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Effects of food restriction on growth, body composition and gene expression related in regulation of lipid metabolism and food intake in grass carp

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ABSTRACT

It is well known that most fish would prefer to use body lipid stores for energy expenditure when receiving a long-term food restriction. However, the mechanism of this is still not clear. In the present study, a growth experiment was carried out to investigate the effects of food restriction on growth performance, gene expression related in regulation of lipid metabolism and food ingestion in grass carp (*Ctenopharyngodon idellus*). Four rations, satiation (S), 80% S, 60% S and 40% S, were adopted in this study. Each treatment was randomly assigned to triplicate net cages of 15 fish (177.3 ± 3.3 g) per cage. The experiment lasted for 49 days at 30.0 ± 3.0 °C. The experimental results showed that a significant increase in feeding rate and weight gain was found in grass carp with the increased ration level. The body lipid and energy content of the grass carp exhibited a significant decrease when receiving food restriction. The transcriptional levels of the genes involved in lipogenesis (*srebp*-1c, *fas*, *pparγ*) were down-regulated at the rations of food restriction. The relative expression of hepatic *fas* (fatty acid synthetase) and *srebp*-1c (sterol regulatory element-binding protein 1c) in the fish at satiation were significantly higher than the restricted-fed groups. Similarly, the expressions of hepatic *pparγ* (peroxisome proliferator-activated receptor-γ) in the fish at the ration of satiation and 80% S were significantly higher than the group at the low ration of 40% S. However, the expression of hepatic *cpt*-1*a* (carnitine palmitoyl transferase I) involved in fatty acid β-oxidation in fish was significantly up-regulated when receiving food restriction. Other hepatic lipolysis genes of *pparα* (peroxisome proliferators-activated receptor α) and *hl* (hepatic lipase) didn't show any significant changes in restricted-fed fish. The transcriptional levels of hepatic *leptin* and hypothalamus *pomc* (proopiomelanocortin) were significantly down-regulated in fish fed with restricted rations. But the hypothalamus *npy* (neuropeptide Y) and *lepr* (leptin receptor) had no change. The present results indicated that a long-term food restriction could cause less accumulation of lipid and could be through a way of down-regulating lipogenesis genes and up-regulating lipolysis genes. Long-term restriction could also activate the appetite of grass carp by down-regulating some anorexigenic genes.

Statement of relevance: Food restriction for some time could lead to a suitable lipid storage, in case of accumulation of fatty acid profile and lipid, in cultured grass carp.

1. Introduction

In nature, almost all animals have to cope with some time of food shortage during their lifetimes (Knapp, 2016). When animals suffer from food shortage, they need internal energy stores to support the life activities. The energy balance between input and output determines the life status of the body and it depends on a number of biotic and abiotic factors such as temperature, light-dark cycle and food (Fauconneau and Paboeuf, 2001; Assis et al., 2004; Johnston, 2006). Hunger is one of the important factors to trigger the physiological and physical changes in juveniles and adults. Besides, the energy status can regulate the appetite of organisms (Gélineau and Boujard, 2001; Gélineau et al., 2001). Animals have evolved a complex and precise metabolic system to adapt to all kinds of nutritional states (Soengas, 2014).

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It has been frequently proved that body composition is significantly affected by food restriction. In fishes, lipid is stored in the liver, viscera, and muscles. Lipids are broken down early in food restriction, and often constitute the main energy source for maintenance of fish during starvation. In the sea bream (*Sparus aurata*), the lipid content of muscle was significantly decreased from 6.77% to 4.78% after a 30-day food restriction. While, the crude protein content of the muscle was invariant with different rations (Suárez et al., 2010). A similar change was found in channel catfish (*Ictalurus Punctatus*) that a 28-day food deprivation markedly decreased the fillet lipid content of the fish (Weber and Bosworth, 2005). And a 2-month food restriction also reduced the body lipid content of juvenile Atlantic salmon (*Salmo salar* L.) from 7.6% to 5.7% (Trombley et al., 2012). In juvenile Atlantic halibut (*Hippoglossus hippoglossus*), the fillet lipid content was significantly lower than the control group after a 16-day starvation. But no change was observed in protein content of the starved halibut (Heide et al., 2006).

Although the lipid depletion during food restriction has been extensively reported in many fish species (Jobling, 1980; Hogendoorn, 1983; Reinitz, 1983; Hung et al., 1997), few studies could clarify the mechanism of the lipometabolism of the restricted-fed fish. The body lipid content depends on the balance of the anabolism and catabolism of lipid. Therefore, in the present study, we hypothesized that the sharp lipid depletion in food-restricted fish is regulated by the inhibited lipogenesis and the promoted lipolysis.

In recent years, many key lipometabolic genes in fishes have been cloned and characterized. The lipogenic genes include sterol regulatory element-binding transcription factor 1 (*srebp*-1) (Burke and Heisler, 2015), fatty acid synthase (*fas*) (Leng et al., 2012) and peroxisome proliferator-activated receptor γ (*pparγ*) (Kaneko et al., 2016). *srebp*-1 plays important regulative roles in lipogenesis, and the protein encoded by *srebp*-1*c* isoform bind to the specific sites in the promoter of different lipogenic genes (Eberle et al., 2004; Jeon and Osborne, 2012). And the *fas* is one of the important *srebp*-1*c* targeted lipogenic genes (Bennett et al., 1995). While the *pparγ* is considered to play a critical role in lipid accumulation (Rosen and Spiegelman, 2001) and adipogenesis in the adipocytes (Farmer, 2005). The lipolytic genes include carnitine palmitoyltransferase I (*cpt*-1) (Morash et al., 2009), peroxisome proliferator-activated receptor α (*pparα*) (Leaver et al., 2005) and hepatic lipase (*hl*) (Burke and Heisler, 2015). The *cpt*1 is a critical gene in mitochondrial fatty acid β-oxidation (Kerner and Hoppel, 2000), and is regulated through a *pparα*-independent pathway (LeMay et al., 2005). *Cpt*-1*a*, one isomeride of *cpt*1, was mainly expressed in liver (Price et al., 2002). The *hl* (hepatic lipase) is one of lipoprotein lipases, a kind of key genes in lipolysis (Leaver et al., 2008).

The present study investigated the effects of food restriction on growth, body composition and the transcriptional expression of lipogenic genes (*srebp*-1*c*, fas, pparγ) and lipolytic genes (*cpt*-1*a*, *pparα*, *hl*) in grass carp (*Ctenopharyngodon idellus*). Moreover, food intake is regulated by hypothalamic arcuate nucleus, which contains the endogenous anorectic melanocortin receptor agonist (POMC cells) and the endogenous orexigenic genes (Heisler et al., 2006), and some peripheral signals, such as *leptin* and other gastrointestinal hormones (Ahima and Osei, 2004). Therefore, the gene expression of the orexigenic gene of neuropeptide Y (*npy*) and the anorexigenic genes of pro-opiomelanocortin (*pomc*), *leptin* and *leptin* receptor (*lepr*) were also analyzed in the present study.

2. Materials and methods

2.1. Experimental design

Four ration levels were adopted in this study: satiation (S), 80% of satiation (80% S), 60% of satiation (60% S) and 40% of satiation (40% S). The satiation group used the feeding platforms for the ad libitum feeding. One cultured net cage was equipped with one feeding platform at the bottom of the cage. The feeding platform is a nylon gauze disc with the diameter of 100 cm. By lifting up the feeding platform, the uneaten feeds could be observed and the daily feeding amounts were adjusted to make sure that fish could receive the ad libitum feeding (Sveier et al., 2001).

2.2. Experimental procedure

The experimental fish were one-year-old grass carp (*Ctenopharyngodon idellus*), which were obtained from the station of the Institute of Hydrobiology, Chinese Academy of Sciences (Huangshi, Hubei, China). Prior to the experiment, all fish were acclimated for 4 weeks in floating net cages (2.0 m × 2.0 m × 2.0 m, water depth: 1.7 m) of a pond and fed with a commercial feed (catalogue number: 1035, Tongwei Group, Shashi, Hubei, China). The crude protein and lipid content of the feed were 296.2 g kg^{-1} and 48.8 g kg^{-1} respectively.

At the beginning of the feeding trial, all fish were fasted for 24 h. Then 15 fish per net cage were bulk-weighed (individual initial body weight of 177.3 ± 3.3 g) and randomly distributed into one of 12 net cages. Each ration level was randomly assigned to triplicate cages. Fish were hand-fed with the commercial feed at one of 4 ration levels. Fish were fed 3 times daily at 8:00, 12:30 and 18:30. Feed consumption was recorded daily. The experiment was executed under ambient temperature (30.0 ± 3.0 °C) and natural photoperiod. During the experiment, ammonia-N was kept under 0.1 mg L^{-1}. Dissolved oxygen was 5.0–7.5 mg L − 1 and pH was 8.2–8.4. Chlorophyll concentration was 121.4 ± 17.6 μg/L. The experiment lasted for 7 weeks. At the end of the experiment, all fish were bulk-weighed to get the final mean weight. All fish were conducted according to the Guiding Principles for Care and Use of Laboratory Animals and were approved by the Institute of Hydrobiology, Chinese Academy of Science (Approval ID: IHB 20140724).

2.3. Sample collection

At the start of the trial, triplicate groups of three fish were sampled randomly for initial body composition analysis. At the end of trial, fish were anesthetized by MS-222 (100 mg L^{-1} tricaine methane sulfonate, Argent Chemical Laboratories Inc., Redmond, WA, USA). Two fish from each cage were sampled and frozen at −20 °C to determine the whole body composition. Another two fish per cage were randomly sampled and the liver and hypothalamus were dissected on ice, frozen immediately in liquid nitrogen and stored at −80 °C until RNA extraction and the analysis of gene expression.

2.4. Chemical analysis

Proximate composition analysis of all samples was conducted using the methods described by AOAC (2003). Moisture content was determined by oven drying at 105 °C to constant weight. Crude protein content (N × 6.25) was determined by Kjeldahl method after acid digestion using 2300 Kjeltec Analyzer Unit (FOSS Tecator, Haganas, Sweden). Crude lipid content was determined by ether extraction in a Soxtec system (Soxtec System HT6, Tecator, Haganas, Sweden) with diethyl ether as extraction liquid. Crude ash content was determined by incineration in a muffle furnace at 550 °C for 12 h. Energy density was determined by combustion in an adiabatic micro bomb calorimeter (Phillipson micro bomb calorimeter, Gentry Instructions Inc., Aiken, SC, USA). At least duplicate analyses were conducted for each sample.

2.5. qRT-PCR analysis

Total RNAs of hypothalamus and liver were extracted by trizol method. Total RNAs were quantified at 260 and 280 nm to evaluate the concentration and purity. One percent agarose gel electrophoresis was also used to assess the quality. Total RNAs with clear ribosomal bands and

high RNA ratios (A260/A280 = 1.8–2.0) were used for further analysis. The isolated RNA was reverse transcribed using an M-MLV First-Strand Synthesis Kit (Invitrogen, Shanghai, China), and the obtained cDNA was used in subsequent polymerase chain reaction (PCR). The PCR primers (Table 1) were designed based on the published sequences from grass carp in GenBankK, and were synthesized by Icongene Company (Wuhan, Hubei, China). The PCR amplification conditions consisted of a denaturation step at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s with different annealing temperatures and extension times corresponding to the primers sequences length. The PCR products were fractionated using 1% agarose gel electrophoresis, then the expected band was observed under the UV light.

Primers for quantitative real-time PCR (qRT-PCR) were designed based on the obtained fragment cDNA sequences. They were given in Table 1 with annealing temperature and size of products. One microgram total RNA per sample was reverse transcribed using an Invitrogen™ M-MLV First-Strand Synthesis Kit (Invitrogen, Shanghai, China). No reverse transcriptase or total RNA replaced by sterile double-distilled water was performed as negative controls for detecting genomic DNA contamination and reagent pollution. Before the quantitative test, a mixture of test templates was used to determine the amplification efficiency of the reference gene and the target genes by serial dilution. QPCR amplifications were carried out in duplicate in a final volume of 20 μL containing 1 μL cDNA, 1000 nM each primer and 10 μL iQ™ SYBR® Green Supermix (Bio-Rad, USA). A negative control was done with the template replaced by sterile double-distilled water. The qPCR was initiated at 95 °C for 2 min, followed by 35 cycles of a three-step amplification program (15 s at 95 °C, 18 s at the specific primer annealing temperature and 12 s at 72 °C). After amplification, a melt curve of 0.5 °C increments from 65 °C to 95 °C was performed to confirm the amplification of a single product. β-actin was chosen as internal references for normalization. Expression levels were calculated according to Vandesompele et al. (2002).

2.6. Statistical analysis

All statistical analyses were carried out using SPSS 13.0 software package for Windows (SPSS, Chicago, IL, USA). Data were expressed as means ± S.E. (standard error of the mean) and checked for normality and homogeneity of variances among groups prior to their comparison and subjected to one-way ANOVA analysis of variance to test the effects of ration level. Duncan's multiple-range test with $P < 0.05$ was subsequently used to identify significant differences. The linear regression analysis was used to analyse the relationships between SGR, WGR, FE and FR.

3. Results

3.1. Growth performance

Growth and feed utilization in grass carp fed with different ration levels are listed in Table 2. Food restriction had serious effects on the growth performance of grass carp. For the experimental fish fed with four different ration levels, the poorest growth performance was found in the fish fed with 40% of satiation. The final body weight (FBW) and feeding rate (FR) of the experimental grass carp increased significantly ($P < 0.05$) with increasing ration level, and they peaked at the satiation group. The WGR and SGR increased linearly with increased FR. Regression analysis showed the following relationship between SGR and FR: $y = 0.4516x + 0.6204$ (Fig. 1a), and the relationship between WGR and FR was: $y = 60.263x - 8.9699$ (Fig. 1b). And the regression is both significant ($P < 0.05$). Protein retention efficiency (PRE) and feed efficiency (FE) decreased significantly ($P < 0.05$) with the increasing ration level. And regression analysis showed that the relationship between FE and FR was: $y = -9.1173x + 91.051$ (Fig. 1c). Numerically the lowest FE was for the satiation group but 80% S and S were not different statistically. For PRE, the 80% S treatment was numerically lowest but not different from the S treatment.

3.2. Body composition

The whole body composition exhibited different changes in grass carp when receiving a long-term food restriction (Table 3). No obvious change was found in the crude body protein content at the different ration levels after the 49-day experiment. However, when receiving the food restriction, the whole body lipid concentration of the grass carp had a significant ($P < 0.05$) declined from 6.0% of the 80% S group to 3.2% of the 40% S group. The energy density showed a similar pattern of change in the present study. Moisture content decreased significantly ($P < 0.05$) with the increasing ration levels. Ash content of the fish fed with 40% of satiation is markedly higher than the other three high ration levels.

Table 1
Primers used for quantitative RT-PCR (qPCR).

Gene name	Accession no.	Primer sequence (5′–3′)	Annealing temperature (°C)	Extension time (s)	Product (bp)
srebp-1c	KJ162572.1	F: AGAAACTGCCCATCAATCGC R: GTCAATGGCTTTCCTCAACACT	62	30	200
pparγ	EU847421.1	F: CGGGCTTTGTCAACCTGG R: GAGCGTGCCGTCTTTGTTC	60	25	110
fas	HM802556.1	F: GTCCACAGGGTGTCGTTCC R: CGAGGTCTTGGGCTCTTTATT	58	30	223
hl	FJ436064.1	F:CATTTATTCACATTTAGCCCAGTAC R: CAGGCAGTAGCCCTTATCG	57	25	169
pparα	FJ231987.1	F: TGTCAATACTGCCGTTTCCG R: GTGTTTGAGGTAGGCTTCGTG	60	25	195
cpt-1a	JF728839.1	F: TCCCACCTGAGTGCCAAAC R: AGTGCGAGCTGGATGAAGG	60	25	157
npy	KC342202.1	F: CGCCTGCTTGGGAACTCTTAC R: TTTGCCATACCTCTGCCTTGT	62.5	25	142
lepr	JQ080547.1	F: AGGACTCTGGGACCTGGACAAC R:ATATCCTCATCTTCTTCCTGCTTCTC	61.5	25	195
leptin	EU719623.1	F: CCACCCACCACATTACTT R: GCAACATTTCTGGCTTTCT	55	30	250
pomc	FJ692322.1	F: CGGCGTAGAGGAGGAATC R: GCCGTCCTTCTTCTGCTGATTT	57	25	118
β-actin	DQ211096.1	F: CGTGACATCAAGGAGAAG R: GAGTTGAAGGTGGTCTCAT	57	25	150

Table 2
Growth performance of grass carp fed at satiation or restricted rations.

	Ration			
	40% S	60% S	80% S	Satiation (S)
IBW(g)[a]	173.1 ± 1.5	176.5 ± 1.4	178.0 ± 2.0	175.1 ± 2.4
FR (% BW day^{-1})[b]	1.76 ± 0.02[a]	2.81 ± 0.02[b]	3.80 ± 0.05[c]	4.41 ± 0.08[d]
FBW(g)[c]	340.4 ± 6.4[a]	467.1 ± 4.6[b]	545.8 ± 13.7[c]	641.1 ± 15.7[d]
PRE (%)[d]	37.7 ± 2.7[b]	34.7 ± 1.4[b]	26.7 ± 1.0[a]	28.0 ± 1.0[a]

Values are presented as mean ± SEM of triplicate groups. Mean values sharing a common superscript letter in the same row are not significantly different ($P > 0.05$). The actual feeding rates of four groups were 41% of satiation, 64% of satiation, 86% of satiation and satiation, respectively.

[a] IBW: initial body weight (g).

[b] FR: feeding rate (% body weight day^{-1}) = 100 × (feed intake in dry matter) / [days × (initial body weight + final body weight) / 2].

[c] FBW: final body weight (g).

[d] PRE: protein retention efficiency (%) = 100 × (retained protein) / (protein intake).

3.3. Genes expression

Transcriptional levels of the genes involved in lipogenesis were significantly down-regulated at the rations from satiation to 40% of satiation (Fig. 2). The expression of hepatic sterol regulatory element-binding protein 1c (*srebp-1c*) in fish fed at satiation was significantly ($P < 0.05$) higher than the group with the low ration level of 40%, 60% and 80% of satiation (Fig. 2a). Similarly, the expressions of hepatic fatty acid synthase (*fas*) in fish at the ration of satiation was significantly ($P < 0.05$) higher than all other groups with the low ration levels (Fig. 2b). And there was a quite similar significant change ($P < 0.05$) was found in the expression of another lipogenesis related gene of peroxisome proliferators-activated receptor γ (*pparγ*) (Fig. 2c).

Meanwhile, the transcriptional levels of the genes involved in lipolysis were up-regulated at the rations from satiation to 40% of satiation. The expression of hepatic carnitine palmitoyl transferase 1A (*cpt-1a*) involved in fatty acid β-oxidation in fish at the ration of 40% of satiation was significantly ($P < 0.05$) higher than all other high ration groups (Fig. 2d). The other lipolysis related genes of peroxisome proliferators-activated receptor α (*pparα*) (Fig. 2e) and hepatic lipase (*hl*) (Fig. 2f) didn't show any changes in grass carp.

The expression of orexigenic gene of hypothalamus neuropeptide Y (*npy*) had no significant difference with the increasing rations (Fig. 3a). However, the transcriptional levels of anorexigenic genes of hepatic *leptin* (Fig. 3b) and hypothalamus *pomc* (Fig. 3c) were significantly ($P < 0.05$) down-regulated in restricted-fed fish. The expression of another anorexigenic gene of *leptin* receptor (long form) in the hypothalamus of grass carp fed with food restriction had no significant difference (Fig. 3d).

4. Discussion

In the present study, the relationship between specific growth rate (SGR) and ration levels (RL) of grass carp (*Ctenopharyngodon idellus*) is linear: y = 0.4516x + 0.6204 ($n = 12$, $R^2 = 0.9636$). This result agrees well with the previous study in grass carp (Cui et al., 1994), juvenile white sturgeon (*Acipenser transmontanus*) (Cui et al., 1996) and juvenile yellow grouper (*Epinephelus awoara*) (Sun et al., 2007). Nevertheless, in most carnivorous and omnivorous fishes, the relationship between growth and ration levels is decelerating curves (Brett

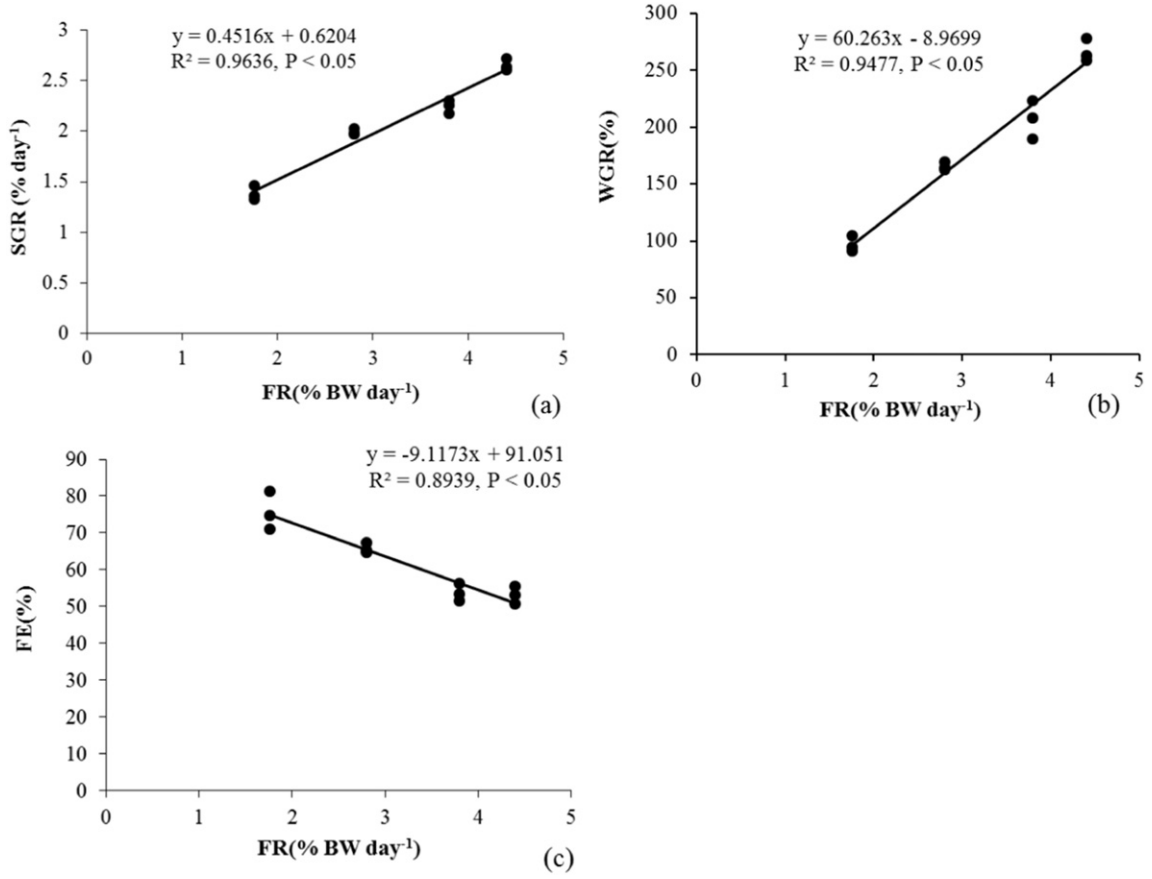

Fig. 1. Regression analysis between FR and a) SGR, b) WGR or c) FE in grass carp after a 49-day food restriction. WGR: weight gain rate (%) = 100 × (final body weight − initial body weight) / (initial body weight). SGR: specific growth rate (% day^{-1}) = 100 × [Ln (final body weight) − Ln (initial body weight)] / days. FE: feed efficiency (%) = 100 × (wet weight gain) / (feed intake in dry matter).

Table 3
Whole body composition of grass carp fed at satiation or restricted rations.

Whole body composition (% fresh weight)	Initial	Ration			
		40% S	60% S	80% S	Satiation (S)
Moisture	77.1 ± 0.4	77.2 ± 0.4^{c}	75.7 ± 0.7^{b,c}	74.8 ± 0.5^{a,b}	74.0 ± 0.4^{a}
Ash	3.6 ± 0.1	3.8 ± 0.1^{b}	3.3 ± 0.1^{a}	3.3 ± 0.04^{a}	3.4 ± 0.1^{a}
Crude protein	15.1 ± 0.5	14.9 ± 0.2	15.5 ± 0.3	15.0 ± 0.3	15.6 ± 0.7
Crude lipid	2.7 ± 0.5	3.2 ± 0.1^{a}	4.2 ± 0.3^{b}	6.0 ± 0.3^{c}	6.2 ± 0.1^{c}
Energy content (MJ kg^{-1})	4.7 ± 0.1	4.8 ± 0.1^{a}	5.4 ± 0.2^{b}	5.8 ± 0.2^{bc}	6.1 ± 0.1^{c}

Values are presented as mean ± SEM of triplicate groups. Mean values sharing a common superscript letter in the same row are not significantly different ($P > 0.05$).

and Groves, 1979). In juvenile *Pangasius bocourti*, it is an asymptotic curve described as SGR = 1.6913 + 0.3505 RL − 0.0146 RL^2 (Wirat, 2010); while it is a logarithmical equation: SGR = 3.8759 ln (RL + 1) − 3.7164, in juvenile cobia (*Rachycentron canadum*) (Sun et al., 2006). And these differences in relationships may be caused by species-specific differences, temperature differences or feed types (Brett and Groves, 1979). There is no obvious difference in FE, PRE, body lipid content and energy density between 80% S group and satiation group; while 40% S group and 60% S group are significantly lower than satiation group in various parameters about growth performance and body composition. From this perspective, it indicates that the efficiency of sub-satiation group (80% S) is pretty befitting.

In this study, the whole body lipid concentration of the grass carp was markedly reduced from 6.2% to 3.2% when receiving a food restriction of 40% satiation. This result has been verified in some other fishes. In juvenile gibel carp (*Carassius auratus gibelio*), a one/two-week deprivation could reduce the ratio of lipid to lean body mass (Xie et al., 2001). Significant reductions both in body lipid and protein content after a 2-week feed deprivation were found in *Labeo rohita* fingerling (Yengkokpam et al., 2014). After a 48-day starvation, there was a significant decrease in body lipid content from 4.31% to 0.93% in rainbow trout (Jezierska et al., 1982). Similarly, during a 15-day food restriction experiment, the body lipid content of juvenile cobia (*Rachycentron canadum*) was decreased from 3.89% to 1.92% with the decreasing rations from ad libitum to starvation (Sun et al., 2006).

The body lipid content is the balance between the lipogenesis and lipolysis. The large decline of fish body lipid content could be resulted from (1) stable lipogenesis and increased lipolysis, (2) decreased lipogenesis and increased lipolysis, (3) decreased lipogenesis and stable lipolysis, (4) slight increased lipogenesis and large increased lipolysis.

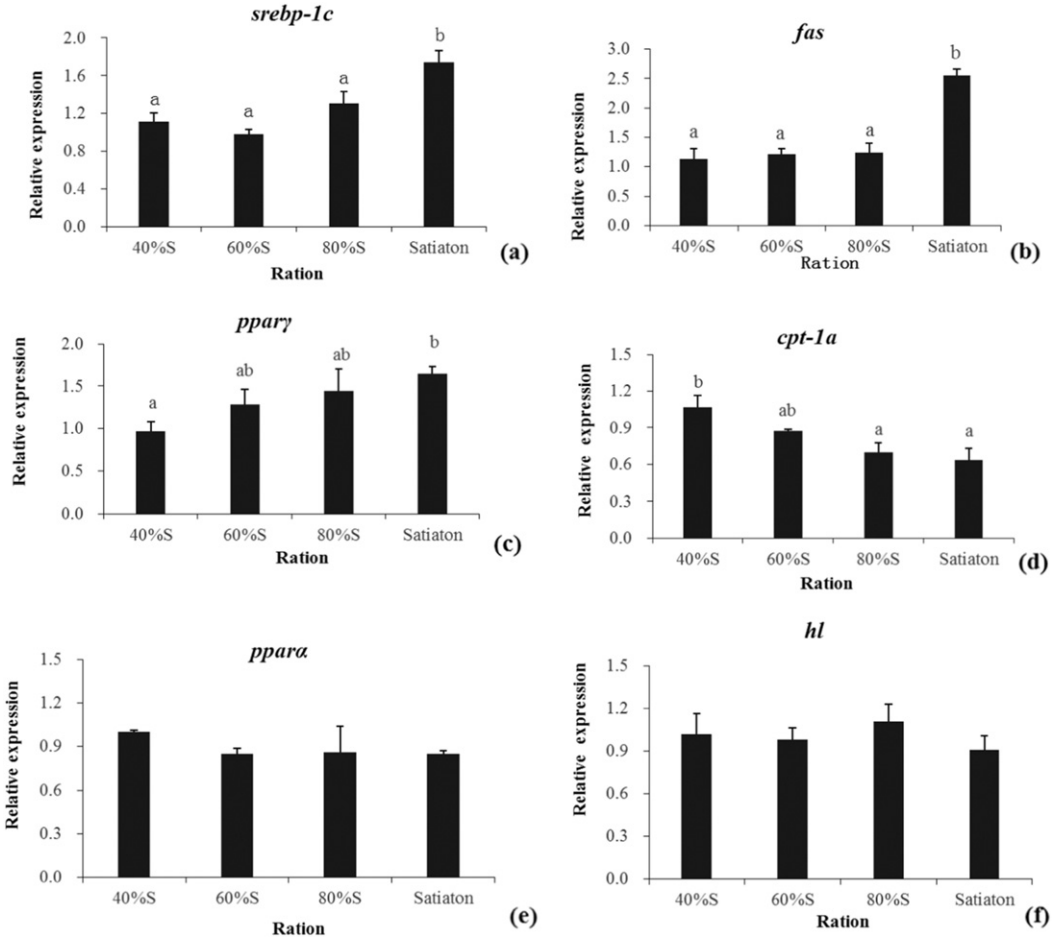

Fig. 2. Transcriptional expression of lipogenic genes (*srebp*-1c, *fas*, *pparγ*) and lipolytic genes (*cpt*-1*a*, *pparα*, *hl*) of liver tissue of grass carp fed at satiation or restricted rations. Columns represented the mean ± SE ($n = 3$) and gene expression levels are normalized against β-actin as reference gene and standardized to the group of 40% of satiation (40% S). Columns sharing a common superscript letter were not significantly different ($P > 0.05$).

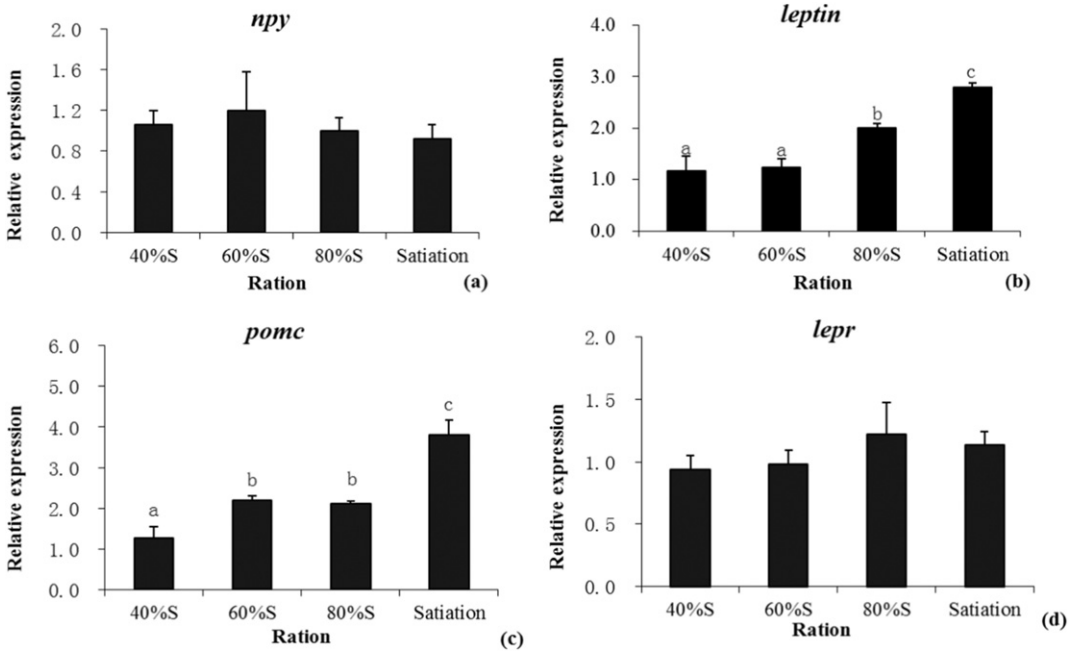

Fig. 3. Relative expression of hypothalamus *npy* (a), hepatic *leptin* (b), hypothalamus *pomc* (c) and hypothalamus *lepr* (d) genes of grass carp fed at satiation or restricted rations. Columns represented the mean ± SE ($n = 3$) and gene expression levels are normalized against β-actin as reference gene and standardized to the group of 40% of satiation (40% S). Columns sharing a common superscript letter were not significantly different ($P > 0.05$).

However, few previous literatures could elaborate the mechanism of lipometabolism of fish when receiving a time of food restriction. In the present study, we found that the inhibited lipogenesis (*srebp*-1*c*, *fas*, *pparγ*) and promoted lipolysis (*cpt*-1*a*) could lead to a less accumulation of lipid in grass carp (*Ctenopharyngodon idellus*) after a 49-day food restriction (Fig. 4). This indicated that the lipometabolism homeostasis was disturbed in grass carp suffering from food restriction.

In this study, the genes involved in lipogenesis and lipid accumulation (*srebp*-1*c*, *fas*, *pparγ*) were significantly down-regulated in the restricted-fed grass carp. The present results were supported by many previous studies. In rat, the transcriptional level of *srebp*-1*c* was down-regulated significantly after a 48 h-fasting, as well as *fas* (Gosmain et al., 2005). In Nile tilapia (*Oreochromis niloticus*), it was found that the expression of *fas* markedly decreased during a 28-day fasting (Tian et al., 2013). The *pparγ* makes the adipose tissue swell mainly by increasing the adipocyte numbers in Nile tilapia (*Oreochromis niloticus*) (He et al., 2015).

The significant up-regulation of the gene involved in lipolysis and fatty acid β-oxidation of *cpt*-1*a*, but not the other two lipolysis genes of *pparα* and *hl*, was found in grass carp when receiving a 49-day of food restriction. In juvenile grass carp (*Ctenopharyngodon idellus*), the expression of liver *cpt*1 was up-regulated when fed with a high-fat diet (Li et al., 2016). In sea bream (*Sparus aurata*), the expression of hepatic *pparα* was up-regulated after a 72-h fasting (Leaver et al., 2005). When rainbow trout (*Oncorhynchus mykiss*) were treated with mammal *pparα* activator, the peroxisome β-oxidation in hepatic was enhanced (Du et al., 2004). In sea bream (*Pagrus major*), the transcriptional level of *hl* in starved fish was significantly higher than that the fed fish

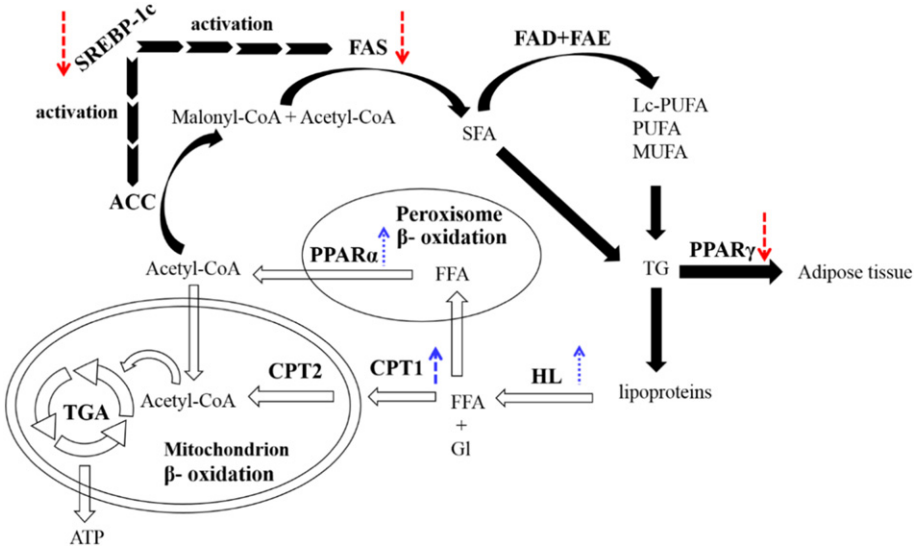

Fig. 4. The proposed metabolism of lipids in grass carp liver after 49-day food restriction, based upon the expression of lipogenic genes (*srebp*-1*c*, *fas*, *pparγ*) and lipolytic genes (*cpt*-1*a*, *pparα*, *hl*).

(Liang et al., 2001). In tilapia (*Oreochromis niloticus* × *O. areus*) and Nile tilapia (*Oreochromis niloticus*), the mRNA level of *hl* increased significantly during a 28-day starvation (Han et al., 2011; Tian et al., 2013).

Fish body composition has a close relationship with the regulation of food intake. Pharmacological inhibition of *fas* results in decrease in food intake and body weight (López et al., 2005). In the hypothalamus, many evidences support that circulating long chain fatty acids act as nutrient abundance signals. The recent study suggested that the increased dietary lipid level might induce the appetite suppression through the modulation of *leptin* expression in grass carp (*Ctenopharyngodon idellus*) (Li et al., 2016).

In addition, food restriction can also alter the appetite of fish, which could be regulated by both central nervous system and peripheral organs (Gao et al., 2015). In the present study, the transcriptional levels of the anorectic genes of *leptin* and *pomc* were significantly down-regulated, while the orexigenic gene of *npy* had no effect was observed in the feed restricted fish. The present results indicated that the anorectic genes would act more in fish fed at satiation than the feed restricted fish. The *leptin* plays an anorexigenic role in regulation of food intake (Robertson et al., 2008). In zebrafish, lower transcriptional levels of hepatic *lepb* (a *leptin* subtype) were found in starved fish after a 7-day food deprivation compared with the fed fish (Gorissen et al., 2009). The gene expression of *leptin* was increased in fed common carp (*Cyprinus carpio*), but not fasted fish (Huising et al., 2006). In goldfish (*Carassius auratus*), the expression of *npy* was increased gradually during a 72-h absolute fasting (Narnaware et al., 2000). Weeks or 24 h of fasting significantly increasing the *npy* expression in mice hypothalamic arcuate nucleus (Lauzurica et al., 2010; Yasrebi et al., 2015).

In conclusion, the present results suggested that when suffered from a long-term food shortage, feed-restricted grass crap would decrease body lipid accumulation by promoting the adipose tissue decomposition and inhibiting the lipid synthesis. The present study further demonstrated that food restriction caused the differences in the lipometabolism, including the down-regulation of lipogenesis genes and the up-regulation of lipolysis genes. Moreover, the anorexigenic genes were in low expressions caused by food restriction.

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