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RESEARCH PAPER

The Effect of Green Tea Waste on Growth and Health of Grass Carp (*Ctenopharyngodon idellus*)

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Abstract

To investigate the effects of green tea waste on growth and health of grass carp (*Ctenopharyngodon idellus*), Green tea waste (GTW) and green tea (GT) were added in the basal diet (BD) of fish by replacing wheat at an inclusion level of 5% to form three diets. 60 grass carp (43.8±2.8 g) were divided into three groups (each group having two replicates) and fed for 66 days. After which growth and health of fish were determined. The results showed that final weight of fish and feed efficiency ratio in GTW group were between BD and GT groups and they were not different compared with BD or with GT groups. Expression of muscle myf5 and myf6 in GTW were between BD and GT group, being significantly lower than BD group. Serum TP, ALB, GLO, HDL, LDL and T-AOC in GTW group were significantly higher than BD group. Expression of hsp 70 and ubiquitin in hepatopancreas of fish in GTW group were significantly higher than BD group. The results suggest that GTW supplementation has positive impact on health of fish without affecting the growth of fish.

Keywords: Green tea waste, growth related genes, serum biochemical indices, nonspecific immunity capacity, grass carp *(Ctenopharyngodon idellus)*.

Introduction

Green tea (*Camellia sinensis*) is one of the herbs traditionally used to make tea-style beverages in Asia. The major active ingredients in green tea are polyphenolic compounds known as catechins (Balentine *et al.*, 1997). These compounds are reported to have high antioxidant activity in humans (Meng *et al.*, 2008; Kaushik *et al.*, 2011), rabbit (Eid *et al.*, 2010) and in rainbow trout (Thawonsuwan *et al.*, 2010). These compounds were reported to stimulate fat oxidation in obese Thais (Auvichayapat *et al.*, 2008) and decrease the immune potency of fish species (Thawonsuwan *et al.*, 2010).

Green tea waste (GTW) is obtained through the production of green tea in beverage factories and is disposed of as compost or incinerated by an industrial waste disposal contractor, which causes both an economical and environmental problem. In the commercial industry the green tea waste and by-products that remain after processing still contain large amounts of protein (20-80 % CP), carbohydrates and phenolic compounds (Cai *et al.*,, 2001; Tsubaki *et al.*,, 2008, 2010; An *et al.*,, 2011; Toh *et al.*,, 2010). Several previous studies had suggested that green tea waste could be used as potential source of natural anti-oxidants or functional nutrients in animal feed; broiler and laying hens (Jung 2001; Yang *et al.*, 2003a), goats (Kondo *et al.*, 2004a), sheep (Xu *et al.*, 2003, 2004), pig (Ko *et al.*, 2008), cattle (Nishida *et al.*, 2006), lactating cow (Kondo *et al.*, 2004b), broiler chicks (Yang *et al.*, 2003b), mouse and chicken (Lee *et al.*, 2012) and even kids (Saikia *et al.*, 2005).

Materials and Methods

Diet Preparation

Green tea (GT), being purchased in a supermarket of Yangling (Shaanxi, China) was soaked in boiling water for three times and then dried in 70 ^{o}C to get the residue (green tea waste, GTW) . The GT and GTW were ground into powder and added into the basal diet replacing 5 % wheat flour respectively, then the feed mixture were manufactured by pellet mill to get three diets. The basal diet and GT diet were both the control. Formulation and proximate composition of the 3 diets are presented in Table 1.

Experimental Fish and Feeding

A total of 60 juvenile grass carp (43.0±2.8 g), purchased from Xing Pin fish farm (Xian Yang, Shaanxi, China) were randomly divided into three groups (replicate per group) in 6 fiberglass tanks, each containing 180 L water.

The fish were respectively fed three diets described above for 66 days. The fish were hand fed to satiation four times a day (8:30, 11:30, 14:30 and 17:30). Each 20-23 days fish in each tank was weighed. The water in each tank were aerated 24 h each day during the whole feeding experiment and water temperature and dissolved oxygen were 18-23 ºC and 8-10 mg/L respectively.

Growth Performance, Feed Utilization and Biological Parameters of Grass Carp

At the termination of the experiment, the fish were sedated in a water containing 0.1 g L^{-1} MS222 (metacain), and weight and length of each fish and the feed intake in each group were determined to calculate growth related indices and feed utilization by the following equations. Meanwhile viscerosomatic, hepatopancreas, and muscle weights and the length of intestine were obtained to calculate the biological indices by the following computational formula.

Feed efficiency ratio, FER,(%)=(Final weight of fish - Initial weight of fish)/Feed intake×100%;

Condition factor (g/cm^3) = body weight/(body length)^3;

Viscera ratio (%) = Viscera weight/body weight×100%;

Hepatosomatic index (%) = Hepatopancreas weight/ body weight×100%;

Relative intestine length = intestine length/ body length;

Muscle ratio (%) = muscle weight/body weight×100%.

Analysis of serum biochemical parameters

After 66 days of feeding, blood from the caudal peduncle vein was sampled and the serum was obtained by firstly storing the blood at 4 C° for 8 h and then centrifuging at 2000 rpm for 10 min (4 C°).

Table 1. Ingredient composition and proximate composition of experimental diets (air-dry basis, g/100g)

Ingredients	Basal diet (BD)	Green Tea (GT)	Green tea waste (GTW)
Wheat flour	25.96	20.96	20.96
Soy bean meal	33.92	33.92	33.92
Rape seed meal	19	19	19
Cotton seed meal	12	12	12
Fish meal	3	3	3
Vegetable oil	3	3	3
Salt	0.3	0.3	0.3
$Ca(H_2PO_4)_2$	1.5	1.5	1.5
Premix *	1.3	1.3	1.3
Capsulated VC	0.02	0.02	0.02
Green tea		5	
Green tea waste			5
Total	100	100	100
proximate composition			
Crude protein	33.92±0.82	35.33±2.55	34.67±1.94
Crude lipid	4.19±0.03	4.19±0.24	3.87±0.07
Moisture	11.17±1.76	10.95±0.71	12.03±0.54
Ash	11.17±1.76	9.95±0.71	12.03±0.54

* Premix was composed of minerals mix and vitamins mix. Mineral premix(% mixture): $KAl(SO_4)_2$:0.159, $CaCO_3$:18.101, $Ca(H_2PO_4)_2$:44.601, $CoCl_2$:0.070, $MgSO_4$:5.216, $MnSO_4.H_2O$:0.070, KCl:16.553, KI:0.014, $ZnCO_3$:0.192 , NaH_2PO_4:13.605, Na_2SeO_3:0.006, $CuSO_4.5H_2O$:0.075 , Ferric Citrate.$5H_2O$:1.338;
Vitamin premix (mg or IU/kg mixture):VA:3000IU, VD_3:1500IU, VE:50IU, VK_3:10, VB_1.HCl:10, B_2:20, Nicotinamide:50, Calcium pantothenate:40 (pantothenate), B_6.HCl:10, B_{12}:0.02, Folic Acid:5, Biotin:1.0, VC:200, inosito:400, choline chloride:2000 .
This followed the requirements of common carp according NRC(1993).

Glutamic pyruvic transaminase, total protein, albumin, globulin, glucose, blood urea nitrogen, cholesterol, total glycerol, high density lipoprotein and low density lipoprotein in serum were determined using automatic biochemical analyzer (Hitachi 7180, Tokyo, Japan). Total antioxidant capacity and maleic dialdehyde in serum were determined using kit (Jian Cheng, Nan Jin, China).

Proximate Composition in Muscle of Grass Carp

Muscle of fish were sampled and dried in 70°C. Crude protein of muscel was determined by the Kjeldahl method, crude lipid by ether-extraction; moisture was determined by drying in 105 °C and ash was determined using a muffle furnace (TMF-3100, EYELA Co., Tokyo, Japan) at 550 °C for 4 h.

Analysis of Expression of MRFs and Nonspecific Immunity Response Genes in Muscle and Hepatopancreas of Grass Carp by Quantitative real-time RT-PCR

Trizol™ reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from muscle and hepatopancreas according to the manufacturer's instructions. RNA was purified using the RNAwiz protocalTM (Ambion), subjected to DNase treatment (DNA-freeTM, Ambion) and the quality and quantity were determined by denaturing gel electrophoresis and spectrophotometry (A260/280).

RNA (1 μg) was reverse-transcribed in a 20 μl reaction volume using random primers (Fermentas Life Science, Hanover, MD, US), and reverse transcription was carried out at 42°C for 50 min and 72°C for 15 min. Subsequently, 2 μl of the cDNA product, 10μl of SYBR® Premix Ex Taq TM (2×) (TaKaRa, Dalian, China), 0.5 μL of 10 μmol/L of each gene-specific primer and 8.5 μL ddH_2O were used to perform PCR using a fluorescence temperature cycler (Bio-Rad, Hercules, CA, USA). The threshold cycle (CT) was analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). RT-PCR were as follows: pre-denaturation of the synthesized cDNA at 95°C for 5min was followed by 38 cycles of denaturation at 95°C for 45 s, annealing at each gene-specific primer Tm (°C) for 1 min, and extension at 72°C for 1 min. Proper amplification of the genes was verified by melting point analysis and 1.2 % agarose gel electrophoresis. The PCR primers sequences, GenBank accession number and amplicon size of the assays used are shown in Table 2.

Statistical Analyses

Data are shown as mean±S.D. Data were subjected to one-way ANOVA and Tukey's post-hoc test. All statistical analyses were performed using SPSS8.0 for Windows Software (SPSS, Chicago, IL, USA). Results were considered significant at $P < 0.05$.

Results

Growth Performance of Grass Carp and Feed Efficiency Ratio

Table2: The sequence information and primer-design in the relative gene expression. All sequences are presented as 5'-3'

Gene	Direction	Primer	Accession number	Annealing Temperature (C°)
Muscle regulation factors (MRFs)				
Myf5	F	GGAGAGCCGCCACTATGA	AB012883	63.5
	R	GCAGTCAACCATGCTTTCAG		
Myf6	F	GAAAATCTGCTCCAACCGA	NM_001003982	60
	R	CGCTGCGTAAAATCTCCA		
MyoG	F	AGAGGAGGTTGAAGAAGGTC	AB012881	59
	R	GTTCCTGCTGGTTGAGAGA		
*Myo*D	F	TGAGGGAGAGGAGACGACT	gi:119947342	54
	R	GCTCCAGAACAGGGTAGTAGT		
β-actin 1)	F	ATCCTCCGTCTGGACTTGG	Endogenous control	55.5
	R	TCCGTCAGGCAGCTCATAG		
Stress related genes				
GSH-Px	F	GCAACCAGTTCGGACATCAGGAG	EU828796	58
	R	GGCGTTCTCACCATTCACTTCCA		
HSP 70	F	AGGCTGAGAAGTACAAGGCTGAAGA	EU816595	58
	R	TGAAGGCATAGGACTCCAGACCATT		
metallothionein	F	GCTGCTGTCAATGAGGAGGTCAA	KC256783	58
	R	CAGAAGACTGAGAACAACTGGAGGT		
ubiquitin	F	CAAGATCCAGGACAAGGAAGGCATT	JK849127	58
	R	TGAGGCGGAGCACCAGATGAA		
β-actin 2)	F	TCGTGATGGACTCTGGTGATGGT	DQ211096	58
	R	TGGTGGTGAAGCTGTAGCCTCT		

1) It means the β-actin is used for assessing the expression of MRFs; 2) It means the β-actin is used for assessing the expression of lipid metabolism related genes and genes of GSH-Px, HSP 70, metallothionein and ubiquitin.

After 66 days of feeding, mean weight of fish and feed efficiency ratio in GT group was significantly lower than those in BD group, while these growth parameters in GTW group were not different compared with GT or with BD groups (Figure 1, Figure 2).

Relative Expression of MRFs in Muscle of Grass Carp

MyoD, *myoG*, *myf5* and *myf6* are growth-related genes, which control muscle formation, muscle differentiation and skeletal muscle growth (Campos *et al.*, 2010; Francetic and Li. 2011; Valente *et al.*, 2012). Expression of *myf5* and *myf6* in GTW group were significantly lower than those in BD group, being not different from the expression of *myf5* in GT group and being significantly higher than GT group in *myf6* expression. Relative expression of *myoD* and *myoG* genes in three groups was not significantly different (Figure 3).

Biological Parameters and Proximate Composition of Grass Carp

The condition factor, including viscera ratio, hepatosomatic index, relative intestine length, muscle ratio of grass carp in GTW group, were not significantly different among these groups (Table 3).

Crude protein, crude lipid and crude ash of muscle in GTW group were not significantly different between these groups (Table 4).

Serum Biochemical Indices of Grass Carp

Serum GPT in GT group was significantly lower

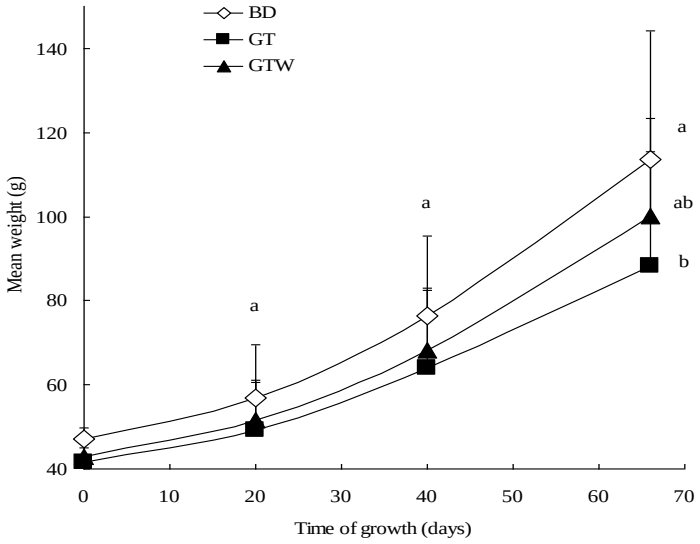

Figure 1. Effect of GTW on growth performance in grass carp (*Ctenopharyngodon idellus*). Data are presented as mean ±S.D. (n=20). Different letters mean P<0.05.

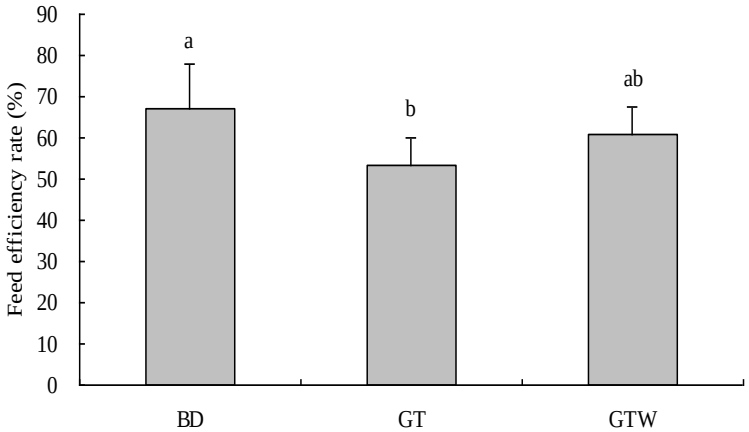

Figure 2. Effect of GTW on feed intake and feed efficiency of grass carp (*Ctenopharyngodon idellus*). Feed efficiency rate, FER, (%) = (Final weight of fish - Initial weight of fish)/Feed intake×100%. Feed intake in BD, GT and GTW groups were 1627 g, 1544 g and 1577 g respectively. Data are presented as mean ±S.D (n=6, FER in 3 growth stage were sampled and gathered together). Different letters mean P<0.05.

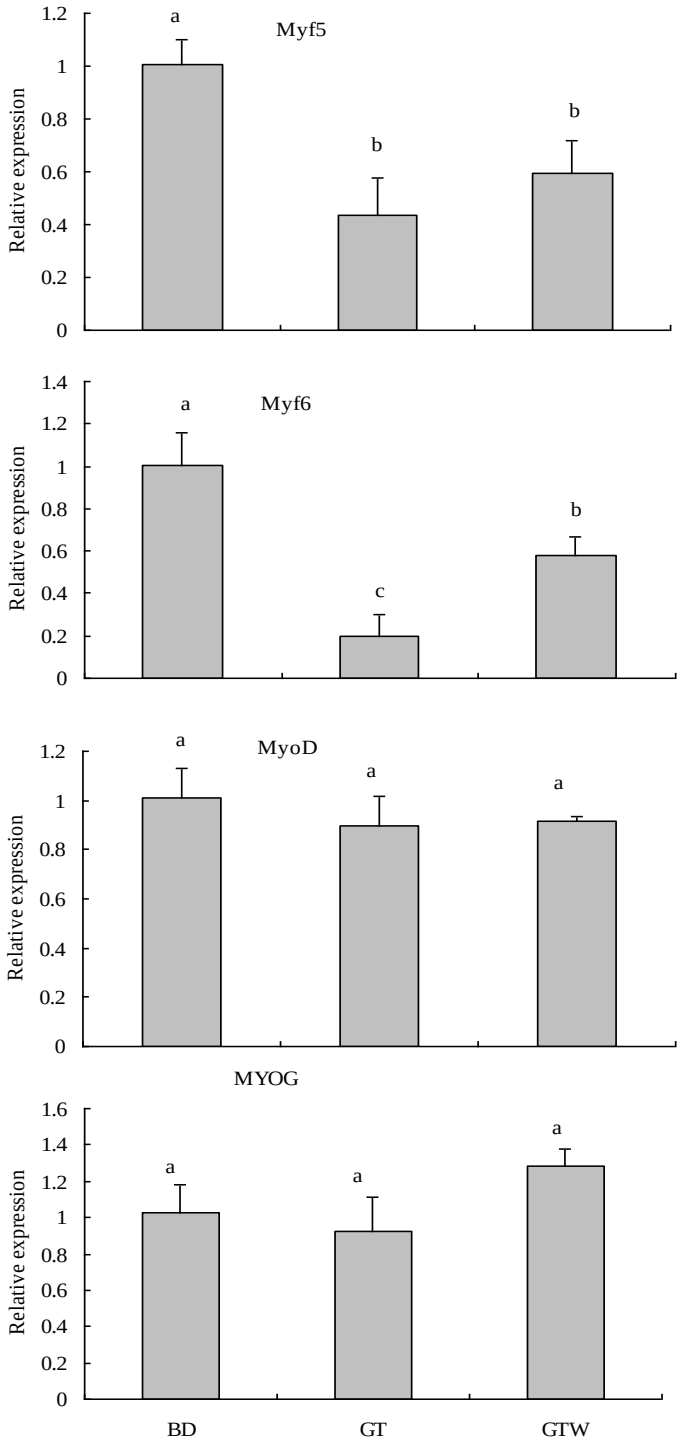

Figure 3. Effect of GTW on relative expression of MRFs in muscle of grass carp (*Ctenopharyngodon idellus*). Data are presented as mean ±S.D (n=6). Different letters mean P<0.05.

Table3: Effect of GT and GTW on biological parameters of grass carp (n=20)

Biological parameters	BD	GT	GTW
Condition factor (g.cm^{-3})	1.96±0.30$^{ns\ 1),\ 2)}$	1.82±0.22	1.95±0.20
Viscera ratio (%)	7.61±1.05^{ns}	8.28±1.37	7.74±0.84
Hepatosomatic index (%)	1.55±0.40^{ns}	1.36±0.32	1.53±0.34
Relative intestine length	1.80±0.18^{ns}	1.84±0.18	1.88±0.17
Muscle ratio (%)	37.21±6.67^{ns}	41.26±7.57	38.84±4.49

1) Data are mean±standard deviation (SD) (n=20). Values with different superscripts are significantly different (P<0.05).
2) ns means not significantly different (P>0.05).

than in BD group, which were not different form that in GTW group. Serum TP, ALB and GLO in GTW group were significantly higher than those in BD or GT groups respectively. Serum TG in GTW group was significantly higher than that in GT group, being not different from that in BD group. Serum HDL and LDL in GTW group were significantly higher than those in BD group respectively, being not different from those in GT group. Serum GLU in GTW group were significantly lower than those in BD group, being not different from that in GT group. Serum T-AOC in GTW group was significantly higher than those in GT or BD groups. Serum A/G, BUN, Chol and MDA in three groups respectively were not significantly different. Serum biochemical indices of grass carp were showed in Table 5.

Relative Expression of Nonspecific Immune Response Related Genes in Hepatopancreas of Grass Carp

Expression of *hsp70* and *ubiquitin* in GTW group were significantly higher than those in BD group, being not different from those in GT group. Expression of *GSH-Px* and *metallothionein* were not significantly different among three groups (Figure 4).

Discussion

Effect of GTW on Growth and Feed Efficiency Ratio of Grass Carp

Because of phenolic compounds in tea, green tea has been proposed as a strategy for weight loss and maintenance (Westerterp-Plantenga 2010). Previous report showed that growth was found decreased in fish of gilthead sea bream fed white tea supplementation (Pérez-Jiménez *et al.*, 2013). In the present study fish in GT group had significantly lower mean weight and feed efficiency rate, meanwhile the expression of growth related genes, *myf5* and *myf6*, were also significantly lower than those in GTW or BD groups, which were in accordance with previous results.

Like green tea, green tea waste also contains phenolic compounds (Cai *et al.*, 2001; Tsubaki *et al.*, 2008, 2010; An *et al.*, 2011; Toh *et al.*, 2010), while the content of phenolic compound would be much

Table 4. Proximate composition of muscle in grass carp (*Ctenopharyngodon idellus*) fed the test diets for 66 days (n=6; 105°C dry matter, %)

	BD	GT	GTW
Crude protein	86.03±0.80 $^{ns\ 1),\ 2)}$	85.36±0.73	84.25±2.27
Crude lipid	7.21±0.81 ^{ns}	6.72±0.79	7.84±1.04
Crude Ash	7.64±0.36 ^{ns}	6.66±0.53	7.51±0.28

1) Data are mean±standard deviation (SD) (n=6). Values with different superscripts are significantly different (P<0.05).
2) ns is not significantly different (P>0.05).

Table 5. Effect of GTW on serum biochemical parameters in grass carp (*Ctenopharyngodon idellus*). (n=6)

Serum biochemical index	*BD*	*GT*	*GTW*
Serum biochemical indexes[1)]			
GPT (U/L)	5.65±1.74$^{a3),\ 4)}$	3.52±1.04^{b}	5.46±1.06^{ab}
TP (g/L)	24.37±4.04^{b}	24.32±3.10^{b}	31.00±3.25^{a}
ALB (g/L)	11.33±1.95^{b}	11.80±1.51^{b}	14.45±1.29^{a}
GLO (g/L)	13.03±2.19^{b}	12.52±1.72^{b}	16.55±2.84^{a}
A/G	0.87±0.07^{ns}	0.95±0.07	0.89±0.13
BUN (mmol/L)	0.75±0.14^{ns}	0.64±0.14	0.84±0.18
Chol(mmol/L)	4.84±1.15^{ns}	5.45±1.03	5.87±0.74
TG(mmol/L)	2.92±0.36^{ab}	2.80±0.28^{b}	3.50±0.64^{a}
HDL-c(mmol/L)	0.49±0.11^{b}	0.57±0.10^{ab}	0.65±0.04^{a}
LDL-c(mmol/L)	1.36±0.24^{b}	1.98±0.48^{a}	2.13±0.23^{a}
GLU (mmol/L)	5.12±1.86^{a}	2.79±1.59^{b}	2.83±1.73^{b}
Serum antioxidative indexes[2)]			
MDA (nmol/ml)	11.49±1.20^{ns}	11.84±1.17	12.21±0.52
T-AOC (U/ml)	5.19±1.58^{b}	5.46±1.00^{b}	9.97±2.58^{a}

1) ALB: albumin; A/G: ratio of albumin and globulin; BUN: blood urea nitrogen; Chol: cholesterol; GLO: globulin; GLU: glucose; GOT: glutamic oxaloacetic transaminase; GPT: glutamic pyruvic transaminase; HDL: high density lipoprotein; LDL, low density lipoprotein; TG: total glycerol; TP: Total protein;
2) MDA: maleic dialdehyde, T-AOC: total antioxidant capacity
3) Data are mean±SD (n=6). Values with different superscripts are significantly different (P<0.05)
4) ns means there is no significant difference among these groups (P>0.05).

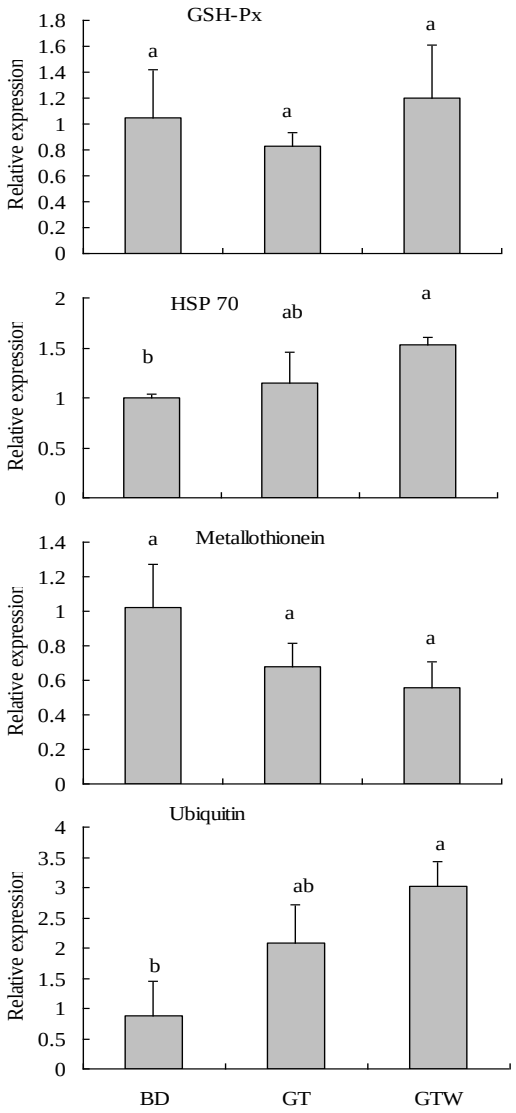

Figure 4. Effect of GTW on relative expression of stress response genes, *GSH-Px* (*glutathione peroxidise*), *heat shock protein 70* (*HSP 70*), *metallothionein* (*MTA*) and *ubiquitin*, in hepatopancreas of grass carp (*Ctenopharyngodon idellus*). Data are presented as mean ±S.D (n=6). Different letters mean P<0.05.

lower than green tea. Previous reports showed that green tea extract improved growth performance in juvenile black rockfish (*Sebastes schlegeli*) (Hwang *et al.*, 2013) and supplementation with tea waste increased daily weight gain in calves (Begum *et al.*, 1996). While in other reports, it showed that green tea extracts and ground green tea reduced growth rate in yellow tail fish and ayu (Kono *et al.*, 2000). The present results showed that growth rate of fish and feed efficiency ratio in GTW group were not different compared with BD group or with GT group, indicating that replacing wheat at an inclusion level of 5% with green tea waste did not affect growth of grass carp. Although the expression of *myf5* and *myf6* in GTW group were lower than those in BD group, the expression of *myoD* and *myoG* were in accordance with the present result in growth of fish, where they were not significantly different between BD and GTW groups.

In the present study muscle crude protein and crude lipid were not different among groups, which were consistent with previous findings where no changes in body composition, whole-body lipid concentration or muscle lipid concentration were observed in rainbow trout fed on EGCG (epigallocatechin-3-gallate, Thawonsuwan *et al.*, 2010) or in juvenile olive flounder fed on various sources of green tea (Cho *et al.*, 2007).

Effect of GTW on Serum Biochemical Parameters of Grass Carp

Biochemical parameters in blood or serum show the nutritional status health of the fish (Patriche *et al.*, 2011). Previous report showed that the activities of GPT in the serum was a good indicator of liver damage and could reflect the degree of liver damage and necrosis (Liu *et al.*, 2008). In the present study

serum GPT in GTW group was not significantly different from that in BD or GT groups, indicating that 5% replacement of wheat meal with GTW in diet of grass carp did not affect the health of fish.

The function of HDL and LDL-C is to transport extra-hepatic fatty acid and cholesterol into liver (Babin and Vernier 1989) and previous reports showed that tea components had important effects on serum lipoproteins (Basu and Lucas 2007; Kao *et al.*, 2006; Nie and Xie 2011; Sajilata *et al.*, 2008). In the present study serum HDL, LDL in GTW or GT groups were significantly higher than that in BD group, which were in accordance with previous reports in rats (Kuo *et al.*, 2005) and in fish (Pérez-Jiménez *et al.*, 2013), indicating fish in GT or GTW groups probably transported more fatty acid or cholesterol into hepatopancreas and had cleaner blood than in BD group, which were more healthier in fish of GTW or GT groups than in fish of BD group.

Previous studies in man (Kono *et al.*, 1996; Tokunaga *et al.*, 2002) and in rats (Raederstorff *et al.*, 2003) showed that tea decreased plasma total cholesterol concentrations, while in fish it was found that dietary tea supplementation did not reduce plasma total cholesterol in olive flounder (Cho *et al.*, 2007). In the present study serum Cholesterol among three groups was not significantly different, being in line with previous report in olive flounder, indicating that the effect of tea or tea waste on fish was probably different from on mammals.

A number of previous studies have demonstrated that tea has a hypolipidemic effect, decreasing plasma triglycerides (Kono *et al.*, 1996; Raederstorff *et al.*, 2003; Tokunaga *et al.*, 2002). In the present study, serum triglyceride in GT group was the lowest, which was in agreement with the previous results.

Serum ALB is synthesized and secreted by hepatocytes and ALB is important transporters in serum; serum GLO is one of the important components in the immune system of fish, which correlates with the health of the animals (Chen *et al.*, 2011 ; Zhou *et al.*, 2005). In the present study, higher serum ALB and GLO in GTW group suggests that fish fed GTW was healthier than fish fed GT or BD.

Blood or serum GLU in fish is easily affected by water temperature, feed intake, movement and photoperiod (Yang *et al.*, 2007). In the present study serum GLU levels in GTW and GT groups were significantly lower than in BD group, which is in accordance with previous result that gilthead sea bream juveniles fed white tea-supplemented diets had lower serum GLU (Pérez-Jiménez *et al.*, 2013). Pérez-Jiménez *et al.*, (2013) speculated that lower serum GLU in white tea supplemented group was due to lower feed intake. In the present study the fish were hand fed to satiation four times a day and the feed intake in BD, GT and GTW groups were 1627 g, 1544 g and 1577 g respectively (note in figure 2), indicating that fish in GT or GTW group had less feed intake than BD group, which probably resulted in lower serum GLU in fish of the two groups.

Catechin is reported to be an antioxidative and hepato-protective agent that improves liver function in rats (Byun *et al.*, 1994; Ikeda *et al.*, 1992). The effects of flavonoids, including the catechins found predominantly in tea, have been studied on a wide range of biological activities along with their effects on the promotion of health and prevention of disease in rats (Yokozawa *et al.*, 2002; Kang *et al.*, 2008) and in humans (Yamamoto *et al.*, 1997; Dufresne and Farnworth 2001; Nijveldt *et al.*, 2001; McKay and Blumberg 2002). Dietary tea inclusion in feeding could be an important source of Mn with metabolic repercussions on antioxidant mechanisms in fish (Pérez-Jiménez *et al.*, 2012). Polysaccharides from green tea, *Huangshan Maofeng*, have also been found to have antioxidant effects (Lu *et al.*, 2013).

Tea waste or tea residue also contains polyphenols, which was reported to have strong antioxidant activity (Tsubaki *et al.*, 2008). The present result shows that serum T-AOC in GTW group are higher than that in BD group, indicating that fish fed GTW had higher antioxidant capacity, which is consistent with previous result of Nishida *et al.*, (2006), who found higher plasma anti-oxidative activity in cattle fed with green tea waste silage.

Effect of GTW on Relative Expression of Nonspecific Immunity Response Related Genes in Grass Carp

Oxidative stress occurs when the production of reactive oxygen species (ROS) surpasses the ability to remove them. Glutathione peroxidise (GSH-Px) functions to remove ROS and protect from the damage caused by oxidative stress (Halliwell and Gutteridge 1999). Many markers, *hsp70*, *metallothionein*-Aisoform and *ubiquitin*, had been used to evaluate the perturbations in cell function resulting from increased heat stress and inflammatory stress respectively in mammals (Maiorino *et al.*, 1991; Arai *et al.*, 1999; Aufricht *et al.*, 2005; Bremner and Beattie 1990; Oarada *et al.*, 2007), which are nonspecific immune response related genes.

Oxidative, inflammatory and heat stress are the factors that induce the stress response (Bartelme 2004), which will affect the nonspecific immunity in body. Previous report that decaffeinated green tea can enhance immunity of rainbow trout (Sheikhzadeh *et al.*, 2011) and that catechins increased nonspecific immunity in grass carp (Sun *et al.*, 2012). In the present study expression of these nonspecific immunity response related genes, *GSH-Px*, *hsp70*, *MTA* and *ubiquitin*, were not significantly affected by GT compared with BD, while expression of *hsp70* and *ubiquitin* in hepatopancreas of fish in GTW significantly increased compared with BD, suggesting that grass carp in GTW had higher immune ability to overcome stress, which was in line with previous result. The mechanism of immuno-stimulation by

dietary GTW is not clear but may be attributed to one or more of its components, in particular catechins, glycosides, flavonols, flavanones, phenolic acids and the aglycones of plant pigments (Pan *et al.*, 2003; Farhoosh *et al.*, 2007). These results suggested that supplementation with GTW was beneficial to the immunity of grass carp.

Conclusion

It is assumed that supplementation of GTW in diet of grass carp is appropriate to improve the health of grass carp by enhancing serum biochemical indices, serum antioxidant ability and nonspecific immunity response, without affecting the growth of grass carp.

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