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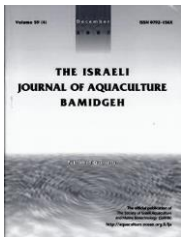
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

The Israeli Journal of Aquaculture - Bamidgeh, IJA_70.2018.1496, 7 pages



Effect of Dietary Supplementation of Glycyrrhetinic Acid and Tea Polyphenols on Depuration of 2-methylisoborneol from Blunt Snout Bream, *Megalobrama amblycephala*

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Keywords: *Megalobrama amblycephala*; glycyrrhetinic acid (GA); tea polyphenols (TP); 2-methylisoborneol (MIB); depuration

Abstract

Off-flavor is a serious issue in cultured fish. It affects marketability and texture of fish and causes great economic losses in the aquaculture industry. Holding fish in a clean-water environment for some time is an effective method to depurate them although it involves weight loss. This study investigates the potential effects of dietary supplementation of glycyrrhetinic acid (GA) and tea polyphenols (TP) on the depuration of the strong odors of 2-methylisoborneol (MIB) from blunt snout bream, *Megalobrama amblycephala*. 560 fish, initial average weight of 48.75±0.48 g were distributed into 28 tanks at a rate of 20 fish per tank. Three diets, namely the control diet, a GA supplemented diet (0.3 g/kg), and a (TP) supplemented diet (0.3 g/kg) were used in this study. The control diet was randomly assigned to 20 tanks, 4 tanks with clean water (control), or with water containing 1μg/L MIB. During the first 28-day the fish in each treatment were hand-fed to apparent satiation three times daily. In the second phase (29-42 day), the control, GA, and TP treatments were divided into four groups: MIB, low density (LD), high density (HD), and dynamic sampling (DS) with twenty fish in DS, and fifteen fish in the others. Fish in LD, HD, and DS in all treatments were starved in clean water. Fish in the DS treatment were sampled at 0, 24, 48, 72 hours, at 7 and 14 days. No differences were observed in body weights between groups within the 28-day feeding trial ($P>0.05$). Fish in GA, LD, and HD groups had significantly lower hepatosomatic index (HSI) and intraperitoneal fat ratios (IPF) than the control, MIB, and TP groups ($P<0.05$). After 28 and 42 days, fish in the MIB group had higher MIB concentrations than the other groups ($P<0.05$). Scores of sensory characteristics were similar, with no statistical differences between the control, GA, and TP groups ($P>0.05$). MIB content decreased significantly in relation to longer depuration times ($P<0.05$). Fish weight in LD and HD treatments declined by 10% and 12% respectively after 14-day fasting. In conclusion, clean water depuration for fourteen days is an effective method to purge off-flavored flesh although it involves weight loss and dietary supplementation of GA and TP which also depurate MIB from flesh of *Megalobrama amblycephala.*

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Introduction

In intensive aquaculture, fish fillets often have an earthy/musty flavor which usually derives from two highly odorous molecules, 2-methylisoborneol (MIB), and/or geosmin. This flavor does not endanger consumers however it deters them because of poor meat texture and quality (Robertson et al. 2005). MIB is present in aquatic environments including fishponds and recirculating systems. It is recommended that MIB in aquatic water should be under 0.035 μg/L, and less than 0.7 ng/g in fish tissues (Persson 1980). Excessive MIB is deposited into fish flesh via gills, guts, and skin. MIB can be detected in fish after two or three hours of exposure and it increases dramatically within a period of 24 h (Johnsen and Lloyd 1992). Off-flavors in fish caused by MIB are dependent on water temperature, tissue fat content, and water microbial abundance. The depuration process requires more than 12 days days to completely eliminate this unpleasant off-flavor from fish (Persson 1980).

Due to its critical role in the development of off-flavor, MIB (1,2,7,7-tetramethyl-exo-bicyclo [2.2.1.]heptan-2-ol) has received considerable attention. Three general approaches have been adopted to depurate MIB from fish tissue:

(1) improving aquaculture management to eliminate odor-producing bacteria from pond water by regulating N/P ratios in the water (Krishnani 2008), the ingestion process of fish, and the restraint of heavy metals (Tucker 2000). Although simple in theory, putting this into practice is quite difficult due to the various chemicals, water volume, and the possibility of secondary pollution.

(2) Holding fish in clean water to depurate MIB naturally (Robertson et al. 2005). This method is an established and feasible way, although time-consuming and costly, to solve the taint problem.

(3) Depurating of off-odor from fish fillets after slaughter through absorption (Masaki et al. 2005), oxidation-reduction (Varlet et al. 2007), and sonication approaches (Song and Kevin 2007). However, previous studies focused mainly on aquaculture management and processing technologies. To the best of our knowledge, limited reports are available concerning the depuration of MIB through feed additives in the breeding stage.

Blunt snout bream (*Megalobrama amblycephala*) is a widely cultivated and favored fish in Asia, Europe, Africa, and North America, characterized by fast growth and tender flesh (Lu et al. 2014). Recently, aquaculture of this species has decreased because of compromised fillet quality, especially the earthy/musty flavor. The present study was conducted to evaluate the beneficial effects of glycyrrhetinic acid (GA) and tea polyphenols (TP) on the fillet quality of blunt snout bream (*Megalobrama amblycephala*), with special reference to MIB depuration. The results obtained here will provide new insights on possible improvements in the flavor.

Materials and Methods

Fish, diet, and experimental design

Megalobrama amblycephala fingerlings were obtained from the fish hatchery of Yangzhou (Jiangsu, China), and were acclimated to laboratory conditions for 15 days by gradually weaning them in a recirculating water system from a commercial to a basal diet with 30% protein and 6% lipid. A total of 560 fish (initial average weight of 48.75±0.48 g) were distributed into 28 tanks (250 L) at a rate of 20 fish per tank. Three diets, namely a control diet (C), glycyrrhetinic acid (GA) diet (0.3 g/kg), and tea polyphenols (TP) diet (0.3 g/kg) (Wu et al. 2013) were adopted in this study. The GA and TP used in this study were purchased from Nanjing ZeLang Medical Technology Company (Nanjing, China). The control diet (C) was randomly assigned to 20 tanks of fish reared either in clean water (4 tanks) or in MIB water (16 tanks). MIB, purchased form Sigma Chemical Co., (St. Louis, MO, USA), was added to the water at a concentration of 1μg/L. The GA and TP diet was randomly assigned to eight tanks with fish held in MIB water (the GA and TP treatment), respectively, each with four replicates. The whole feeding trial consists of two phases: days 1-28, and days 29-42. During the first 28 days, fish in each treatment were hand-fed to apparent satiation three times daily. After this, five fish per tank were randomly sacrificed for biometric analysis of whole body and dressed carcass. In the second phase from days 29-42, the control, GA, and TP treatments were as per the original conditions, however there were fewer fish per tank (15 fish/tank). The MIB treatment was further divided into four groups: MIB (four tanks with 15 fish/tank), LD (low density, four tanks with 15 fish/tank), HD (high density, two tanks with 30 fish/tank) and DS (dynamic sampling, three tanks with 20 fish/tank). Fish in the later MIB treatment were kept under the original conditions, however fish in LD, HD, and DS treatments were starved in clean water. Furthermore, fish in the DS treatment were sampled at different time intervals, three fish each time. The experimental design is shown in Table 1.

Table 1. Experimental design.

Date	*Factor*	*Control*	*MIB*				*GA*	*TP*
1-28 day	MIB	-	+				+	+
	Diet	Control	Control				0.3g/kgGA	0.3g/kgTP
	Fish/tank	20	20				20	20
			MIB	LD	HD	DS		
29-42 day	MIB	-	+	-	-	-	+	+
	Diet	Control	Control	-	-	-	0.3g/kgGA	0.3g/kgTP
	Fish/tank	15	15	15	30	20/tank	15	15

Control; MIB: 2-methyl-iso-borneol; GA: glycyrrhetinic acid; TP: tea polyphenols; LD: low density; HD: high density; DS: dynamic sampling.

Sampling and analytical procedures

After the 28th and 42nd day of feeding, fish from each tank (apart from the DS treatments), were fasted for 24 h, and then anesthetized in diluted MS-222 tricaine methanesulfonate, SigmaChemical Co., St. Louis, MO, USA) at a concentration of 100 mg/L. Total number and weight of fish in each tank were later determined. The proximate composition of diets, whole body, and dressed carcass of fish was determined in duplicate according to the procedures detailed previously (Li et al. 2014).

After 42 days of feeding, 3 fish per tank (in control, MIB, GA, and TP groups) were sacrificed by blunt trauma to the head for muscle samples. Fish in the DS group were sampled at 0 h, 24 h, 48 h, 72 h, 7 d, and 14 d after being transferred from MIB water to pure water. Samples were frozen in liquid nitrogen and stored at -20°C until analysis. MIB concentration was determined according to the methods detailed by Guttman et al. (2008).

Sensory analysis

Fish fillets, 50 g per sample from the abdomen, were rinsed with distilled water, sealed in a zip-lock bag, and later cooked in a beaker with boiling water for 1.5 min. Then, samples were scored by a pool of 15 panelists who were trained as sensory descriptors for cooked fish by the NCMQSC (National Center of Meat Quality and Safety Control, China). The panelists received samples just after cooking and graded the musty off-flavor attributes on a continuous scale from low (0) to high intensity (5) (Table 2).

Table 2. The standard sensory grades of musty off-flavor used in the present study.

Degree of smell	*Score*
None	0
Quite light	1
Light	2
Medium	3
Strong	4
Terrible	5

Statistical analysis

All data were subjected to one-way ANOVA analysis using SPSS 16.0 (SPSS 16.0, Michigan Avenue, Chicago, IL, USA), after the test of equality of error variances using the Levene's test. Multiple comparisons were conducted using the Dancan test. The effect of all treatments were considered significant at a P value of 0.05. All data were presented as the mean of 4 replicates.

Results

Growth performance

At the end of the 42 day feeding trial, no mortality was observed in all treatments throughout the whole feeding period. No difference was observed in body weight between all the groups after the 28th day of feeding ($P>0.05$). However, after the 42nd day into the feeding trial, fasting groups (LD and HD) had significantly lower ($P<0.05$) FBW, WG, and SGR values than those of the other treatments (see Table 4). After the 14th day of fasting, body weight in LD and HD group declined by 6% and 8%, respectively. In addition, FBW, WG, and SGR, in HD group was lower than that in the LD group, but no statistical difference was observed ($P>0.05$).

Table 3. Growth performance of blunt snout bream subjected to different treatments

Date	Parameters	Control	MIB	GA	TP	LD	HD
0-28d	IBW (g)	48.75±0.48	48.25±0.81	48.35±0.98	48.61±0.68	48.63±0.61	48.44±0.76
	FBW (g)	63.73±4.78	63.58±3.41	63.28±2.86	63.41±3.48	63.21±2.88	63.96±3.99
	WG (%)1	30.72±8.96	31.77±3.21	30.88±1.90	30.38±2.12	31.09±3.72	31.61±6.11
	SGR2	0.96±0.08	0.99±0.05	0.96±0.02	0.98±0.06	1.03±0.05	0.96±0.06
0-42d	FBW(g)	77.75±2.27a	74.57±0.81a	77.38±5.54a	80.49±5.03a	59.43±5.05b	58.82±4.76b
	WG (%)1	59.49±3.73a	54.55±0.81a	60.04±4.65a	65.06±6.40a	22.15±5.43b	21.77±4.36b
	SGR2	1.13±0.03a	1.03±0.08a	1.12±0.03a	1.19±0.04a	0.48±0.04b	0.46±0.03b

Values are presented as means of 4 replications, excepted the HD group (2 replications in 29-42 d). Means in the same line with different superscripts are significantly different (P<0.05).
IBW: Initial body weight; FBW: Final body weight.
1 Weight gain (WG, %) = (Final body weight-initial body weight) ∗100/ initial body weight.
2 Specific growth rate (SGR)=(Ln Wt−Ln W0)∗100/T where W0 and Wt are the initial and final body weights, and T is the culture period in days.

Biometric parameters

After feeding for 28 days, no statistical difference (P>0.05) was observed in CF, DP, HIS, and IFR among all the treatments (Table 5). After 42 days of rearing, CF and DP showed no significance (P>0.05) between the different treatments. However, HSI of GA, LD and HD treatments was significantly lower than in the control, MIB, and TP groups (P<0.05). In addition, fish in LD and HD had obviously lower IPF ratio than that in other groups (P<0.05).

Table 4. Biometric parameters of blunt snout bream subjected to different treatments

Training (days)		Control	MIB	GA	TP	LD	HD
28	CF (%)1	1.82±0.08	1.67±0.27	1.77±0.24	1.77±0.22	-	-
	DP (%)2	68.46±0.81	69.58±1.18	70.18±0.40	67.07±0.9	-	-
	HSI (%)3	1.46±0.06	1.40±0.04	1.36±0.04	1.47±0.07	-	-
	IPF(%)4	4.53±0.20	4.26±0.19	3.97±0.14	4.01±0.11	-	-
42	CF (%)1	1.56±0.12	1.55±0.10	1.32±0.13	1.61±0.21	1.48±0.25	1.48±0.23
	DP (%)2	66.28±0.80	67.30±1.31	69.33±1.52	67.45±1.24	68.56±0.48	67.30±1.21
	HSI (%)3	1.78±0.13a	1.77±0.07a	1.43±0.07b	1.74±0.12a	1.32±0.04b	1.37±0.22b
	IPF (%)4	5.68±0.24a	5.49±0.28a	4.96±0.21b	5.39±0.25ab	4.13±0.16c	4.19±0.25c

Values are presented as means of 4 replications. Means in the same line with different superscripts are significantly different (P<0.05).
1 Condition factor (CF)=(body weight×100)/total body length3.
2 Dressout percentage (DP)=(carcass weight×100)/body weight.
3 Hepatosomatic index (HSI)=(liver weight×100)/body weight.
4 Intraperitoneal fat ratio (IPF)=(intraperitoneal fat weight×100)/wet body weight.

Sensory characteristics

The scores of sensory characteristics of fish fillets were shown in Fig. 1. At the end of the 28th day of feeding, fish in MIB group had significantly higher (P<0.05) scores than those of the other groups (Fig. 1A). No significant difference (P>0.05) was observed in the control, GA, and TP groups. At the termination of the 42 day rearing period, fish in MIB group had significantly higher (P<0.05) scores of sensory characteristics than the control (Fig. 1B) but exhibited little difference for the other treatments. Scores of sensory characteristics in the DS group decreased significantly (P<0.05) with increasing depurated time (Fig. 1C).

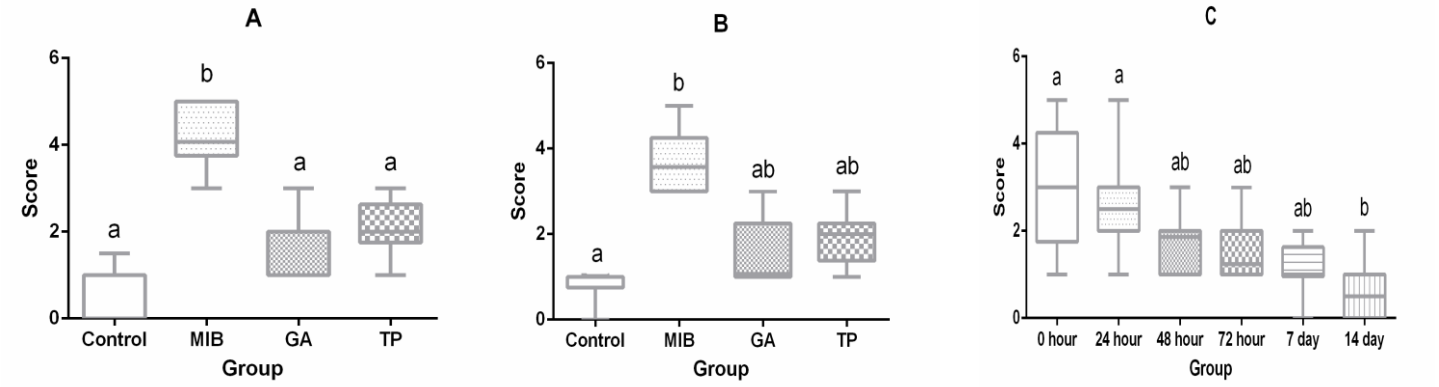

Figure 1. Sensory property of blunt snout bream subjected to different treatments
A: the scores after a 28 day feeding period;
B: the scores after 42 days of rearing;
C: the scores of DS treatment at different time intervals.

MIB concentrations

The MIB concentrations of fish fillets from different treatments are shown in Fig. 2. At the end of the 28 day feeding period, fish in MIB group had significantly higher ($P<0.05$) MIB concentrations than the other groups (Fig. 2 A). There was no significance ($P>0.05$) between control, GA, and TP groups. After the 42nd day of rearing, MIB concentration of fish in MIB group was significantly higher ($P<0.05$) than in the other groups (Fig. 2 B). There was no statistical difference ($P>0.05$) in MIB concentration for the two rearing periods, namely 28th day and 42nd day of culture. MIB concentration in the DS treatment decreased significantly with increasing depurated time ($P<0.05$) with the lowest value seen on the 14th day (Fig. 2C).

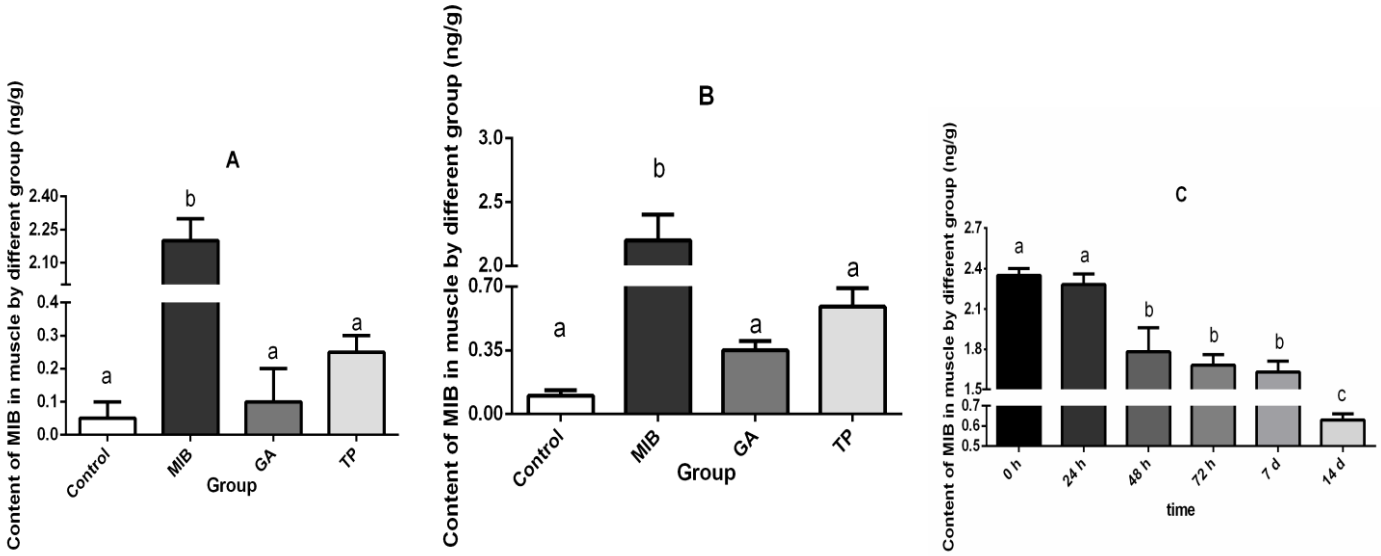

Figure 2. The MIB concentrations in the muscle of blunt snout bream subjected to different treatments
A: the MIB content in muscle after a 28 day feeding period;
B: the MIB content in muscle after 42 days of rearing;
C: the MIB content in muscle of DS treatment at different time intervals.

Discussion

Holding fish in clean water without feeding after breeding is an effective method to purge chemicals such as antibiotics and off-flavors from fish naturally (Robertson et al. 2005), or to improve body lipid profile (Navarro and Guti´errez 1995). However, fish farms are often reluctant to do this because of production loss, increased time, and costs. Compensatory growth may be observed after short-term food deprivation followed by unrestricted refeeding (Jobling et al. 1993). However, this does not apply when starvation time is short without refeeding. In addition, if this benefits the fillet quality, it could be profitable. When taking cost into consideration, fish farms often keep fish together in a small pool with clean water. In the present study, body weight in both LD and HD groups declined by 6-8% after a 14 day fasting period. That is to observation in this species in practical production (about 5-8%) for 15 days. It is also similar to results from a previous study which showed that blunt snout bream in 4 repeated cycles (3 d of no feeding, followed by 12 d of refeeding) had lower weight gain than in unrestricted groups (Lu et al. 2014). Furthermore, weight loss was less in fish kept under high density than fish in low density groups, suggesting that density of depuration might be sufficient when considering the balance between cost and weight loss. In the present study, HSI of fish decreased in fasting groups both in LD and HD groups. This decline is reversible, but requires unrestricted refeeding, which can potentially cause liver glycogen deposition (Navarro and Guti´errez 1995). This finding was supported by the fact that HSI is a dependable index for feed deprivation and re-alimentation, which generally increases after compensatory growth (Lu et al. 2014).

Glycyrrhetinic acid (GA), an active principal constituent of liquorice and has anti-inflammatory and antioxidant effects in vertebrates (Sen et al. 2011). However, its effects on lipid metabolism is little understood. In this study, HSI and IPF decreased with dietary supplementation of GA, which is in line with other studies on rats (Wu et al. 2008), and fish (Jiang et al. 2012). This result is plausible since GA was reported to promote lipolysis (Panneerselvam et al. 2009), increase plasma cortisol levels (Dodds et al.

1997), and improve lipid deposition in fish with no negative affect on growth performance (Jiang et al. 2012). The results obtained here suggested that GA could be used as a non-nutritive fat deposition regulator in aquafeed.

Consumers are very sensitive to MIB content in fish muscle, although values vary in many species. For example, the MIB concentrations in *Chrysophrys* major, *Sphyraenus,* and *Oncorhynchus mykiss* were 0.095, 0.075, and 0.55 ng/g, respectively (Persson 1980). This is many times higher than that found in water. The results of this study clearly demonstrate that the detection threshold of MIB in blunt snout bream is 0.7 ng/g, which is similar to that of *Oncorhynchus mykiss* (Persson 1980). The deposition of MIB in fish flesh is affected by many factors, including body lipid content of fish, and MIB concentration in water (Howgate 2004). Our data showed that the content of MIB in flesh was quite stable from 28-42 days of exposure of MIB. This result differs from observations in trout where maximum concentration of MIB was reported after a 6 day exposure period of MIB in ambient water (Howgate 2004). It is therefore suggested that when water temperature is 28°C and MIB concentration is 1μg/L water, detection threshold of MIB in blunt snout bream is approximately 2.2-2.35ng/g.

To purge off-flavored flesh, abatement strategies focused on clean water before marketing (Tucker and Ploeg 1999). Using a 14C-labelled MIB, asymptotic uptake was found to occur over a short time (12 h), but elimination required more than 50 h (Perkins and Schlenk 1997). In this study, holding fish in clean water for 14 days was found to be an effective method of purging MIB naturally from the flesh of blunt snout bream under 0.7 ng/g, but this also resulted in weight loss. Economic analysis showed that clean-harvesting ponds were found to be more profitable than stocked ponds (Tucker and Ploeg 1999). In practical aquaculture, fish farms must hold fish in clean water after breeding. Results further indicate that clean water depuration (14 days) is an effective approach to purge MIB containing 0.07 ng/g. Antioxidants are commonly used in aquaculture to handle off-flavor in flesh after culture (Yamprayoon and Noomhorm, 2000). Vitamin C and/or tea polyphenols (TP), were reported to eliminate much of the earthy-musty off-odour compounds that formed on silver carp *Hypophthalmichthys molitrix,* slices (Fu et al. 2015). The present study indicated that dietary supplementation of TP decreased MIB concentration in the flesh of blunt snout bream after a 42 day period, suggesting that TP could depress the MIB concentration in flesh and depurate off-flavor in this species. According to previous studies, this result could be explained as follows: (1) excessive MIB in water passes into fish tissues mainly through gills and gut (Howgate 2004); (2) MIB may be degraded by redox reaction for hydroxyl (Sagehashi et al. 2005); (3) TP has high anti-oxidative activities (Wu et al. 2013), thus blocking the MIB deposition through gut and combatting its deposition in tissues. In addition, dietary supplementation of GA also benefited depuration of MIB in fish muscle in this study. The MIB concentration in muscle of the GA group was lower than that of the TP group, indicating that GA is more effective in cleaning MIB in fish tissue than TP. In fact, licorice extract, which is composed of GA, is well known for its role in immunomodulation (Xu et al. 2013), anti-oxidation, and lipolysis (Jiang et al. 2012). The antioxidant properties of GA in depuration off-flavor may be similar to that of TP. In addition, GA reduces fish fat mass and with it MIB deposition (Johnsen and Lloyd 1992). This combined action of lipolysis and anti-oxidation might lead to improved MIB elimination by GA, compared with TP.

In conclusion, this study has demonstrated that a 14-day period of clean water depuration is an effective approach to purge off-flavored flesh of blunt snout bream, despite weight loss which was observed. In addition, dietary supplementation of 0.3 g/kg GA or 0.3 g/kg TP could efficaciously depurate MIB in fish flesh, respectively.

Acknowledgements

This research was funded by the National Technology System for Conventional Freshwater Fish Industries of China (CARS-46-20), and the National Science and Technology Ministry (2013BAD20B05).

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