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Lipid accumulation in grass carp (*Ctenopharyngodon idellus*) fed faba beans (*Vicia faba* L.)

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Abstract Feeding faba beans (*Vicia faba* L.) to grass carp (*Ctenopharyngodon idellus*) increases muscle compactness but decreases growth and motility. The lipid metabolism of grass carp was examined to assess potential effects of feeding faba beans on physiological properties using a total of 180 fish. The treatment group was fed faba beans for 120 days and a commercial diet for another 30 days. The control group received a commercial diet for 150 days. Fish were sampled every month. Weight gain was significantly lower in the treatment group than in the control. Hardness, springiness, chewiness, cohesiveness, and gumminess of the dorsal muscle increased significantly with the feeding faba beans from 30 to 120 days, which was not reversed by the subsequent feeding of commercial diet. Fat accumulation increased significantly in the treatment group as suggested by the condition factor, viscera index, hepatopancreatic index, and intraperitoneal fat index (IPFI), hepatopancreas, and muscle fat content but was not affected by subsequent feeding with the commercial diet. Serum triglyceride and total cholesterol levels were significantly reduced in the experimental diet group. In the hepatopancreas and intraperitoneal fat IPF, monounsaturated fatty acids showed significantly higher content in faba bean feeding fish, whereas polyunsaturated fatty acid content showed the reversed pattern. In the hepatopancreas, the activities of the lipogenic enzymes malate dehydrogenase and glucose 6-phosphate dehydrogenase were higher in the treatment than in the control group. Moreover, the treatment group showed lower mRNA levels of carnitine palmitoyltransferase-1. Overall, our results clearly demonstrate increasing lipid accumulation in the viscera of faba bean-fed grass carp.

Keywords Crisp grass carp · Lipid metabolism · Faba bean · *Ctenopharyngodon idellus*

Abbreviations

ATGL	Adipose triglyceride lipase
CF	Condition factor
CPT-1	Carnitine palmitoyltransferase-1
FAS	Fatty acid synthase
G-6-PHD	Glucose 6-phosphatedehydrogenase
HI	Hepatopancreas index
IPF	Intraperitoneal fat
IPFI	Intraperitoneal fat index
LPL	Lipoprotein lipase
MDH	Malate dehydrogenase
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid

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SFA Saturated fatty acid
TG Triglyceride
VSI Visceral somatic index

Introduction

Freshwater aquaculture is an important part of the entire global aquaculture industry, including that of China. Approximately 32.9 million tons of freshwater fish were produced in China in 2015, of which 17.23% (5.67 million tons) were grass carp (*Ctenopharyngodon idellus*) (Fisheries Bureau of Ministry of Agriculture, China 2016), which is considered high-quality animal protein. However, increasing economic development produced a rising demand of fish products of higher quality. Thus, the improvement of fish meat quality is attracting increasing research interest. The "crisp" grass carp was an incidental product of aquaculture farmers in the early 1970s who found that the muscle structure of grass carp increased substantially in muscle hardness and crispness after they were fed with faba bean (*Vicia faba* L.) for 90 to 120 days. Crisp grass carp is becoming more popular on the Chinese market, and grass carp products have been exported to the USA and various countries in Southeast Asia and Latin America in recent years (Yang et al. 2015). Moreover, increased muscle hardness after feeding with faba bean has also been observed in other fish species such as channel catfish (Zhu et al. 2012), crucian carp (Li et al. 2007), and tilapia (Lun et al. 2007). Similar effects were reported in birds (Przywitowski et al. 2016) and mammals (Cutrignelli et al. 2008), suggesting consistent effects of faba beans on muscle properties in animals.

Previous studies on crisp grass carp focused on textural characteristics (Lin et al. 2012, 2016; Yang et al. 2015), processing and storage (Lin et al. 2009, 2013; Zhu et al. 2013), as well as the causality of the altered muscle structure (Yu et al. 2014a, b, 2017). However, a few studies have examined the physiological characteristics of faba bean–fed grass carp. Evidence of reduced growth performance and motility was found in crisp grass carp unlike in ordinary grass carp; however, differences in energy metabolism have not been investigated thus far. Interestingly, faba beans have a lower lipid content than conventional diets but faba bean feeding can induce excess lipid accumulation in the viscera of grass carp (Wang et al. 2015). This phenomenon is counterintuitive, as physical lipid accumulation should correlate positively with dietary lipid content in fish (Du et al. 2005; Gao et al. 2010; Salmerón et al. 2016). Lipid content is known to affect numerous processes, including absorption, lipogenesis, and lipid catabolism and turnover (Tian et al. 2016a; Todorčević and Hodson 2015); therefore, it would be necessary to investigate which of those processes is affected by a faba bean diet. Excessive lipid accumulation affects the health status of fish and can cause tremendous economic losses in the aquaculture industry (Tian et al. 2015), as indicated by the high mortality in crisp carp. Thus, information on the specific factors affecting the regulation of lipid metabolism processes may be of use for the aquaculture industry in order to improve the productivity of crisp grass carp.

In this study, we examined the lipid metabolism of faba bean–fed grass carp. According to the industrial production process, grass carp were fed faba beans for 120 days to increase muscle crispness, and after that, a commercial diet was provided for another 30 days to improve immunity. Growth, lipid accumulation, fatty acid composition, lipid metabolism-related gene expression, and enzyme activities were analyzed to provide reference for the culture of crisp grass carp and general fish lipid metabolism.

Material and methods

Experimental setup

Grass carp were obtained from a commercial farm in Guangzhou (Guangdong, China). Feeding experiments were carried out at the Pearl River Fisheries Research Institute. Fish were reared in concrete pools and fed a control diet (Table 1) for 2 wks to allow acclimatization to the experimental environment. Before the feeding experiment, the fish were fasted for 24 h. A total of 180 fish (of about 700 g body weight) were randomly assigned to 6 pools (2.5 × 2.5 × 1.2 m; 30 individuals per pool). Three pools were randomly assigned to one of two groups: the control group was fed a commercial diet (Tongwei Company, China) for 150 days; the treatment group was fed faba beans for 120 days, and after that, a commercial diet for another 30 days (Fig. 1a). Faba beans were purchased from a local market in Guangzhou and were soaked in a saline solution for 24 h before feeding. The crude dietary composition including fatty acid content of commercial diet and faba beans are shown in Table 1. Fish were fed an amount of 3% of their body weight twice per day (at 8:00 and 16:00). The

feed intake was recorded. To maintain water quality, one-third of the water was renewed daily during feeding. The water temperature was maintained at 27–31 °C. Dissolved oxygen was maintained approximately at saturation (7 mg L^{-1}) through continuous aeration. The photoperiod was 12 h light/12 h dark.

Sample collection

After 30, 60, 90, 120, and 150 days from the start of the experiment, all of the fish were weighed, and five fish were randomly sampled from each pool. Before sampling, the fish were fasted for 24 h and then anesthetized with tricaine methanesulfonate (MS222). Two fish of each pool were used for blood collection from the caudal vein. The blood sample was allowed to clot at 4 °C for 6 h, and serum samples were collected after centrifugation of the clotted blood for 10 min (at 825×*g* and 4 °C). Serum samples were pooled per sampling day, frozen in liquid nitrogen, and stored at − 80 °C until analysis. The remaining fish were killed and dissected after the end of the feeding experiment. First, the viscera were weighed, then the hepatopancreas and intraperitoneal fat (IPF) were removed and weighed. Samples were then frozen in liquid nitrogen and then stored at − 80 °C until analyses of fatty acid composition, enzyme activities, and gene expression. Dorsal muscle samples (20 × 20 × 20 mm) were collected for immediate texture analysis. All experimental animal procedures were approved by the institutional animal care and use committee and performed in accordance with national and institutional regulations on the care and use of experimental animals.

Condition factor (CF), viscera index (VI), hepatopancreas index (HI), and intraperitoneal fat index (IPFI) were calculated using the following formulae:

$$\mathrm{CF}\left(\mathrm{g}/\mathrm{cm}^3\right) = \text{body weight} \times 100/\text{body length}^3$$

$$\text{Feed conversion ratio(FCR)} = \text{amount of intake}/\text{weight gain (g)}$$

$$\mathrm{VI}(\%) = \text{viscera weight} \times 100/\text{body weight}$$

$$\mathrm{HI}(\%) = \text{hepatopancreas weight} \times 100/\text{body weight}$$

$$\mathrm{IPFI}(\%) = \text{IPF weight} \times 100/\text{body weight}$$

Table 1 Proximate composition and fatty acid composition of commercial diet and faba bean

Items	Commercial diet (control)	Faba bean (FB)
Proximate composition (%)		
Moisture	9.21	11.79
Crude protein	29.62	27.48
Crude lipid	3.56	0.75
Crude ash	7.83	2.6
Fatty acid composition		
14:0	0.78	0.51
16:0	12.55	14.62
18:0	4.62	2.59
Σ SFA	15.95	17.72
16:1n-7	3.56	1.9
18:1n-9	28.52	30.14
20:1n-9	0.11	0.55
MUFA	32.19	32.59
18:2n-6	34.87	42.37
18:3n-6	n.d.	0.43
20:4n-6	5.12	0.68
22:4n-6	0.89	0.26
Σn-6 PUFA	40.88	43.74
18:3n-3	7.59	3.82
20:5n-3	0.51	0.41
22:6n-3	0.79	0.28
Σn-3 PUFA	8.89	4.51

SFA, saturated fatty acid; *MUFA*, monounsaturated fatty acid; *PUFA*, polyunsaturated fatty acid

Diet composition analyses

The crude composition of diets and tissues (hepatopancreas and muscle for crude fat) were determined according to the methods of Association of Official Analytical Chemists (AOAC) Procedures (1995). Briefly, samples were dried to a constant weight to determine moisture at 105 °C. Crude protein was determined by measuring nitrogen (N × 6.25) of the samples using the Kjeldahl method. Crude lipid content was measured by ether extraction using the Soxhlet method. Crude ash was determined by combustion at 550 °C in a muffle furnace.

Texture analysis of dorsal muscle

The texture of the dorsal muscle, including hardness, springiness, chewiness, cohesiveness, and gumminess, was determined with a CT3 Texture Analyzer (Brookfield Engineering Laboratories,

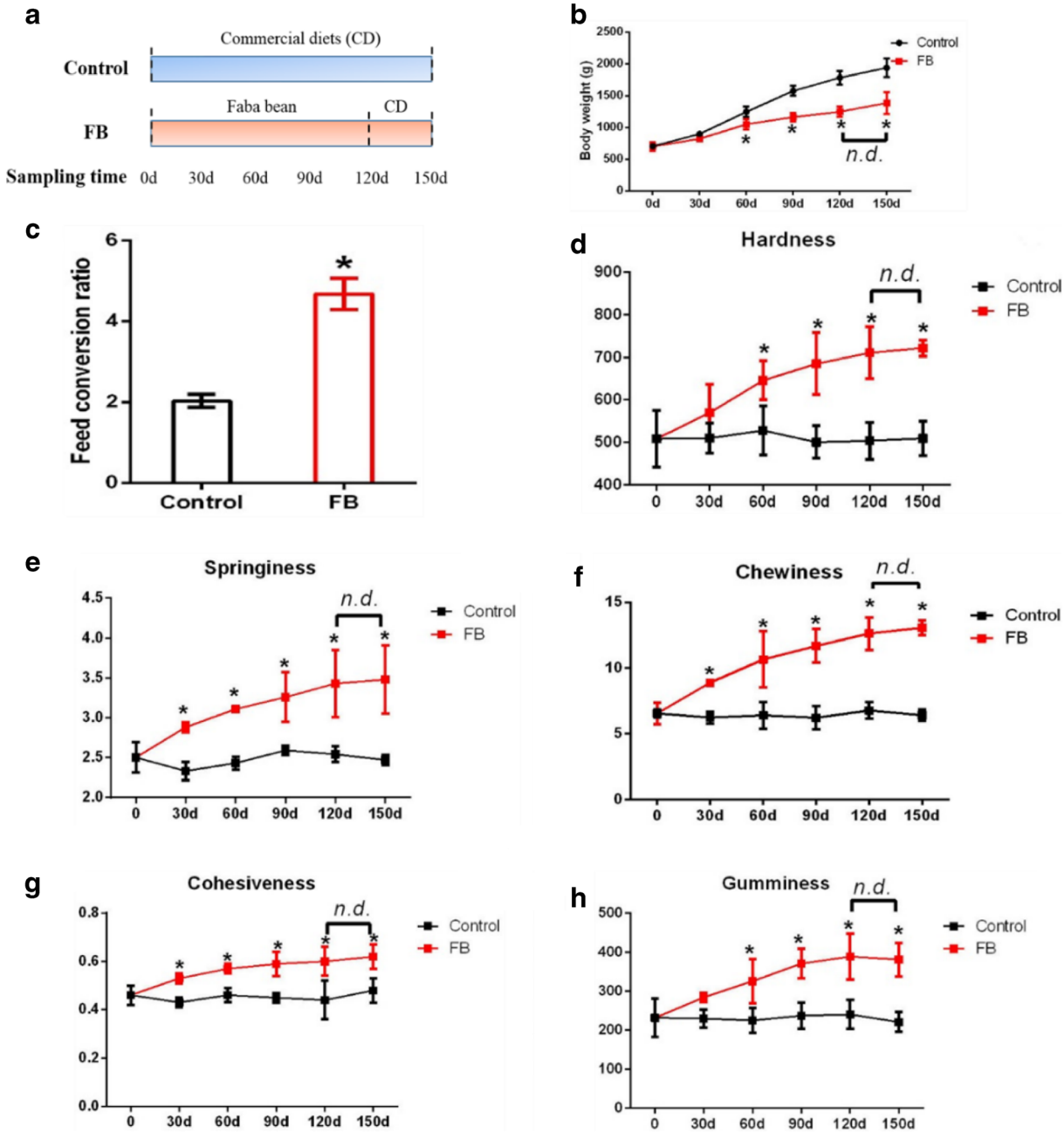

Fig. 1 Growth and TPA values of grass carp fed faba beans. **a** Schematic representation of the feeding experiment and sampling time. **b** Growth performance. **c** Feed utilization of grass carp after 150 days. **d**–**h** Hardness, springiness, chewiness, cohesiveness, and gumminess of the muscle in grass carp fed faba beans at different times. All results are presented as the means ± SD (error bars) ($n = 3$). Statistical significance at $P < 0.05$ is indicated by asterisks. No significant difference between fish fed faba beans for 120 days and fish fed subsequent commercial diet for 30 days is indicated by 'n.d.'

Inc., Brookfield, USA). A portion of grass carp back muscle (30 mm × 30 mm × 15 mm, at the junction of the fifth dorsal fin and the lateral line scales) was collected. A P35 cylindrical probe of the CT3 Texture Analyzer was used to test the compression speed at a pre-test speed of 2 mm s^{-1}, a post-test speed of 5 mm s^{-1}, and a test speed of 1 mm s^{-1}. The compression interval was 2 s, with a

compression ratio of 25%. Three replicates of each sample were collected, and each replicate was measured three times.

Serum triglyceride and total cholesterol analysis

Serum triglyceride (TG) and total cholesterol (T-chol) levels were assayed enzymatically using an automatic biochemical analyzer (Hitachi 7180, Tokyo, Japan) with the respective assay kits (Elikan Biological Technology Co., Ltd., Zhejiang, P.R. China).

Fatty acid composition analysis

Lipid extractions from tissues (hepatopancreas and IPF; three individuals per pool) and diets were performed based on the method of Folch et al. (1957). The preparation of fatty acid methyl esters (FAME) was performed based on the method described previously (Tian et al. 2014; Tian et al. 2016b). Briefly, 1 mL hexane was added to dissolve the lipid fractions, and methyl esterification was performed for 1 h after adding 1 mL 0.4 M potassium hydroxide methanol. Then, 2 mL of distilled water was added to produce two separate layers. The top layer was removed and used for GC analysis. FAME were determined using an Agilent 7820a Series GC device (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector and a capillary column (HP-88, Agilent, USA; length 100 m, internal diameter 0.25 mm, film thickness 0.20 μm). Helium was used as a carrier gas; the split rate was 1/50; the injection and detection temperatures were 250 and 280 °C, respectively. The thermal gradient program started at 175 °C for 10 min, then the temperature increased to 220 °C within 20 min at a rate of 3 °C/min. The final temperature of 240 °C was reached by an increase rate of 4 °C/min in 10 min. A sample volume of 1 μL was injected for analysis. Individual methyl esters were identified by a comparison with known standards (47015-U, Sigma-Aldrich, Inc., St. Louis, USA). The results of identified fatty acids were presented as percentage of total fatty acids.

Hepatopancreatic lipid metabolism enzymes activities

Lipid metabolism-related enzymes, including lipoprotein lipase (LPL), malate dehydrogenase (MDH), and glucose 6-phosphate dehydrogenase (G-6-PDH) in the hepatopancreas were measured using commercially available assay kits (Jiancheng Biotech Co., Nanjing, China) according to manufacturer's instructions.

Real-time quantitative RT-PCR

Hepatopancreatic tissue samples of three fish per pool were used for gene expression analyses. RNA extraction, cDNA synthesis, and gene expression measurements were performed as described previously (Tian et al. 2014). The primer sequences for the β-actin, *lpl*, fatty acid synthase (*fas*), adipose triglyceride lipase (*atgl*), and carnitine palmitoyltransferase-1 (*cpt-1*) gene fragments are listed in Table 2. After the RT-PCR reactions, melting curves were analyzed to confirm specific amplification. A relative quantification method, the comparative CT method ($2^{-\Delta\Delta Ct}$), was used to calculate gene expression values (Livak and Schmittgen 2001; Pfaffl 2001).

Statistical analyses

All data are shown as means ± standard deviation (SD). Percentages were arcsine-transformed for analysis. An independent samples *t* test was used to compare differences between the two experimental groups. The analyses were conducted using the PASW Statistics 18 software (SPSS, Chicago, IL, USA). Statistical significance is reported at $P < 0.05$.

Table 2 Primers used in real-time quantitative PCR

Primer	Sequence(5′-3′)	Accession number
β-actin-F	GCTTCACCACCACAGCTGAG	M25013
β-actin-R	TGTCCGTCAGGCAGCTCATA	
FAS-F	GTGGTGTATGCCACCGCTTA	GQ466046
FAS-R	CAATAGCAATAGCGGCCTGT	
LPL-F	TGGACCTCACCGACATTGAG T	FJ716100.1
LPL-R	ACATACCGGTGACCGACCAT	
ATGL-F	CTTCCGTGGTGTGCGTTATG	335999282
ATGL-R	CATGGAAGCTGGTG GAACTG	
CPT-F3	TTGGCTGATGATGTGGACTC TC	332650711
CPT-R2	GAGCTGGATGAAGGCATCTG	

Results

Growth and muscle texture

No mortality was observed over the experimental period of 150 days. Fish fed faba beans showed significantly lower body weight than the controls from days 30 to 120, which was not reversed by providing the commercial diet for another 30 days (Fig. 1b). The FCR in the treatment group was significantly lower than in the control group (Fig. 1c). To assess crispness of grass carp fed faba beans, hardness, springiness, chewiness, cohesiveness, and gumminess of the dorsal muscle were measured (Fig. 1d–h). Only springiness, chewiness, and cohesiveness were significantly increased in the experimental group after 30 days. After 60 days, hardness, springiness, chewiness, cohesiveness, and gumminess were significantly increased by 27.04, 24.40, 62.90, 23.91, and 40.73%, respectively. After 120 days, this increase was 39.76, 37.20, 92.82, 30.43, and 68.17%, respectively. The additional 30 days of commercial diet did not affect any of these parameters significantly.

Lipid accumulation

The parameters CF, VI, HI, and IPFI increased in faba bean–fed fish compared with those in the control, which was significant after 120 days (CF), 60 days (VI), 90 days (HI), and 90 days (IPFI) (Fig. 2a–d). However, no variation in these parameters was observed in the treatment group from 120 to 150 days. The hepatopancreatic fat content in faba bean–fed fish increased significantly with time (Fig. 2e). Muscle fat content produced no obvious pattern, but significantly lower levels were observed in the treatment group after 30 and 150 days (Fig. 2f). Serum TG and T-chol were significantly lower in fish fed faba beans, and the difference in serum TG concentrations was significant from 120 to 150 days (Fig. 2g, h).

Fatty acid composition

Primary fatty acid composition of the hepatopancreas and IPF are shown in Fig. 3. Saturated fatty acids (SFA) were significantly higher after 60 days but significantly lower after 120 and 150 days in the hepatopancreas of fish fed faba beans (Fig. 3a). In contrast, this type of fatty acids did not show a decrease in IPF until 60 days of feeding faba beans (Fig. 3e). Monounsaturated fatty acid (MUFA) content increased significantly in the hepatopancreas and IPF of fish fed faba beans (Fig. 3b, f). The proportion of n-6 polyunsaturated fatty acids (PUFA) and n-3 PUFA in the hepatopancreas and IPF showed significantly lower values in the treatment group than in the control (Fig. 3c, d and g, h).

Lipid metabolism-related enzyme activities in the hepatopancreas

The LPL activities in the hepatopancreas were significantly lower in the treatment than in the control group, and this trend remained after the treatment group received the commercial diet for another 30 days (Fig. 4a). In contrast, MDH and G-6-PDH showed significantly higher activities in faba bean–fed fish than in the control (Fig. 4b, c). After feeding the commercial diet for another 30 days, the G-6-PDH activity decreased to its initial levels (Fig. 4c).

Lipid metabolism-related gene expression in the hepatopancreas

Lipid metabolism-related genes, including *lpl*, *fas*, *atgl*, and *cpt-1*, were analyzed (Fig. 5). The mRNA levels of *lpl*, and *fas* were significantly lower in the treatment group than in the control (Fig. 5a). Similarly, the *fas* mRNA levels also decreased in fish fed faba beans compared with the levels in the control but returned to the previous level after feeding the commercial diet for 30 days (Fig. 5b). Regarding lipid catabolism genes, faba bean feeding significantly increased the mRNA levels of *atgl* but significantly decreased mRNA levels of *cpt-1* (Fig. 5c, d). Upregulation of *atgl* gene expression was not reversed by feeding the commercial diet, and *cpt-1* gene expression did not differ significantly between 120 and 150 days.

Discussion

The faba bean is native to north Africa, southwest and south Asia, and is extensively cultivated in other geographical regions. The seeds are rich in protein and energy, which is why faba bean production has a long history of numerous applications in human food and animal feed production (Crépon et al. 2010). Faba beans have been used in aquaculture for decades, particularly

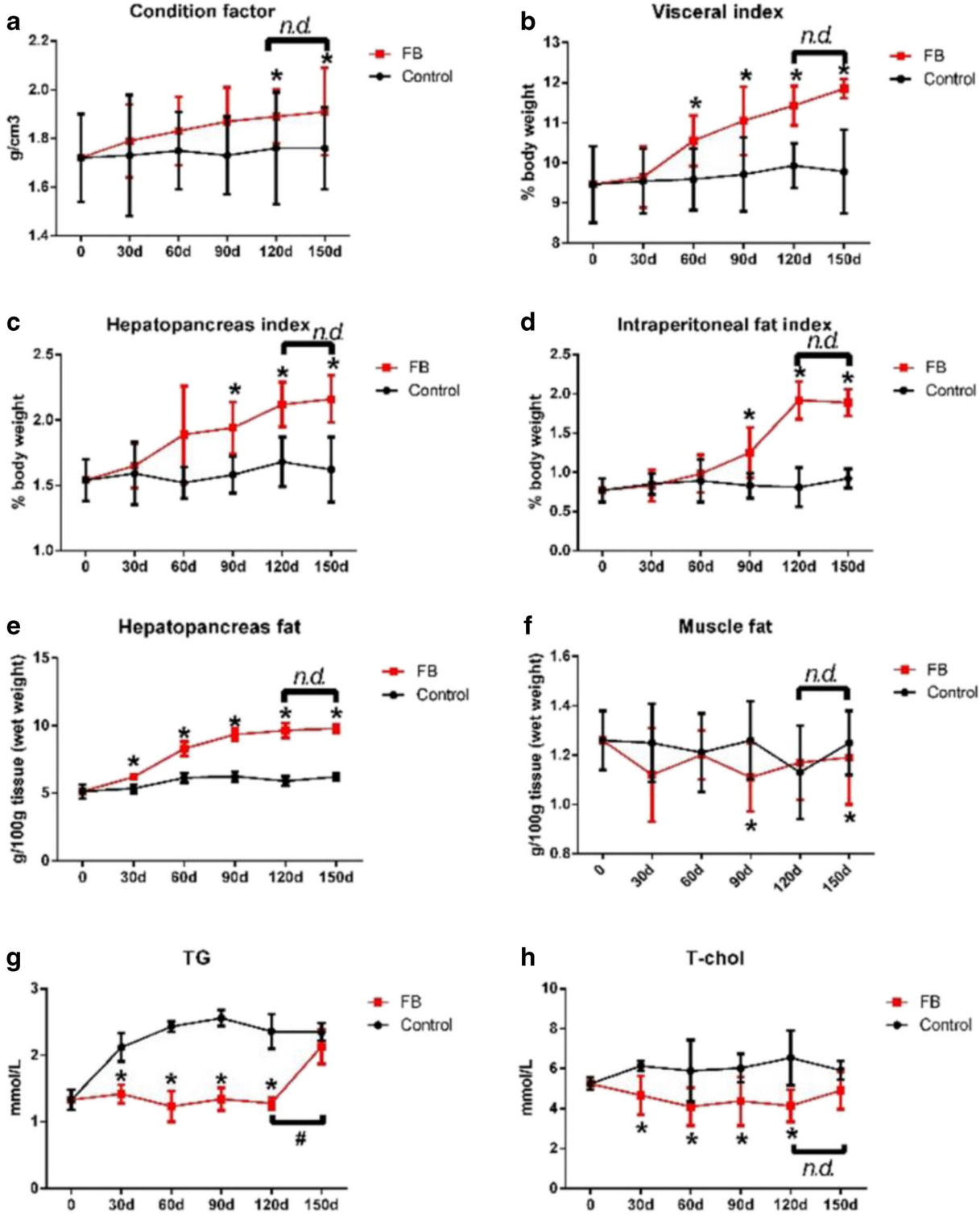

Fig. 2 Biological parameters and lipid accumulation of grass carp fed faba bean at different times. Means ± SD (error bars) ($n = 3$). Statistical significance at $P < 0.05$ is indicated by asterisks or hash symbols. No significant difference between fish fed faba beans for 120 days and fish fed subsequent commercial diet for 30 days is indicated by 'n.d.'

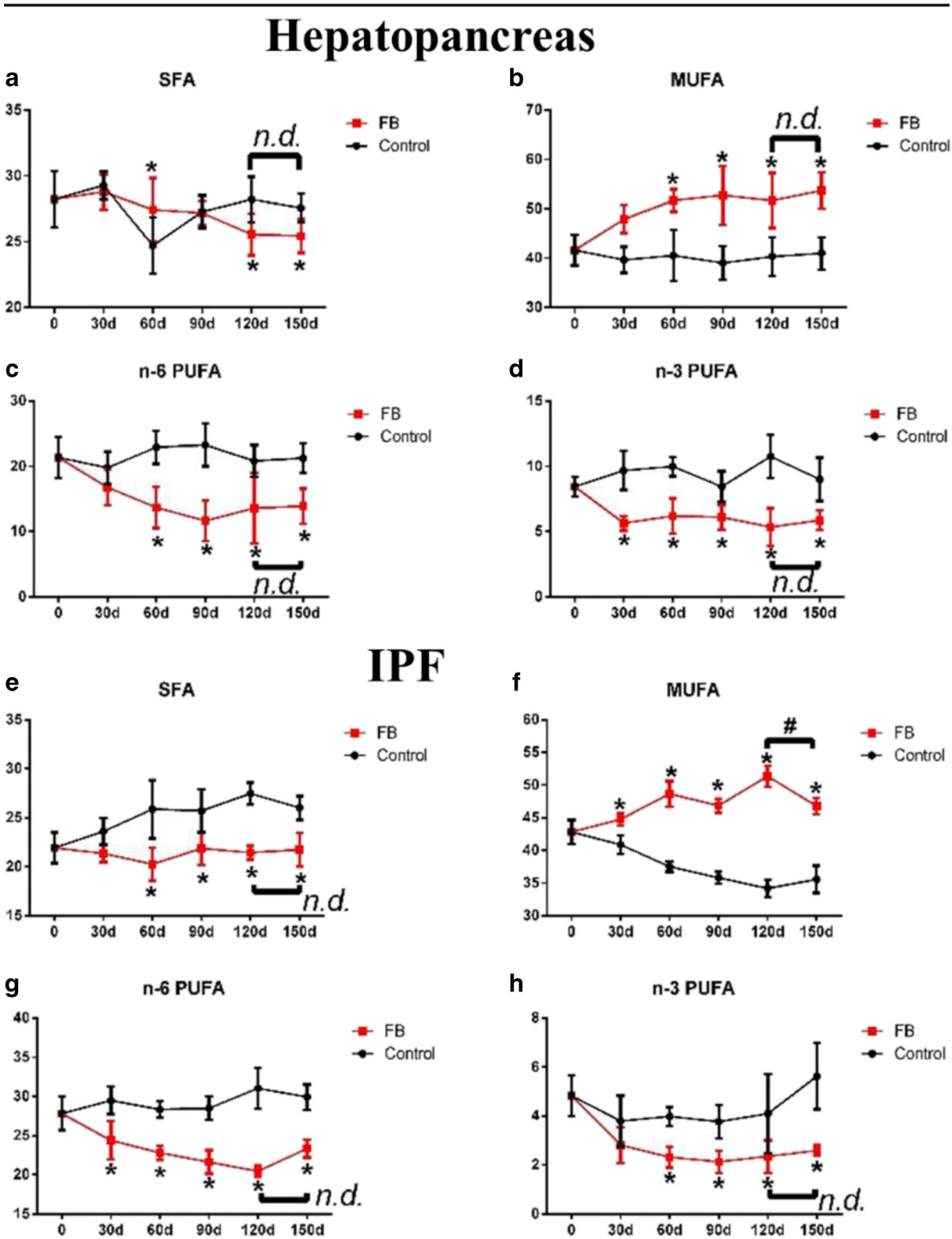

Fig. 3 Fatty acid composition of the hepatopancreas and intraperitoneal fat (IPF) in grass carp fed faba bean, at different times. Means ± SD (error bars) ($n = 3$). Statistical significance at $P < 0.05$ is indicated by asterisks or hash symbols. No significant difference between fish fed faba beans for 120 days and fish fed subsequent commercial diet for 30 days is indicated by 'n.d.'

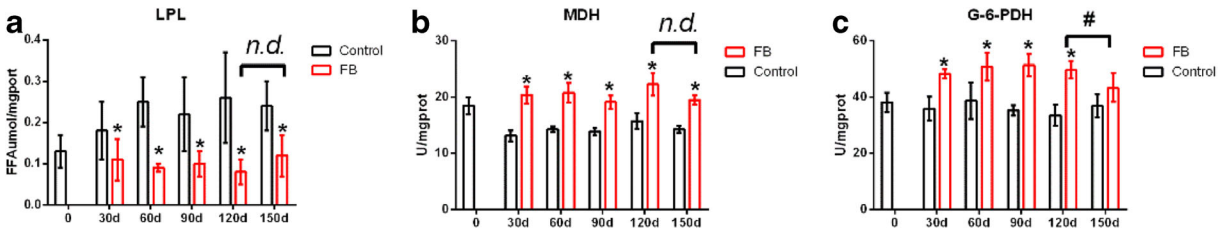

Fig. 4 Activities of lipid metabolism-related enzymes in grass carp fed faba beans, at different times. Means ± SD (error bars) ($n = 3$). Statistical significance at $P < 0.05$ is indicated by asterisks or hash symbols. No significant difference between fish fed faba beans for 120 days and fish fed subsequent commercial diet for 30 days is indicated by 'n.d.'

in order to produce crisp grass carp which achieves a high market value. Currently, however, crisp grass carp shows poor growth, low motility, and overall poor health. In the present study, lipid accumulation in grass carp increased with time of feeding faba bean, and effects of this diet on several key molecular mechanisms involved in lipid metabolism were observed. This study thus provides reference values of several physiological parameters following the use of faba beans in aquaculture.

Faba bean–fed grass carp showed lower weight gains than grass carp fed a commercial diet. Similar results were observed previously in other fish such as channel catfish (Zhu et al. 2012), crucian carp (Li et al. 2007), and gilthead seabream (Adamidou et al. 2011). Faba beans contain 27.48% protein, which nearly equals the protein content of the commercial diet (29.62%). Thus, the lower lipid content of faba beans (0.75%) may partially explain slower growth in fish of the treatment group. This is in line with numerous studies showing that low dietary lipid levels can reduce growth in grass carp (Du et al. 2005; Li et al. 2016). However, anti-nutritional factors in faba beans such as phytic acid, α-galactosides, and tannins may also impede the absorption of nutrients (Crépon et al. 2010; Sharma and Sehgal 1992; Vidal-Valverde et al. 1998), and thereby further slowdown growth. The characteristics of muscle

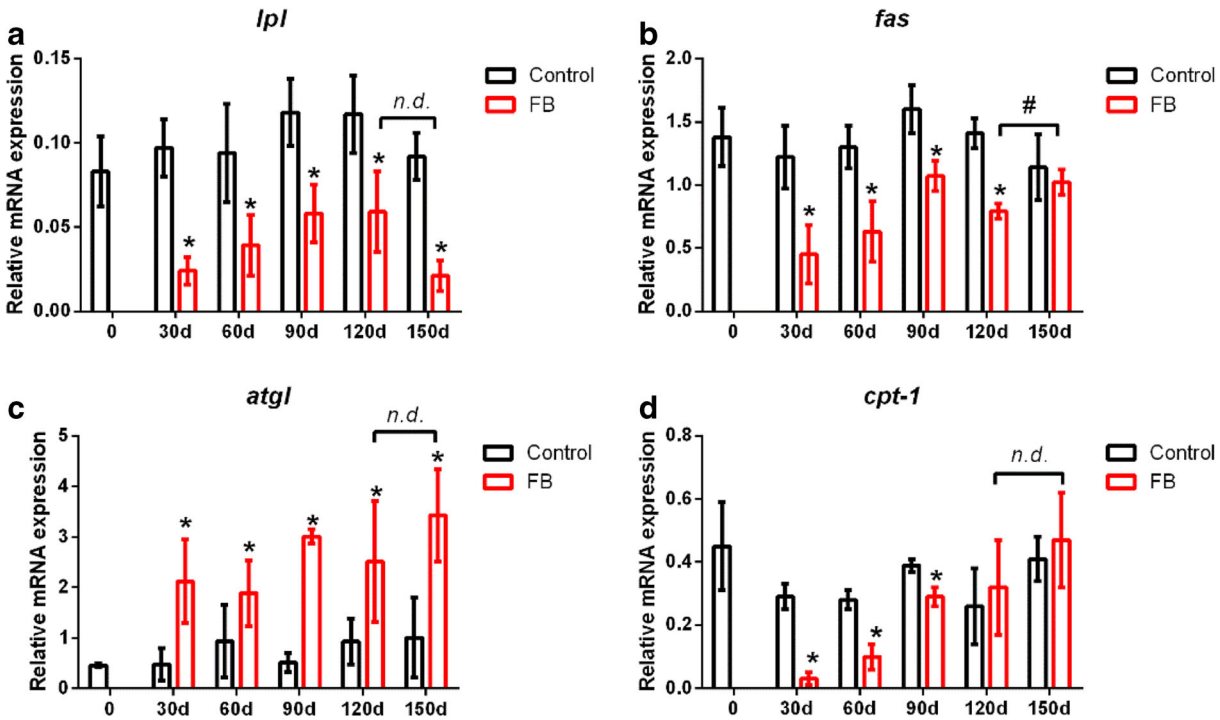

Fig. 5 Levels of mRNA of lipid metabolism-related genes in grass carp fed faba beans, at different times. Means ± SD (error bars) ($n = 3$). Statistical significance at $P < 0.05$ is indicated by asterisks or hash symbols. No significant difference between fish fed faba beans for 120 days and fish fed subsequent commercial diet for 30 days is indicated by 'n.d.'

texture, including hardness, springiness, chewiness, cohesiveness, and gumminess are useful to assess the quality of crisp grass carp (Lin et al. 2016; Yang et al. 2015). In the current study, these characteristics gradually increased with time in the treatment group, which is in line with the results of a previous study (Yu et al. 2017). Crispness of grass carp meat is due to an increase in muscle fiber numbers and decrease in muscle fiber diameters compared with that in ordinary grass carp (Yu et al. 2017), which indicated that a faba bean diet may affect myocyte development. Our results indicated that the trends were not reversed by feeding a commercial diet for another 30 days, suggesting that muscle fiber hyperplasia cannot be reversed for some time.

Despite slower growth, crisp grass carp tended to accumulate fat as indicated by the CF. Furthermore, our results showed that VI and HI increased with time in the treatment group. This may partially be explained by increased fat accumulation in the visceral tissue such as adipose hepatopancreatic tissue (Tian et al. 2017). Muscle tissue, however, showed no obvious lipid accumulation, which was lower in the treatment than in the control group. In general, dietary lipid levels strongly affect lipid deposition in the body of fish as observed in various fish species, including grass carp (Du et al. 2006; Li et al. 2016; Xie et al. 2017; Zhou et al. 2017). However, a reversed pattern was observed in the present study. Low dietary lipid content in the treatment group resulted in higher lipid accumulation in the viscera, suggesting modified lipid metabolism pathways. Moreover, serum TG and T-chol levels were consistent with the dietary lipid content. These results demonstrate that the transport of lipids from the viscera to peripheral tissue may be challenging in crisp grass carp, possibly due to lower growth or motility. Interestingly, the additional feeding of commercial diets for 30 days did not affect lipid accumulation in crisp grass carp, suggesting that the faba bean diet may have long-lasting effects on lipid metabolism.

The increase of lipid accumulation in the viscera was also reflected in the fatty acid composition. In the present study, no obvious difference of the SFA, MUFA, and n-6 PUFA content was observed between diets; however, the content of these fatty acids significantly changed in the hepatopancreas and the IPF. Specifically, SFA and n-6 PUFA content decreased, whereas MUFA percentage increased in these two tissues. A previous study showed MUFA to be the main component of neutral lipids in grass carp, whereas SFA and LC-PUFA are readily retained in polar lipid (Tian et al. 2017). Thus, the change of the levels of fatty acids indirectly suggested an increase of TG levels in the hepatopancreas and in IPF of crisp grass carp.

LPL is considered a key enzyme of lipid accumulation and metabolism in various tissues and is important for lipoprotein TG removal from circulation (Eckel 1989; Nilsson-Ehle et al. 1980). Both the *lpl* mRNA level and the activity of LPL decreased in the hepatopancreas of faba bean–fed grass carp, potentially due to the lower serum TG and T-chol concentrations. However, autoregulation in fatty tissue to decrease lipid absorption cannot be excluded. MDH and G-6-PDH can provide NADPH and NADH which are essential for lipid synthesis, and these enzymes are considered key molecular markers for lipogenesis (Ji et al. 2011; Rincón-Cervera et al. 2016). In the present study, the higher activities of MDH and G-6-PDH suggests that faba bean may increase the lipogenic capacity of grass carp. Fatty acid synthase is a key enzyme in de novo fatty acid synthesis that catalyzes the entire pathway of palmitate synthesis from malonyl-CoA (Smith et al. 2003). The downregulation of *fas* transcription may be a feedback mechanism in response to high lipid accumulation in the hepatopancreas. Similar results were found in other studies on grass carp (Leng et al. 2012; Li et al. 2016). ATGL catalyzes the first step for the sequential hydrolysis of TG (Sun et al. 2016). Although an increase of *atgl* expression can reduce the liver TG levels and promote fatty acid oxidation (Reid et al. 2008), obesity is typically accompanied by higher *atgl* expression and NEFA levels (Hu et al. 2016; Rosen and Spiegelman 2014). In the present study, the upregulation of *atgl* in faba bean–fed carp seemed not induce fatty acid degeneration as suggested by the low *cpt-1* gene expression (Bonnefont et al. 2004), which may be due to obesity. Moreover, low *cpt-1* mRNA levels suggest that lipid catabolism capacity was attenuated in faba bean–fed carp, possibly because lipid accumulation increased in the viscera. Overall, our results indicate that the molecular mechanism of excessive lipid accumulation in the hepatopancreas in faba bean–fed grass carp is a cumulative result of increasing lipogenesis and decreasing lipid oxidation. However, the diet components (or anti-nutritional factors) remain to be identified.

In conclusion, we showed that gradual lipid accumulation in the viscera occurred in grass carp after feeding faba beans, which may be caused by increased lipogenesis, as well as by a reduction of lipid catabolism.

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