, fish fed the antibiotic diet were higher in GSH-Px activity compare to the control, suggesting an index of oxidative stress in fish fed the antibiotic diet. The increase in GSH-Px might be caused by the utilization of non-enzymatic antioxidants to control the enormous number of free radicals. Our study also demonstrated that a higher MDA activity was observed in fish fed antibiotic compared to the lowest in the control group, indicating that OTC diet could indeed lead to the hepatopancreas oxidative stress of blunt snout bream, this was supported by the fact that tissue MDA levels provide direct evidence of lipid peroxidation caused by free radicals or disordered lipid metabolism [32]. We also observed a significant increase in TG content in fish fed antibiotics. It is possible that the increase in TG content in this study could be associated with higher MDA and GSH-Px in the present study.

Since fish growth is influenced by the digestive capacity [33], we analyzed the activities of enzymes involved in digestion in the intestine of blunt snout bream to better interpret the growth performance. The present study revealed that fish fed SMZ diet was significantly higher in AMS, PTS and LPS contents compare to those in the OTC group, but no statistical differences were attributed to those fed the control diet. This result indicates that fish fed SMZ diet could utilize carbohydrate, protein, and lipid without any adverse effect than those in the OTC group. This was supported by the fact that these enzymes are responsible for digesting carbohydrates, protein, and fat for proper metabolism [34]. Although the detailed mechanism in which SMZ improves the activities of digestive enzymes has been still unclear. However, fish fed OTC diet was significantly reduced in the digestive content, indicating that dietary OTC might result in the hampered intestinal functions of blunt snout bream. In this study, no difference was observed in SML, LP, ML, and SL among treatments. However, fish fed the control diet were significantly higher in VD and VL compare to the rest diets. The significant reduction in the antibiotic group might be responsible for the poor growth performance observed in this study. This was supported by the fact that villi length and diameter of intestine generally has a positive correlation with the intestinal digestive and absorptive capability [35, 36], which plays an essential role in the digestion and absorption of nutrients in fish [37]. This result might be due to one or more of the following reasons: elimination of gram-positive bacteria responsible for growth enhancement, inhibitions of nutrient transporters thus interfering with the metabolism of nutrients, as might consequently lead to a decrease in microvilli length and diameter [38]. Moreover, goblet cells were significantly enhanced in the control group compared to the antibiotic groups, suggesting that there was a mucosal barrier that serves as a lubricant and aid in the preservation of the intestinal epithelium. This was supported by the fact that goblet cells are an essential component in this barrier and constitute the majority of the immune system. The significant reduction in the goblet cell might be responsible for the decrease in the IgM activity, which could be linked with the reduced VL and VD in fish fed the antibiotic diets in this study, since intestine plays a crucial role in regulating the nutrition status of fish, as might lead to the modulations of the immune response in fish [37]. In this study, fish fed the antibiotics diets revealed specific nutritional deficiency symptoms, including severe fatty change, cellular swelling, and nuclei aggregation in the liver as compared to those fed the control diet. The histomorphological changes and impairment could be a result of deteriorative changes over a long period of feeding antibiotic diets. However, these pathological changes are significant symptoms of liver toxicity due to antibiotics feeding. Therefore, it is possible that these changes in the liver could be responsible for poor growth performance in the fish fed antibiotic diets since the liver is known as an accessory digestive organ and a good indicator of the nutritional condition of a fish [18]. These results further confirmed that prolong administration of antibiotics could cause inflammatory changes both in the liver and distal intestine. However, this needs further experimental investigations to explain these mechanisms.

5. Conclusion

In conclusion, the present study revealed that SMZ and OTC inclusion into a diet of blunt snout bream fry for 60 days could cause and adverse effects on growth performance, antioxidant capacities, and liver and intestine functions. These might be partly ascribed to the histopathological changes in the liver, and a significant reduction in intestinal VL and VD in these fish fed SMZ and OTC diets. It is pertinent therefore to consider these finding for the future development of diets specific for blunt snout bream under a variety of culture conditions and stages of growth from fry to fingerlings and on-growing to production (harvest) size to better confirm the effect of SMZ and OTC.

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7. Conflict of Interest

The authors declare they have no competing interests.

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