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Effect of Feeding-Intensity Stress on Biochemical and Hematological Indices of Gift Tilapia (*Oreochromis niloticus*)

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Abstract

The effects of feeding-intensity stress on biochemical indices of the GIFT strain of Nile tilapia were investigated in a recirculating freshwater system. Differences in serum and liver biochemistry, routine blood measurements, and growth were examined in fish fed three experimental rations (2%, 4% and 6% of fish biomass daily). Larger rations led to higher SGR but lower FCR, PER and SR. Smaller rations caused higher levels of HSI and cortisol. Fish on the 2% and 6% diets had lower liver levels of C3, LZM, SOD, Glu-GOD, HDL-C, TG, ALP, CAT and higher T4, AST, MDA than those on 4% diets. The 6% group had the lowest levels of LDH, TP, GSH, and GSH-PX. The 2% and 6% groups had lower blood levels of HGB, MCHC, RBC, and WBC than the 4% group; 2% fish had the highest RDW-CV and RDW-SD levels. Therefore, the optimal feeding rate for GIFT tilapia at about 29°C is 4% biomass/day; 6%/day could increase productivity but would be uneconomical; 2%/day would be unsuitable in the absence of natural food. We conclude that high and low feeding intensities would adversely influence the physiological health of tilapia and their resistance to disease, and reduce FCR.

Keywords: Nile tilapia, feeding intensity, growth, biochemical index, serum indicator.

Introduction

Feeding behavior is an important daily activity of cultured fish and has been the focus of many studies. The type of feed and feeding frequency has a direct effect on water quality, growth, fish welfare and responses of the immune system (Riche *et al.*, 2004; Silva *et al.*, 2007; Garcia and Villarroel, 2009; Luthada and Jerling, 2013). Stocking density and ration size also influence growth, survival, and the innate immune system (El-Sayed, 2002; Rowland *et al.*, 2006; Salas-Leiton *et al.*, 2010). Farming factors may cause stress and disturb fish homeostasis. At the same time, non-specific physiological responses may be evoked that cope with the disturbance and restore balance. If challenges to the resting state exceed the range of homeostatic mechanisms, farmed fish are likely to be unhealthy or troubled by disease (Barton and Iwama, 1991; Barton, 2002).

Corticosteroids are recognized as indicators of stress responses within the hypothalamic–pituitary–interrenal axis. They may reduce immune competence by reducing lymphocyte numbers and antibody-production (Barton and Iwama, 1991; Alcorn *et al.*, 2002). The corticosteroid cortisol is the hormonal stress indicator (Lazarus and Folkman, 1984; Ramsay *et al.*, 2006) that has been most studied in fish farming; e.g., in relation to stocking density (Barton *et al.*, 1980; Barcellos *et al.*, 1999; Montero *et al.*, 1999), feeding behavior (Gregory and Wood, 1999; Martins *et al.*, 2011), temperature (Strange, 1980; Alcorn and Murray, 2002; Atwood *et al.*, 2003; Delaney *et al.*, 2005; Davis and Peterson, 2006), salinity, and pH (Avella *et al.*, 1991).

Stress impairs immune function and disease resistance of fish. Temperature changes, handling, crowding and feeding frequency have inhibitory or stimulatory effects on innate immune parameters and may act synergistically (Wise and Johnson, 1998; Jaso-Friedmann *et al.*, 2000; Ortuno *et al.*, 2001; Alcorn and Murray, 2002; Magnadóttir, 2006; Salinas *et al.*, 2006). Complement is an important component of fish innate immunity; it assists antibody function, participates in inflammatory reactions, plays a role in host defense (Holland and Lambris, 2002) and its appearance is an indicator of disease resistance (Cai *et al.*, 2004). High rearing densities are associated with elevated serum cortisol concentrations and suppression of serum IgM concentrations, followed by high mortality (Iguchi *et al.*, 2003). Cold-stress

induces changes in serum levels of IgM, leukocyte count (WBC), phagocytosis, thyroxine (T_4) and catecholamines in tilapia (Chen *et al.*, 2002). Transferring tilapia from 27°C to lower temperatures (19 and 23°C), or to higher temperatures (31 and 35°C), reduces their immune capability, and involve changes to WBC, lysozyme (LZM) and the phagocytic index (Ndong *et al.*, 2007). Plasma LZM level is not correlated with sex, parental origin, rearing length, weight, condition factor or rearing density (Caruso and Lazard, 1999) but handling, transport and toxic water change the levels of LZM (Möck and Peters, 1990; Wu *et al.*, 2007). Hypoxia, viruses and salinity stress change the phenoloxidase (PO) activity of shrimps (Le Moullac *et al.*, 1998; Cheng and Chen, 2002; Li *et al.*, 2010; Salas-Leiton *et al*., 2010). Serum concentration of thyroxine (T4) and PO can be altered by cold-stress (Chen and Sun, 2002). Superoxide dismutase (SOD) has also been used to evaluate the immunological response of fish exposed to oxidative stress. SOD inhibits lipoperoxide formation and relieves or protects against damage caused by oxygen free radicals produced by water contaminants (Almeida *et al.*, 2002; Arican and Kurutas, 2008).

The levels of nutrients and digestive enzymes in serum can indicate the health status of farmed fish. Glutamic-pyruvic transaminase (GPT), alanine transaminase (ALT), glutamic-oxaloacetic transaminase (Duston *et al.*, 2004), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and glucose oxidase (Glu-GOD) have all been used as parameters of disease and health status (Wacker *et al.*, 1956; Abdel-Tawwab *et al.*, 2010; Noor El–Deen *et al.*, 2010). Total protein (albumin and globulin) (TP) and total cholesterol (TC) levels are also good indicators of fish health (Yang and Chen, 2003; Yoneyama *et al.*, 2009). Low density lipoprotein (LDL, the so-called 'bad lipoprotein') and the counteracting high density lipoprotein (HDL, 'good lipoprotein) are present in tilapia, as in mammals. Tilapia have been used as model animals to prescreen anti-atherosclerosis products (Chen and Pan, 2007).

Routine examination of blood parameters, including hemoglobin (HGB), erythrocyte count (RBC), leukocyte count (WBC), hematocrit and cytochemical staining, have been used in earlier studies to assess fish health status and circumstantial stress (Hesser, 1960; Blaxhall, 1972; Blaxhall and Daisley, 1973; Casillas and Smith, 1977). Liver function reflects oxidative stress and heavy metal pollution; accordingly, malondialdehyde (MDA), SOD, glutathione (GSH) and glutathione peroxidase (GSH-PX) are important functional factors (Jos *et al.*, 2005; Farombi *et al.*, 2007) that have been extensively studied and used to evaluate environmental stress when fish are used as pollution indicators. Previous studies on the feeding levels have mainly evaluated growth, feeding efficiency (El-Sayed, 2002) and feeding rate (Silva and Gomes, 2007; Luthada and Jerling, 2013) but there is little information on the effects of feeding intensity on the health of tilapia. In this study we investigated the effects of different feeding intensities on physiological and biochemical parameters in the blood, serum and liver to provide guidelines for tilapia farming and management.

Materials and Methods

Experimental Fish

Fish were raised from eggs collected in June 2013 from the mouth of a female GIFT Nile tilapia mated with a male in a breeding hapa at the Tilapia Breeding Center, Freshwater Fisheries Research Center (FFRC), Wuxi, China. The eggs were hatched at 28°C in the hatchery. After 1 week, approximately 1600 larvae were obtained and cultured for 1 month to the juvenile stage under the same conditions in the hapa. Mean fish body weight was 20.81±3.84 (SE) g.

Feeding-Intensity Stress

Experiments were conducted in a recirculating purification system (Qingdao Zhongke Water Treatment Co., Ltd). Twelve tanks (1 m^3, 750 L aerated tap-water) were used for the experiment with three treatment factors × four replicates. Each tank was randomly stocked with 27 individuals. Daily diets were rationed at 2% (low treatment), 4% (moderate treatment) and 6% (high treatment) of the fish biomass respectively, and adjusted for growth. Fish were fed three times daily, at $8:^{00}$ (40% of daily feed), $12:^{00}$ (30% of daily feed) and $16:^{00}$ (30% of daily feed), on an expanded pelleted feed (28% crude protein, 7% crude lipids, ~3-mm diameter pellets) commonly used in freshwater fish farming in eastern China. During the experiments, unionized ammonia and nitrite were undetectable, dissolved oxygen was >8.2 mg/L, pH was maintained at 7.5–7.6, and temperature was 29.2±0.3°C. Photoperiod was controlled on a 12-h light/dark cycle. The trial system was kept free from disturbance including noise and one third of the volume of water was exchanged with aerated tap-water daily. All pellets offered were consumed within 20 minutes after feeding. The quantities of feed supplied to each tank were recorded daily until the end of the trial, which lasted for 50 days.

Sampling and Measurement of Growth Performance

After the period of experimental feeding-intensity stress, all fish were starved for 1 day prior to sampling. Ten fish were randomly selected from each tank and were immediately exposed to tricaine methanesulfonate (MS-222, 200 mg/l) for deep anesthesia. The fish were quickly weighed, their

abdominal cavities were opened, and the internal organs removed. Each liver was weighed and samples of ~0.1 g were preserved in liquid nitrogen for liver function measurements. The total weight of each fish in each tank was also measured. Blood was sampled from the caudal vein of six fish in each tank. Each blood sample fish was divided into two portions. One portion was held as whole blood in heparinized tubes for hematological tests; the other portion was kept at 4°C overnight and centrifuged at 1006.2 × g for 10 min to separate serum for biochemical analysis (preserved in liquid nitrogen). Individual body weights, liver weights and blood-sample data were recorded for each of the treatments and tanks. The parameters of growth performance were calculated as:

Specific growth rates (SGR, % / d) = $100 \times (\ln W_t - \ln W_0) / t$;
Weight gains (WG, g / fish) = $W_t - W_0$;
Feed conversion ratios (FCR) = $(\sum f_k) / (W_t - W_0)$;
Protein efficiency ratio (PER, %) = $(W_t - W_0) / P \times 100$;
Survival rates (SR, %) = $100 \times (N_0 - N_t) / N_0$;
Hepatosomatic index (HSI, %) = $100 \times W_h / W_b$;

where t is the number of feeding days, W_0 id the initial live weight of fish (g), W_t is the final live weight of fish (g), f_k is the weight of feed consumed by the fish at each feeding (g), P is the protein contain of f_k (g), N_0 is the initial number fish, N_t is the final number fish, W_h is the live weight of the liver, and W_b is the live weight of the body.

Serum Biochemistry Examination

Cortisol was measured using an electrochemical luminescence immunity analyzer (Roche model E170, F. Hoffmann-La Roche AG, Basel, Switzerland). Elisa Kits were purchased from Shanghai Langton Biotechnology Co., Ltd., Shanghai, China. Determinations of C3, IgM, LZM, PO, SOD, T4, Glu-GOD, HDL-C, LDL-C, TC, TG, ALP, ALT, AST, LDH and TP were made using a Roche automatic biochemical analyzer using kits purchased from Shanghai Jun Biotechnology Co. Ltd., Shanghai, China.

Hematological BR Tests

The HCT, MCV, RBC, RDW-CV, RDW-SD and WBC were determined by the Coulter principle using an automatic blood cell analyzer (BC-3000PLUS, Mindray Medical International Ltd., Shenzhen, China); HGB, MCH and MCHC were determined according to Bouguer–Lambert–Beer law using the BC-3000PLUS blood cell analyzer.

Liver Function Examination

Levels of MDA, SOD, CAT, GSH and GSH-PX were determined using a microplate spectrophotometer (Eon, BioTek Instruments Inc., Winooski, VT). Elisa Kits were purchased from Shanghai Jun Biotechnology Co. Ltd.

Data Analysis

The data obtained for this trial were statistically analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Levels of the same indicator among different treatments were analyzed by one-way ANOVA. Means were regarded as significantly different when $P<0.05$. A three-factor post-ANOVA comparison of multiple means was carried out to estimate differences among treatments. Values in graphs, tables and text in this article are presented as means ± the standard of the mean (SE).

Results

Growth Performance

Growth performance data for Nile tilapia are presented in Table 1. Tilapia that received higher feed rations had significantly higher weights than those on lower rations ($P<0.05$). SGR and liver weights showed the same statistical trends among treatments as the weights. There were significant differences between treatments in HSI, FCR, PER and SR ($P<0.05$). The HSI was not significantly different between moderate and high treatments ($P>0.05$) but was higher in lowest feed treatment ($P<0.05$). FCR, PER and SR were not significantly different between low and moderate treatments ($P>0.05$) but were significantly different in the high treatment ($P<0.05$); FCR was highest in the high feed treatment while SR and PER were both lowest in the high feed treatment.

Feeding-Intensity Stress and Serum Biochemistry

Data on feeding-intensity stress and serum biochemistry of GIFT tilapia are presented in Figure 1A, B and C. There were no significant differences among the treatments in IgM, PO, LDL-C, or TC ($P>0.05$). Cortisol in the low treatment was higher than in the other two. C_3, LZM, SOD, Glu-GOD, HDL-C, TG and ALP were all significantly different among treatments ($P<0.05$). In each case, the level was highest in the moderate treatment.

T_4 and AST were significantly different among treatments ($P<0.05$); both were lowest in the moderate treatment.

LDH and TP showed similar trends; significantly lower values were observed in the high treatment ($P<0.05$); there were no differences between low and moderate treatments. ALT was significantly different among treatments with the level in the high treatment significantly higher than in the low treatment ($P<0.05$).

Table 1. Comparison of growth performance at three feeding intensities. Daily diets were rationed at low feeding intensity (2% of the fish biomass), moderate feeding intensity (4% of the fish biomass) or high feeding intensity (6% of the fish biomass)

Growth performance indicators	Low feeding intensity (2% of the fish biomass)	Moderate feeding intensity (4% of the fish biomass)	High feeding intensity (6% of the fish biomass)
Initial weight (g)	21.84±3.73	20.30±3.04	20.3±3.13
Final weight (g)	108.32^{a}±9.34	169.76^{b}±9.07	232.24^{c}±7.41
SGR (%/d)	2.49^{a}±0.23	3.66^{b}±0.24	4.05^{c}±0.26
Liver weight (g)	2.25^{a}±0.67	3.01^{b}±0.50	4.31^{c}±0.84
HSI (%)	3.04^{a}±0.10	2.26^{b}±0.08	2.56^{b}±0.11
FCR	1.62^{a}±0.11	1.58^{a}±0. 09	2.03^{b}±0.13
PER (%)	2.02^{a}±0.04	2.07^{a}±0. 07	1.64^{b}±0.11
SR (%)	98.83^{a}±1.21	99.24^{a}±0.93	95.36^{b}±1.89

Note: Data are means ± SE (n=40). Different alphabetical superscripts indicate significant differences among treatments (P<0.05).

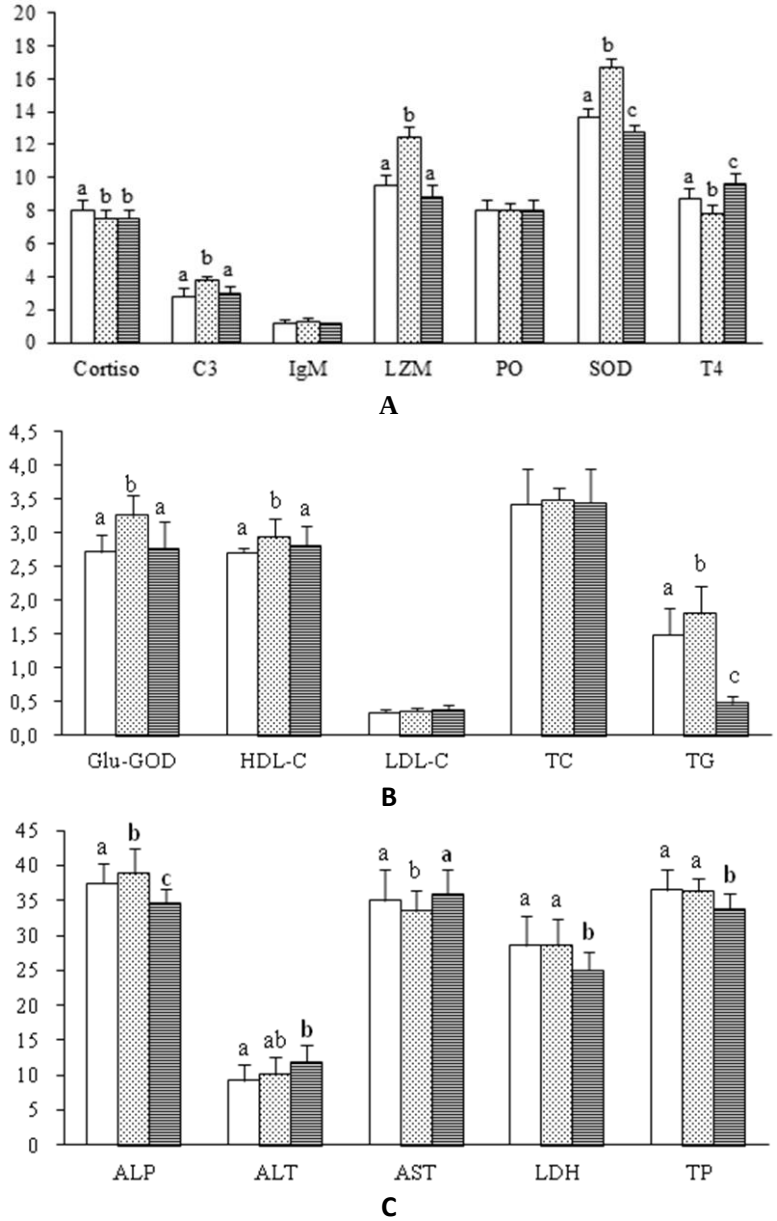

Figure 1. Effects of three feeding intensities on levels of serum biochemical parameters of GIFT tilapia. . daily diets of 2% of the fish biomass, daily diets of 4% of the fish biomass, daily diets of 6% of the fish biomass. Data represent means ± SE (n=24). Different alphabetical superscripts within each treatment group are significantly different at P<0.05.

Feeding-Intensity Stress and Hematological Parameters

Data on feeding-intensity stress and routine blood parameters are presented in Table 2. There were significant differences among treatments ($P<0.05$) in all indicators except for hematocrit (HCT). RBC and WBC showed similar trends; the moderate treatments had the highest values, followed by the low treatment, and then the high treatment. HGB in the moderate treatment was higher than in the other two treatments. Mean corpuscular hemoglobin (MCV) was highest in the high treatment. The mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were highest in the moderate treatments. Red cell distribution width CV (RDW-CV) and red cell distribution width SD (RDW-SD) showed similar trends; values were highest in the low treatment.

Feeding-Intensity Stress and Liver Function

Data on the effects of feeding-intensity stress on indexes of liver function are presented in Figure 2. Significant differences ($P<0.05$) existed among treatments for MDA, SOD, CAT, GSH and GSH-PX. In the moderate treatment of MDA levels was lowest but SOD and CAT were highest. Levels of GSH and GSH-PX were lowest in the high treatment than in the other treatments.

Discussion

Feeding Strategy

In this study, high feeding intensity had a negative influence on SR, FCR and PER. Excess feeding of farmed tilapia might be associated with poor digestion and nutrient absorption while inadequate feeding would lead to poor nutrition and could induce liver tissue abnormalities. Clearly, hunger caused greater stress than overeating did, as indicated by the levels of serum cortisol. Therefore, the 2% feeding intensity is likely to be unsuitable for farming of GIFT tilapia because of fish health considerations, and the 6% level could be uneconomical. Many studies have explored strategies of fish farming for optimal production. Increasing feeding frequency appears to confer a higher resistance to disease in Nile tilapia (Garcia and Villarroel, 2009). The optimal frequency and rate of feeding of *Oreochromis mossambicus* fry were found to be four times daily at a rate of 15% body weight/day (Luthada and Jerling, 2013). Riche and Haley (2004) suggested that 4-h feeding intervals should increase the production efficiency of Nile tilapia. The best feeding strategy for tambaqui during the first growth phase in cages was found to be 10% body weight/day (Silva and Gomes, 2007). The optimum stocking density and feeding level of Nile tilapia fry were reported to be 5 fry/L and 30% (crude protein) daily (El-Sayed, 2002). The optimum commercial stocking density, for survival, fast growth and high production rates of silver perch were examined by Rowland *et al*. (2006).

Feeding Intensity and Serum Biochemistry Indicators

Feeding-intensity stresses did not alter homeostatic state of tilapia, as indicated by the unchanged innate immune and physiological parameters of IgM, PO, LDL-C and TC. However, in tilapia experiencing low and high feeding intensities, the changes with respect to serum levels of C3, LZM, SOD, Glu-GOD, HDL-C, TG, ALP, ALT, AST, LDH and TP indicated that they were under high physiological stress. Such fish could easily contract diseases and would have low resistance to environmental change. The fish at high feeding intensity experienced greater stress than those fed at low intensity, as indicated by the levels of SOD, T4, TG, ALP, ALT, LDH and TP, and their survival.

Our data are consistent with other studies that demonstrated that feeding parameters influence productivity and are associated with physiological

Table 2. Effects of feeding-intensity stress on routine blood indicators. Daily diets were rationed at low feeding intensity (2% of the fish biomass), moderate feeding intensity (4% of the fish biomass) or high feeding intensity (6% of the fish biomass)

Blood parameter	Low feeding intensity (2% of the fish biomass)	Moderate feeding intensity (4% of the fish biomass)	High feeding intensity (6% of the fish biomass)
HCT	36.84±3.12	36.94±2.41	35.83±5.02
HGB	100.11^{a}±10.02	120.78^{b}±14.45	100.77^{a}±11.02
MCV	204.80^{a} ±11.10	205.97^{a}±11.05	218.49^{b}±13.00
MCH	56.24^{a}±4.50	62.99^{b}±6.30	61.84^{b}±5.83
MCHC	271.77^{a}±17.73	311.11^{b}±14.50	282.88^{c}±20.86
RBC	1.76^{a}±0.08	1.90^{b}±0.15	1.64^{c}±0.21
RDW-CV	20.87^{a}±2.37	17.51^{b}±2.11	16.26^{b}±6.24
RDW-SD	174.39^{a}±14.79	141.86^{b}±24.33	141.94^{b}±44.21
WBC	200.21^{a}±16.01	231.99^{b}±40.92	194.16^{c}±17.32

Note: Data are means ± SE (n=24). Different alphabetical superscripts indicate significant differences among treatments ($P<0.05$).

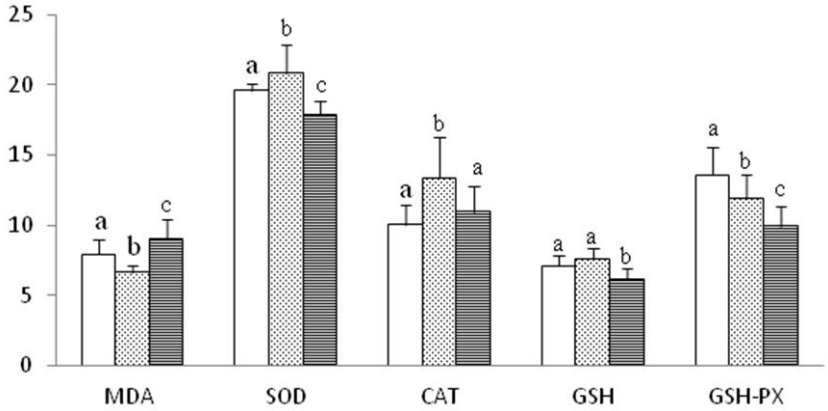

Figure 2. Effects of feeding intensity-stress on indices of liver function of GIFT tilapia, ☐ daily diets of 2% of the fish biomass, ⁙ daily diets of 4% of the fish biomass, ▤ daily diets of 6% of the fish biomass. Data represent means ± SE (n=40). Different alphabetical superscripts indicate significant differences among treatments (P<0.05).

stresses in farmed fish, particularly if they are not optimized for particular species of fish (Avella and Schreck, 1991; Barcellos and Nicolaiewsky, 1999; Davis and Peterson, 2006; Martins and Conceição, 2011).

Feeding Intensity and Routine Blood Analysis

Hematological techniques are regularly used for assessment of the health of fish and for diagnosis of disease and other conditions (Blaxhall, 1972; Blaxhall and Daisley, 1973). In addition, the parameters of health criterion cannot impede assessing the experiment conclusion (Casillas and Smith, 1977; Cnaani *et al.*, 2004). In the present study, fish in the low and high feeding intensity treatments had low erythrocyte counts and inflamed bodies. Presumably, the oxygen transporting capacity of their blood was compromised. The WBC values also indicated that the fish high at feeding intensity experienced higher stress than those at low intensity.

Feeding Intensity and Liver Function Parameters

There have been relatively few studies on the hepatology of fish but changes in liver function can be inferred from knowledge gained from rodents and humans (Brusle and Anadon, 1996; Cnaani *et al.*, 2004). The levels of liver function parameters indicate that fish in the low and high feeding-intensity treatments experienced more stress to the physiological functions of their livers than did fish at moderate feeding intensity. The high feeding-intensity fish appeared to be the most stressed. This conclusion is consistent with information obtained from serum biochemistry and hematological parameters. Interestingly, GSH and GSH-PX were also clearly related to stress but, in this case, the moderate feeding-intensity fish showed more stress than the low-intensity fish. These findings deserve further study.

In conclusion, the optimal feeding intensity is about 4% of tilapia biomass daily at the water temperature of about 29.2°C. Although a higher feeding intensity could increase the productivity of tilapia per unit, there could be greater farming risks and possibly inferior FCR. Low feeding intensities are typically used in semi-intensive farming but where the quantity of natural food is inadequate, pond-cultured tilapia would have poor production and sub-optimal health status.

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