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Compared to fish oil alone, a corn and fish oil mixture decreases the lipid requirement of a freshwater fish species, *Carassius auratus gibelio*

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ABSTRACT

Numerous studies have investigated the use of plant oil to replace fish oil in the formulation of aqua-feeds; however, it is unknown, in general, whether the previously obtained lipid requirement concentrations based on fish oil require adjustments in view of the replacement of fish oil by plant oil. The present study tested the hypothesis that the appropriate dietary lipid concentration for gibel carp, *Carassius auratus gibelio* may change after fish oil is partially replaced by corn oil in the diet. Seven isonitrogenous diets were formulated using a mixture of fish oil and corn oil as the lipid source, with lipid concentrations ranging from 10 to 213 g kg^{-1}. A 70-day growing trial was conducted to evaluate the effects of dietary lipid concentrations on the growth performance, fatty acid changes of the muscle/liver, and mRNA relative expression levels of genes involved in lipogenesis. The results showed that the specific growth rate (SGR) increased markedly with dietary lipid concentrations from 10 to 81 g kg^{-1}, then decreased and got a no statistical change in the 119 to 213 g kg^{-1} groups. In the liver and muscle, 16:0 and 18:0 relative contents exhibited a quadratic regression trend associated with dietary lipid concentrations. MUFA content displayed a logarithmic decrease with increasing dietary lipid concentrations, but a logarithmic increase was observed for LC-PUFA content, with 18:2n−6 and 18:3n−3 contents increasing linearly with dietary lipid concentrations. In the liver, the relative mRNA expression levels of ACC1 and FAS decreased with increasing dietary lipid concentrations. In conclusion, based on a broken-line analysis of the SGR, the recommended dietary lipid concentration for juvenile gibel carp fed with an equal mixture of corn oil and fish oil was found to be 73.2 g kg^{-1}, which represented a 47.9% decrease compared to the value obtained using fish oil as the lipid source. Our results indicate that dietary lipid requirement for freshwater fish species might change markedly when fish oil is replaced by plant oil in the diets.

1. Introduction

Until recently, fish oil was still the main lipid source used in the formulation of commercial aqua-feeds, owing to its ready availability, low price and richness in LC-PUFA (Leaver et al., 2008; Turchini et al., 2009). It is estimated that approximately 90% of fish oil production has been consumed in aqua-feeds. However, as the no-growth annual production of fish oil has been set at approximately 1.1 million metric tons for the past quarter of a century, plant oil is anticipated to play an increasing role in aqua-feeds in the coming years (De Silva et al., 2010; FAO, 2012).

Fish oil is a co-product of the process of fish meal production. The principal species used in this process include Peruvian anchovy, mackerel, sand eel, capelin, menhaden, and herring. Of these species, the Peruvian anchovy strongly dominates fish oil production (De Silva et al., 2010). It is well established that fish oils are highly valued for their high proportions of n−3 LC-PUFA, and taking anchovy oil for example, 20:5n−3 and 22:6n−3 account for about 22% and 9% in total fatty acids (FAs), respectively (Haraldsson and Hjaltason, 2001). Total plant oil production reached approximately 130 million metric tons by 2009. This product was primarily derived from palm, soybean, rapeseed, sunflower, and, to a lesser extent olive, corn, and linseed oils (Gunstone, 2010). Plant oil is typically characterized by a high content of the C_{16} and C_{18} FAs, of which 18:2n−6 represents more than 50% of the total FAs found in soybean oil, sunflower oil, and corn oil (Gunstone and Harwood, 2007). The principal differences between fish oil and plant oil are that FAs with chain length > 20 are virtually absent from plant oil and that low percentage of n−6 PUFAs occur in fish oil (Haraldsson and Hjaltason, 2001).

Abbreviations: △6Fad, Δ6 fatty acid desaturase; ACC1, acetyl-CoA carboxylase alpha; Elovl5, fatty acyl elongase 5; FAS, fatty acid synthase; LC-PUFAs, highly unsaturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

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It is generally considered that freshwater omnivorous, planktivorous or herbivorous species can convert 18:2n−6 and 18:3n−3 into LC-PUFA at a sufficient rate to meet their minimum requirements which play an important role in membrane structure and function, and providing energy during embryonic and early larval development in fish, but the ability is generally much lower, or even absent, in marine species (Sargent et al., 2002; Tocher, 2010). For marine species, fish oil may be the suitable dietary lipid source, but for these freshwater species, plant oil may be the reasonable diet lipid source as well as fish oil (De Silva et al., 2010; Gunstone and Harwood, 2007; Tocher, 2010; Turchini et al., 2009). Numerous studies have investigated the use of plant oil to replace fish oil in the formulation of aqua-feeds of cultured species in recent years; however, dietary lipid requirement data obtained for most freshwater or marine cultured species were based on fish oil as the lipid source (Ghanawi et al., 2011; Pei et al., 2004; Peres and Oliva-Teles, 1999). It is unknown, in general, whether the previously obtained lipid requirement concentrations based on fish oil require adjustments in view of the replacement of fish oil by plant oil. Considering the large difference in the FA composition of fish oil and plant oil, we propose the hypothesis that dietary lipid requirement for freshwater species based on plant oil is much different from that based on fish oil.

The gibel carp is an omnivorous freshwater fish which has become a main cultured species recommended by the Chinese Ministry of Agriculture in China. Dietary lipid requirement reported for 4.5 g gibel carp is 140.5 g kg^{-1} when anchovy oil was used as the only dietary lipid source (Pei et al., 2004). Subsequent research in our laboratory has demonstrated that corn oil could replace 50% of anchovy oil in its diet without any negative effects on growth performance of gibel carp (Chen et al., 2011). Therefore, in this study, we reevaluate dietary lipid requirement for gibel carp based on the mixed lipid source. Such effects were assessed including growth performance, body lipid deposition, tissue FA composition and gene expression of key enzymes involved in lipogenesis (ACC1 and FAS), with the overall aim of verifying whether dietary lipid requirement indicated for this species requires adjustment when fish oil was partially replaced by corn oil in the diet.

2. Materials and methods

2.1. Experimental diets

In the experimental diets, fish meal and casein were used as the protein sources, and corn oil and fish oil (anchovy oil) as the major lipid sources. 0, 35, 70, 105, 140, 175 and 210 g kg^{-1} of mixed oil (corn oil:anchovy oil = 1:1) were added into seven isonitrogenous (350 g kg^{-1} crude protein) diets, and the corresponding dietary lipid concentrations analysed were 10, 46, 81, 119, 150, 183 and 213 g kg^{-1}, respectively. The formulation and FA compositions of the experimental diets were presented in Tables 1 and 2. All ingredients were ground, filtered through 550-μm meshes, and mixed completely. Next, 2-mm (diameter) pellets were produced and then oven-dried at 60 °C. All diets were stored at −20 °C until use.

2.2. Experimental fish

The gibel carp were obtained from the Guanqiao Hatchery of the Institute of Hydrobiology, the Chinese Academy of Sciences (Wuhan, Hubei, China). Before the experiment, the fish were acclimated for two weeks. During the acclimatisation period, fish were fed a mixture of seven diets twice daily (0900 and 1500 h). The experiment was conducted in a recirculation system consisting of 21 fibreglass tanks (diameter: 70 cm, water volume: 130 L). At the beginning of the experiment, the fish were fasted for 24 h and then 25 individuals with an average weight of 3.5 ± 0.1 g ind^{-1} were bulk weighed and randomly distributed into each tank. Each experimental diet was fed to three tanks twice a day (0900 and 1500 h). Uneaten feeds were collected after 1 h, dried at 60 °C and weighed. The faeces were removed *via* siphoning prior to feeding. The experiment lasted for 70 days.

During the experiment, the water flowing rate into each tank was about 2.2 L min^{-1}. The water temperature was between 27 and 31 °C.

Table 1
Formulation and proximate composition of the experimental diets.

Ingredients (g kg^{-1})	Added lipid concentrations (g kg^{-1})						
	0	35	70	105	140	175	210
Fish meal[a]	100	100	100	100	100	100	100
Casein	300	300	300	300	300	300	300
Fish oil[b]	0	17.5	35	52.5	70	87.5	105
Corn oil[c]	0	17.5	35	52.5	70	87.5	105
Corn starch	214	214	214	214	214	214	214
α-Starch	80	80	80	80	80	80	80
Vitamin premix[d]	5	5	5	5	5	5	5
Mineral premix[e]	50	50	50	50	50	50	50
Choline chloride	1	1	1	1	1	1	1
Antioxidant[f]	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium alginate	10	10	10	10	10	10	10
Carboxymethyl cellulose	20	20	20	20	20	20	20
Cellulose	210	175	140	105	70	35	0
Chemical composition (g kg^{-1} dry matter)							
Crude protein	352	351	353	352	352	352	352
Crude lipid	10	46	81	119	150	183	213
Gross energy (kJ g^{-1})	19.1	20.1	20.5	21.6	22.6	23.3	23.7

[a] Pollock fish meal from American Seafood Company, Seattle, Washington, USA.
[b] Anchovy oil from Peru purchased from Coland Feed Co. Ltd., Wuhan, Hubei, China.
[c] Corn plumule oil from Cofco Co. Ltd., purchased from Wuhan, Hubei, China.
[d] Vitamin premix (mg kg^{-1} diet): thiamin, 20; riboflavin, 25; pyridoxine, 20; cyanocobalamine, 2; folic acid, 5; calcium pantothenate, 50; inositol, 100; niacin, 100; biotin, 5; starch, 4322; Vitamin A, 110; Vitamin D3, 20; Vitamin E, 100; Vitamin K3, 10; ascorbic acid, 111.
[e] Mineral premix (mg kg^{-1} diet): NaCl, 500; $MgSO_4 \cdot 7H_2O$, 7500; $NaH_2PO_4 \cdot 2H_2O$, 12,500; KH_2PO_4, 16,000; Ca $(H_2PO_4) \cdot 2H_2O$, 10,000; $FeSO_4$, 1250; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1750; $ZnSO_4 \cdot 7H_2O$, 176.5; $MnSO_4 \cdot 4H_2O$, 81; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 6H_2O$, 0.5; KI, 1.5; starch, 225.
[f] BHT: 2, 6-di-tert-butyl-4-methylphenol.

Table 2
Fatty acid composition of the experimental diets (mg/g diet).

Fatty acids	Added lipid concentrations (g kg^{-1})						
	0	35	70	105	140	175	210
10:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
12:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
14:0	0.6	1.6	3.0	4.0	4.8	5.7	7.0
16:0	2.8	8.9	15.3	21.3	26.2	32.1	36.8
18:0	1.1	2.3	3.4	4.4	5.1	5.9	7.5
20:0	–[a]	0.2	0.3	0.4	0.5	0.6	0.8
22:0	–	0.1	0.1	0.2	0.2	0.2	0.2
24:0	–	0.1	0.1	0.2	0.2	0.3	0.3
∑SFA[b]	4.7	13.3	22.4	30.6	37.2	45.0	52.7
16:1n−7	0.4	1.7	3.0	4.2	5.3	6.4	7.9
18:1n−9	1.5	10.5	18.3	27.6	35.3	44.1	54.2
20:1n−9	0.1	0.4	0.6	0.8	1.0	1.2	1.5
24:1n−9	0.1	0.2	0.2	0.3	0.4	0.4	0.5
∑MUFA[c]	2.2	12.8	22.2	33.0	41.9	52.1	64.1
18:2n−6	0.7	11.4	22.1	34.5	44.7	55.4	61.5
18:3n−6	–	–	–	0.1	0.1	0.1	0.1
20:2n−6	–	0.1	0.1	0.2	0.2	0.3	0.3
20:4n−6	0.5	0.8	1.2	1.4	1.7	1.8	2.0
22:2n−6	–	0.1	0.1	0.1	0.1	0.1	0.1
∑n−6	1.2	12.4	23.4	36.2	46.6	57.7	63.9
18:3n−3	–	0.2	0.4	0.7	0.9	1.1	1.2
20:5n−3	0.7	3.3	5.7	8.6	10.6	12.2	13.7
22:6n−3	0.6	2.0	3.1	4.6	5.5	6.7	7.3
∑n−3	1.4	5.5	9.3	13.9	17.0	19.9	22.2
n−3/n−6	1.2	0.4	0.4	0.4	0.4	0.4	0.4
LC-PUFA[d]	1.9	6.2	10.0	14.6	17.7	20.7	23.0

[a] –: not detected.
[b] SFA, saturated fatty acid: 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, 24:0.
[c] MUFA, monounsaturated fatty acid: 16:1n−7, 18:1n−9, 20:1n−9, 24:1n−9.
[d] LC-PUFA, long chain polyunsaturated fatty acid: 20:4n−6, 20:5n−3, 22:6n−3.