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Lipid deposition patterns among different sizes of three commercial fish species

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Abstract

This study was conducted to compare lipid deposition pattern of three fish species among fish size, Large yellow croaker (*Larmichthys crocea*), Japanese seabass (*Lateolabrax japonicus*) and Turbot (*Scophthalmus maximus* L.), Using magnetic resonance imaging technology for adult fish, results showed that lipid of large yellow croaker mainly deposits in abdominal cavity wall, while for Japanese seabass mainly deposit in visceral adipose tissue and for turbot lipid mainly distribute subcutaneous tissue. Three sizes for each species were selected: S1 (small size), S2 (intermediate size) and S3 (big size), to examine chemical analysis. Results of chemical analysis indicated that whole body lipid content of large yellow croaker significantly increased with the increase in body weight, but Japanese seabass and turbot significantly decreased ($p < .05$). Lipid content of muscle and intestinal tract in large yellow croaker significantly increased with the increase in body weight ($p < .05$), but lipid content of adipose tissue, kidney, heart and skin in S2 group were higher than S1 and S3 groups ($p < .05$). Lipid content of liver, eye, kidney and brain in Japanese seabass significantly increased with the increase in body weight ($p < .05$), but lipid content of stomach and heart showed an opposite trend. Lipid content of liver, adipose tissue, skin and eye in turbot significantly decreased ($p < .05$), but lipid content of brain significantly increased with the increase in body weight ($p < .05$). The results indicated that lipid content of different tissues in fish presented different trends, which was species-dependent.

KEYWORDS
Japanese seabass, large yellow croaker, lipid deposition pattern, turbot

1 | INTRODUCTION

Lipids play a major role in providing a source of energy and essential fatty acids, especially for carnivorous fish as these species have a limited ability to utilize carbohydrates as an energy source (Nanton et al., 2007, Weil, Lefèvre, & Bugeon, 2013). Also, lipids, particularly muscle lipid, have an influence on fish flesh organoleptic quality (Weil et al., 2013).

Lipids store in different parts of body tissues in mammal and fish, such as liver, muscle and adipose tissue, which was species-dependent (Hauser, Mourot, De Clercq, Genart, & Remacle, 1997; Kouba, Bonneau, & Noblet, 1999; Leaver et al., 2008; Sheridan, 1988, 1994; Weil et al., 2013). There are mainly two kinds of adipose tissue in mammal: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is mainly used to store energy distributed in subcutaneous and perivisceral tissues, while BAT is mainly used to produce heat distributed in mediastinum, axilla and perirenal tissues (Hausman, DiGirolamo, Bartness, & Martin, 2001). Hauser et al. (1997) reported that lipids in different tissues in mammal had different developing speed so that these tissues had different lipid content in each growth stage. Development of intermuscular lipid and subcutaneous lipid was later than other tissues and perirenal fat rapidly develop (Hauser et al., 1997; Kouba et al., 1999). For fish, the structural differences in different adipose tissues were detected using light and electron microscopy. For example, visceral adipose tissue consists almost entirely of adipocytes, while adipocytes in muscle are included in the myoseptum (Nanton et al., 2007; Zhou, Ackman, & Morrison, 1995). Muscle lipid that store in the myoseptum occupy 40% white muscle lipid content in Atlantic salmon (*Salmo salar* L.), and the rest of lipid store in myocyte as type of lipid droplets (Nanton et al., 2007).

Previous studies reported that lipids deposition at different fish tissues are modulated by different extrinsic (diets, culture water quality) and intrinsic (life cycle status, selective breeding, fish age or size) factors (Weil et al., 2013). The lipid content of diets (fish oil or vegetable oil based diets, low lipid level or high lipid level diets) affected the lipid content of teleost fish body (Henderson & Tocher, 1987; Nanton et al., 2007; Torstensen, Frøyland, & Lie, 2004; Torstensen, Lie, & Frøyland, 2000; Torstensen, Nanton, Olsvik, Shundvold, & Stubhaug, 2009). Little work was conducted to research the lipid deposition pattern among different fish sizes. However, fish in different growth stage have different physiological reaction and substrate so that they have different body composition (Kiessling, Kiesling, Storebakken, & Asgard, 1991).

Currently, researchers often use biochemical methods to detect fish lipid distribution (Folch, Lees, & Sloane-Stanley, 1957). However, the method is limited to research lipid distribution in tissues and small fish, and the lipid distribution of whole fish body is ambiguous. Magnetic resonance imaging has been utilized ubiquitously to detect fat tissue in clinical application, which also provides the possibility for detecting fish fat distribution (Mathiassen, Misimi, Bondø, Veliyulin, & Østvik, 2011; Wu et al., 2015). Therefore, we firstly detected lipid deposition of adult fish in these three fish species using magnetic resonance imaging technology, then detected lipid deposition of whole body and some tissues among different fish sizes using chemical analysis in this study.

Large yellow croaker (*Larmichthys crocea*), Japanese seabass (*Lateolabrax japonicus*) and Turbot (*Scophthalmus maximus* L.) are important commercial cultured fish species in China. Recently, numerous studies have been carried out to investigate the nutritional requirements about protein, lipid and other nutrients of these fish species (Ai et al., 2006, 2008; Peng et al., 2013, 2014). Knowing the nutritional composition of themselves is also a necessary job, however, little study has focus on the nutritional composition of these fish. Thus, the objective of present study aimed to compare lipid content of body and some tissues among different size of these fish species to enrich background information of these species, and to provide basic data for making feeding standards and formulating compound feed reasonably for different growth stages fish species.

2 | MATERIALS AND METHODS

2.1 | Animals and sampling

These three fish species were chosen and purchased from some aquaculture farms, but different specifications of the same fish species were from the same farm (Large yellow croaker and Japanese seabass were from Aquatic Fingerings Limited Company of Xiangshan Harbour, Ningbo, and turbot was from Qingdao Great Seven Bio-Tech, Qingdao). These fish dieted with commercial feed before this study: Large yellow croaker (43% protein, 12% lipid), Japanese seabass (42% protein, 12% lipid) and Turbot (50% protein, 12% lipid). Every fish species have three specifications: large yellow croaker (S1: 54.18 $\pm$ 0.74 g, S2: 146.30 $\pm$ 0.96 g and S3: 240.86 $\pm$ 0.96 g), Japanese seabass (S1: 13.30 $\pm$ 1.13 g, S2: 207.81 $\pm$ 1.84 g and S3: 296.1 $\pm$ 1.39 g), turbot (S1: 54.95 $\pm$ 1.05 g, S2: 158.56 $\pm$ 1.63 g and S3: 243.31 $\pm$ 1.35 g). Fish tissues used to study includes liver, muscle, adipose tissue, kidney, intestine, brain, skin, etc.

2.2 | Magnetic resonance imaging

Fat study extremely important in fish study, we could further study on the position of fat accumulation if fat distribution of the whole fish was accurately detected. Magnetic resonance imaging has been utilized ubiquitously to quantify fat tissue in clinical application, which also provides the possibility for detecting fish fat distribution. In this study, Magnetic resonance imaging (MRI) was carried out at the Shanghai Key Laboratory of Magnetic Resonance, East China Normal University, Shanghai, China, and the parameters were cited as Wu et al. (2015).

2.3 | Chemical analyses

The lipid of the liver, muscle, adipose tissue and other tissues was assayed according to operational approach (Folch et al., 1957) with some modification. In brief, about 100.00 mg (dry weight) sample (w0) was added to 4 ml chloroform: methanol (C–M) (2:1, v/v) in tube A, and then tube A was settled at rest for over 24 hr (sometimes shaken). Two microliters of C–M was still added to tube A, and tube A was again settled at rest for 4 hr. After centrifugation (3,000 *g* for 10 min at 4°C) (Hitachi Centrifuge CT15RE), the supernatant was transferred to tube B (constant weight, w1). Two microliters of C–M was added to the residue of tube A, settled for over 2 hr, and centrifuged (3,000 *g* for 10 min at 4°C) later, and the supernatant was transferred to tube B. 1.2 ml of 1.6% $CaCl_2$ was added to the supernatant of tube B, then mixed, and tube B was settled at rest over 12 hr. The upper phase was removed. The lower phase of tube B had been dried under a pure nitrogen steam. Tube A was evaporated at 75°C and reweighed (w2). Therefore, the lipid content of the sample (%) is (w2−w1)/w0 $\times$ 100. Duplicate analyses were conducted for each sample.

2.4 | Statistical analysis

Software SPSS 19 was used for all statistical evaluations. All data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were regarded as significant when $p < .05$. The results are presented as mean values with their standard errors (n = 3).

FIGURE 1 Magnetic resonance imaging about adult fish lipid deposition of Large yellow croaker. The region in which the arrow is referred to is the fat distribution area

3 | RESULTS

3.1 | Lipid deposition of adult fish in these three species using Magnetic resonance imaging method

In magnetic resonance imaging, the bright region shows the distribution area of lipid. Lipid deposition pattern of large yellow croaker is shown as Figure 1, which shows that its lipid mainly deposits in abdominal cavity, and adipose tissue adheres to the abdominal cavity wall; For Japanese seabass in Figure 2, its lipid mainly deposits in visceral adipose tissue; however, turbot's lipid mainly distributes subcutaneous tissue around its body (Figure 3), which locates connected position between dorsum and dorsal fin or belly and pelvic fin.

3.2 | Lipid deposition of whole body in these three fish species using chemical analysis

Lipid content of whole body of large yellow croaker, S2 was higher significantly than S1 and S3 (Table 1); For turbot and Japanese seabass, the highest lipid content both emerged in S1, and the whole body lipid content of these two fish species decreased with the

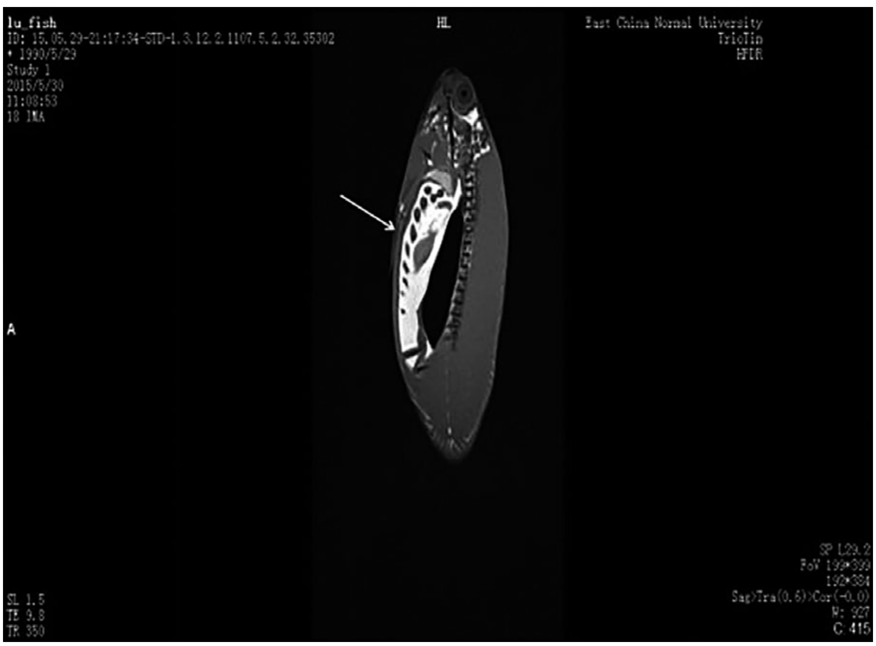

FIGURE 2 Magnetic resonance imaging about adult fish lipid deposition of Japanese seabass. The region in which the arrow is referred to is the fat distribution area

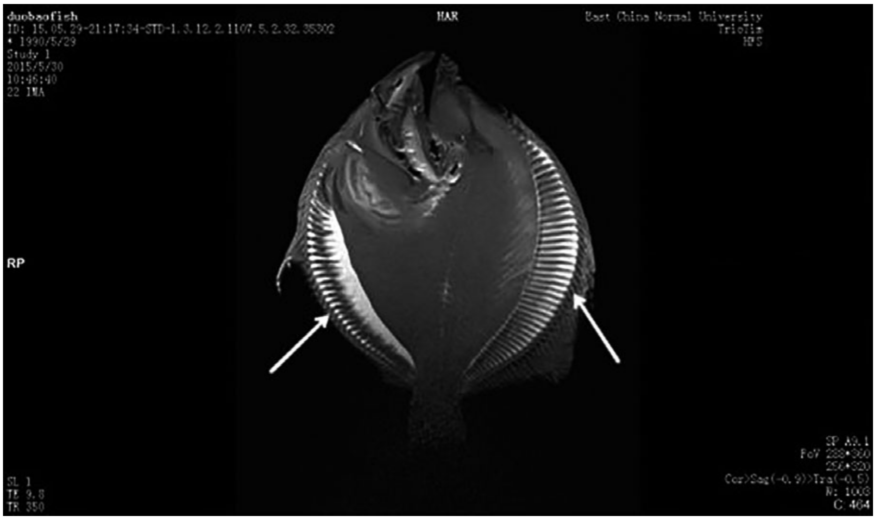

FIGURE 3 Magnetic resonance imaging about adult fish lipid deposition of Turbot. The region in which the arrow is referred to is the fat distribution area

TABLE 1 Lipid content (% wet weight) in three fish species of whole body

Species	S1 (%)	S2 (%)	S3 (%)
Large yellow croaker	6.62 ± 0.25[c]	16.06 ± 0.21[a]	12.87 ± 0.37[b]
Japanese seabass	5.59 ± 0.27[a]	3.99 ± 0.08[b]	3.30 ± 0.08[b]
Turbot	6.51 ± 0.45[a]	3.34 ± 0.02[b]	3.60 ± 0.03[b]

Data are expressed as means ± *SEM* ($n = 3$). Different letters in each row show significant differences among dietary treatments by Tukey's test ($p < .05$).
S1, small size; S2, intermediate size; S3: big size.

increase in body weight, but no significant difference were observed between S2 and S3 ($p > .05$).

Interestingly, result showed that lipid content of whole body in large yellow croaker was higher than other two fish species at S1, S2 and S3. In addition, lipid content of Japanese seabass was relatively lower than turbot at S1 and S2, but was higher at S3 group.

3.3 | Lipid deposition patterns among different size of three fish species using chemical analysis

Lipid content of liver, spleen, eye and brain in large yellow croaker was not significantly different with the increase in body weight (Table 2); lipid content of muscle and intestinal tract significantly increased with the increase in body weight ($p < .05$), but lipid content of adipose tissue, kidney, heart and skin in group with the middle of three sizes was higher than other groups ($p < .05$).

There was no significant difference in lipid content of muscle, intestinal tract and spleen in Japanese seabass with the increase in body weight (Table 3). Lipid content of liver, eye, kidney and brain significantly increased with the increase in body weight ($p < .05$), but stomach and heart showed an opposite trend.

Lipid content of liver, adipose tissue, skin and eye in turbot significantly decreased with the increase in body weight ($p < .05$) (Table 4), but brain lipid level significantly increased with the increase of body weight ($p < .05$), and in other tissues of turbot no significant difference among body weight was observed ($p > .05$).

TABLE 2 Lipid content (% wet weight) in each tissue among different size of large yellow croaker

Tissues	S1 (%)	S2 (%)	S3 (%)
Muscle	6.46 ± 0.45[b]	7.64 ± 0.74[b]	13.08 ± 0.67[a]
Liver	17.26 ± 0.01[a]	15.72 ± 1.86[a]	10.81 ± 1.46[a]
Adipose tissue	3.65 ± 0.31[b]	8.89 ± 0.14[a]	4.84 ± 0.13[b]
Intestine tract	3.31 ± 0.25[b]	3.79 ± 0.15[b]	6.88 ± 0.10[a]
Stomach	3.4 ± 0.37a[b]	4.03 ± 0.15[a]	2.28 ± 0.05[b]
Kidney	8.29 ± 0.04[b]	12.86 ± 0.39[a]	7.47 ± 0.03[b]
Heart	2.78 ± 0.10[b]	3.17 ± 0.17[a]	2.38 ± 0.02[b]
Spleen	3.10 ± 0.01[a]	3.36 ± 0.25[a]	3.83 ± 0.47[a]
Gill	1.31 ± 0.28[b]	4.21 ± 0.02[a]	2.80 ± 0.56a[b]
Skin	11.30 ± 0.18[b]	25.06 ± 0.17[a]	11.09 ± 1.72[b]
Eye	4.19 ± 0.51[a]	6.29 ± 0.12[a]	7.08 ± 1.32[a]
Brain	9.03 ± 0.55[a]	9.24 ± 0.12[a]	9.25 ± 0.10[a]
Pyloric caecum	5.91 ± 0.01[a]	6.31 ± 0.08[a]	2.77 ± 1.04[b]

Data are expressed as means ± *SEM* ($n = 3$). Different letters in each row show significant differences among dietary treatments by Tukey's test ($p < .05$).
S1, small size; S2, intermediate size; S3: big size.

4 | DISCUSSION

As lean fish, fat content in muscle is below 1%, while more than 10% fat in their muscle is fat fish (Lie, Lied, & Lambertsen, 1986; Nanton, Lall, & McNiven, 2001). Therefore, both Japanese seabass and turbot are not fat fish except that big specification large yellow croaker had 13.08% muscle fat. Also, the small size Japanese seabass is lean fish to some extent with 0.73% fat in muscle.

TABLE 3 Lipid content (% wet weight) in each tissue among different size of Japanese seabass

Tissues	S1 (%)	S2 (%)	S3 (%)
Muscle	0.73 ± 0.09[a]	1.06 ± 0.17[a]	1.16 ± 0.09[a]
Liver	5.00 ± 0.10[a]	11.73 ± 0.25[a]	12.90 ± 0.13[a]
Adipose tissue	21.17 ± 1.78[b]	30.81 ± 0.91[a]	24.32 ± 0.33[ab]
Intestine tract	3.66 ± 0.11[a]	3.40 ± 0.08[a]	4.53 ± 0.37[a]
Stomach	2.85 ± 0.28[a]	2.47 ± 0.33[ab]	1.21 ± 0.01[b]
Kidney	3.76 ± 0.22[a]	5.87 ± 0.16[b]	6.00 ± 0.06[b]
Heart	4.12 ± 0.03[a]	4.06 ± 0.02[a]	2.84 ± 0.18[b]
Spleen	3.97 ± 0.27[a]	5.69 ± 0.82[a]	5.49 ± 0.09[a]
Gill	2.97 ± 0.08[a]	1.77 ± 0.36[a]	2.08 ± 0.01[a]
Skin	2.39 ± 0.01[b]	3.94 ± 0.07[a]	2.19 ± 0.01[b]
Eye	1.57 ± 0.06[c]	2.18 ± 0.11[b]	8.10 ± 0.08[a]
Brain	8.12 ± 0.40[b]	10.29 ± 0.01[b]	15.29 ± 0.60[a]
Pyloric caecum	5.59 ± 0.16[a]	6.09 ± 0.20[a]	6.30 ± 0.16[a]

Data are expressed as means ± *SEM* (n = 3). Different letters in each row show significant differences among dietary treatments by Tukey's test ($p < .05$).
S1, small size; S2, intermediate size; S3: big size.

Fatty liver symptom was frequently observed in cultured fish, and the truth that environmental factors affected lipid content of fish liver was reported by many researchers (Arellano, Storch, & Sarasquete, 1999; Handy, Sims, Giles, Campbell, & Musonda, 1999; Ptashynski, Pedlar, Evans, Baron, & Klaverkamp, 2002; Yan et al., 2015). Dietary lipid or energy exceed the capacity of the hepatic cells to oxidize fatty acids, or when protein synthesis is impaired, the result is the large synthesis and deposition of lipid in vacuoles, so the lipid content of liver is higher than other tissues (Caballero, Izquierdo, Kjorsvik, Fernandez, & Rosenlund, 2004; Caballero et al., 2002, 2003; Ruyter, Moya-Falcon, Rosenlund, & Vegusdal, 2006). In this study, lipid content of liver was apparently higher than other tissues in three specification of every fish species except Japanese seabass, and this result was related to the structure of its adipose tissues (Nanton et al., 2007; Zhou et al., 1995). Lie et al. (1986) reported that Japanese seabass liver normally contains 10%–12% of fat. The value of fat content of small size Japanese seabass in the present study was much lower (5.0%), and the value of another two size Japanese seabass are within this range.

TABLE 4 Lipid content (% wet weight) in each tissue among different size of turbot

Tissues	S1 (%)	S2 (%)	S3 (%)
Muscle	3.15 ± 0.21[a]	2.97 ± 0.09[a]	3.45 ± 0.21[a]
Liver	19.47 ± 0.07[a]	6.91 ± 0.17[c]	11.54 ± 0.56[b]
Adipose tissue	8.98 ± 1.78[a]	2.35 ± 0.27[b]	2.64 ± 0.11[b]
Intestine tract	4.87 ± 0.05[a]	4.38 ± 0.36[a]	4.09 ± 0.67[a]
Stomach	1.44 ± 0.01[a]	1.48 ± 0.16[a]	1.48 ± 0.05[a]
Kidney	5.62 ± 0.54[a]	4.45 ± 0.32[a]	4.85 ± 0.15[a]
Heart	4.77 ± 0.41[a]	4.09 ± 0.48[a]	4.10 ± 0.02[a]
Spleen	2.79 ± 0.01[a]	1.49 ± 0.01[b]	2.08 ± 0.22[ab]
Gill	1.54 ± 0.33[a]	1.56 ± 0.12[a]	1.31 ± 0.08[a]
Skin	2.11 ± 0.31[a]	1.12 ± 0.01[b]	0.80 ± 0.12[b]
Eye	1.39 ± 0.01[a]	0.82 ± 0.01[b]	0.91 ± 0.01[b]
Brain	6.91 ± 0.01[b]	8.62 ± 0.01[a]	8.63 ± 0.08[a]
Pyloric caecum	2.31 ± 0.04[a]	1.79 ± 0.16[a]	2.01 ± 0.20[a]

Data are expressed as means ± *SEM* (n = 3). Different letters in each row show significant differences among dietary treatments by Tukey's test ($p < .05$).
S1, small size; S2, intermediate size; S3: big size.

Interestingly, the lipid content of adipose tissue in Japanese seabass was higher than turbot and large yellow croaker in this study, which was related to the structural differences in adipose tissues. Visceral adipose tissue consist almost entirely of adipocytes, but adipose tissues include myocytes in another two species (Nanton et al., 2007; Zhou et al., 1995), the adipose tissue of Japanese seabass mainly consist of adipocytes,while another two adipose tissues contain adipocytes and myocytes. Therefore lipid content in adipose tissues had difference.

In this study, the whole body lipid content of large yellow croaker had nothing to do with the increase in fish size, and whole body lipid content of turbot and Japanese seabass had negative correlation with fish size. In the previous study (Huang, Zhang, Wang, Wang, & Luo, 2013), the lipid content of grass carp (*Ctenopharyngodon idella*) is closely relative to the fish size, which showed that lipid quality fraction was positively correlated with fish body length. Results of this study showed that lipid content of body tissues in these three fish had no constant trend with fish size: some tissues emerged positive correlation, for instance, muscle and intestinal tract for large yellow croaker, brain for turbot, liver, eye, kidney and brain for Japanese seabass; some tissues emerged negative correlation, for instance, adipose tissue, kidney, heart and skin for large yellow croaker, stomach, heart and fin for Japanese seabass, liver, adipose tissue, skin and eye for turbot; and some tissues had no significant difference with increasing body weight. Beyond that, the lipid content of whole body among three fish species at the same size was compared, and result showed that large yellow croaker was higher than other two fish species among S1, S2 and S3, which may be depended on its type property, as previously mentioned, large yellow croaker is fat fish (Lie et al., 1986; Nanton et al., 2001).

The research indicated that different fish species have different lipid deposition patterns. Results of the present study suggested that adipose tissue in turbot is edible and the increasing percentage of adipose tissue could improve the dressing percentage of turbot, but in large yellow croaker and Japanese seabass, the adipose tissue would be discarded, because former's adipose tissue adheres to the visceral mass and latter's adipose tissue adheres to the abdominal membrane. However, visceral mass and abdominal membrane are both inedible in eating habits of human being. Therefore, lipid content of fish body bring different effects for fish species, and different tissues in fish showed different trends with the increase in body weight, which was species-dependent.

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