Postprint: Effects of Complete Replacement of Fish Oil with Linseed Oil-Soybean Oil Blends at Different Ratios on Growth of Hybrid Sturgeon

Authors: Liu Caixia, Xing Wei, Li Tieliang, Ma Zhihong, Jiang Na, Li Wentong, Luo Lin, Liu Yang

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Abstract

The primary objective of this experiment was to investigate the effects of complete replacement of fish oil with mixed oils of different flaxseed oil to soybean oil ratios on growth performance, muscle fatty acid composition, serum liver function, and antioxidant indices in hybrid sturgeon. Four isonitrogenous, isolipidic, and isoenergetic experimental diets were formulated: diet A was supplemented with 8% fish oil, while diets B, C, and D replaced all fish oil in diet A with mixed oils of 75% flaxseed oil + 25% soybean oil (flaxseed oil to soybean oil ratio of 3:1), 50% flaxseed oil + 50% soybean oil (ratio of 1:1), and 25% flaxseed oil + 75% soybean oil (ratio of 1:3), respectively. Each experimental diet was fed to three culture tanks (replicates), with each tank stocked with 40 hybrid sturgeon with an initial body weight of (70.8$±$0.5) g, and the feeding trial lasted for 12 weeks. The results showed that group B had the highest final body weight (FBW), weight gain rate (WGR), and specific growth rate (SGR) among the four groups, which were significantly different from group A ($P<0.05$). The crude lipid content in muscle and liver of hybrid sturgeon in group B was significantly higher than that in group A ($P<0.05$), but not significantly different from groups C and D ($P>0.05$). Serum total antioxidant capacity (T-AOC) in groups A and B was significantly higher than that in groups C and D ($P<0.05$); serum high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) contents in group B were the highest and significantly higher than those in other groups ($P<0.05$); serum triglyceride (TG) content was lowest in group B, significantly lower than that in group C ($P<0.05$), but not significantly different from groups A and D ($P>0.05$). Furthermore, the contents of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in muscle of hybrid sturgeon in group B were 65.7% and 74.5% of the corresponding fatty acids in group A, respectively, and the contents of EPA and DHA in muscle did not decrease dramatically due to replacement of fish oil with mixed oil. Based on

these results, complete replacement of fish oil in feed with mixed oils of different flaxseed oil to soybean oil ratios yielded better growth performance in hybrid sturgeon when the flaxseed oil to soybean oil ratio in the mixed oil was 3:1 (i.e., 75% flaxseed oil + 25% soybean oil).

Full Text

Effects of Total Replacement of Fish Oil by Mixed Oils with Different Ratios of Linseed Oil and Soybean Oil on Growth of Hybrid Sturgeon (Acipenser baeri Brandt ×A. schrenckii Brandt)

LIU Caixia[1], XING Wei[1], LIU Yang[2], LI Tieliang[1], MA Zhihong[1], JIANG Na[1], LI Wentong[1], LUO Lin[1]*

[1]Beijing Fisheries Research Institute, Beijing 100068, China
[2]Beijing Chaoyang Hatchery and Aquaculture Farm, Beijing 100018, China

Abstract: This study investigated the effects of total replacement of fish oil by mixed oils with different ratios of linseed oil and soybean oil on growth performance, muscle fatty acid composition, and serum liver function and antioxidant indices in hybrid sturgeon (Acipenser baeri Brandt ×A. schrenckii Brandt). Four isonitrogenous, isolipidic, and isoenergetic experimental diets were formulated. Diet A contained 8% fish oil, while diets B, C, and D replaced the fish oil completely with mixed oils at ratios of 75% linseed oil + 25% soybean oil (3:1 ratio), 50% linseed oil + 50% soybean oil (1:1 ratio), and 25% linseed oil + 75% soybean oil (1:3 ratio), respectively. Each diet was fed to three replicate groups of 40 hybrid sturgeon with an initial body weight of (70.8$±$0.5) g for 12 weeks. The results showed that group B exhibited the highest final body weight (FBW), weight gain rate (WGR), and specific growth rate (SGR) among all groups, with significant differences compared to group A ($P<0.05$). The crude lipid content in muscle and liver of group B was significantly higher than that of group A ($P<0.05$), but showed no significant differences compared to groups C and D ($P>0.05$). Fish in groups A and B had significantly higher serum total antioxidant capacity (T-AOC) than those in groups C and D ($P<0.05$). Group B displayed the highest serum high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) contents, which were significantly higher than other groups ($P<0.05$). Moreover, the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents in muscle of group B reached 65.7% and 74.5% of those in group A, respectively, indicating that EPA and DHA levels did not decline sharply following fish oil replacement. In conclusion, total replacement of fish oil by mixed linseed and soybean oils at a 3:1 ratio (75% linseed oil + 25% soybean oil) yielded favorable growth performance in hybrid sturgeon.

Keywords: hybrid sturgeon (Acipenser schrenckii Brandt ×A. baeri Brandt); fish oil; linseed oil; soybean oil; growth performance; fatty acids

Introduction

Lipids serve as crucial sources of energy and essential fatty acids for fish and constitute structural components of biological membranes that transport fat-soluble vitamins. Traditionally, fish oil has been considered an optimal lipid source in commercial aquafeeds due to its suitable digestibility and abundant polyunsaturated fatty acids (PUFA). However, with the continuous expansion of aquaculture, global consumption of fish oil for aquaculture has increased dramatically, and future supply will likely fail to meet demand. Consequently, identifying appropriate alternative lipid sources that do not compromise fish growth and flesh quality represents a major research focus. Partial or total replacement of fish oil with alternative lipid sources has been successfully achieved in various fish species. Among these alternatives, vegetable oils have gained popularity due to their stable supply and pricing, as well as their near absence of dioxins and other organic contaminants.

Sturgeon (Acipenser) has become increasingly important in aquaculture because of its delicate flesh and muscle rich in n-3 highly unsaturated fatty acids (HUFA) with high n-3/n-6 PUFA ratios. Hybrid sturgeon, in particular, has emerged as the primary cultured variety in China due to its rapid growth, strong disease resistance, and high nutritional value. Previous studies have demonstrated that partial or total replacement of fish oil with vegetable oils in diets for white sturgeon (Acipencer transmontanus), Russian sturgeon (Acipencer gueldenstaedtii), and hybrid sturgeon (Acipenser baeri Brandt ×A. schrenckii Brandt) did not significantly impair growth, feed efficiency, or reproduction. Furthermore, dietary PUFA are essential for sturgeon health. Although vegetable oils lack PUFA with more than 20 carbons, many freshwater fish, including sturgeon, possess the enzymatic pathways to convert linoleic acid (LA, C18:2n-6) and linolenic acid (LN, C18:3n-3) into arachidonic acid (AA, C20:4n-6), eicosapentaenoic acid (EPA, C20:5n-3), and docosahexaenoic acid (DHA, C22:6n-3).

Notably, linseed oil contains the highest linolenic acid content among all vegetable oils, serving as the primary precursor for long-chain n-3 HUFA synthesis in freshwater fish, while soybean oil is rich in linoleic acid, the main precursor for n-6 HUFA synthesis. Both oils thus represent promising alternative lipid sources for sturgeon feeds. However, research on linseed oil application in hybrid sturgeon diets remains limited. Li et al. reported that a mixed oil of linseed, sunflower, and tallow maintained satisfactory growth performance and health status in Russian sturgeon, while Zhu et al. found that sunflower oil was more suitable than linseed oil for replacing fish oil in Russian sturgeon diets based on growth performance. Given the current high market price of linseed oil, this study aimed to investigate the effects of total fish oil replacement by mixed linseed and soybean oils at different ratios, seeking to identify an optimal formulation to guide practical sturgeon feed production.

Materials and Methods

1.1 Feed Formulation and Preparation

Four isonitrogenous (38.7% crude protein), isolipidic (13.4% crude lipid), and isoenergetic (180 MJ/kg gross energy) experimental diets were formulated using fish meal as the basal ingredient and supplemented with 8% different lipid sources. Fish meal and anchovy oil were purchased from TripleNine Fish Protein A/S (Denmark), wheat flour from Beijing Guchuan Group, premix from Beijing Enhalor Biotech Co., Ltd., soybean lecithin from Yihai Kerry Investment Co., Ltd. (Shandong), linseed oil from Xilingol League Hongjingyuan Oil & Fat Co., Ltd., and soybean oil from Fortune Oil. Diet A contained 8% fish oil (anchovy oil), while diets B, C, and D replaced the fish oil completely with mixed oils at ratios of 75% linseed oil + 25% soybean oil (3:1 ratio), 50% linseed oil + 50% soybean oil (1:1 ratio), and 25% linseed oil + 75% soybean oil (1:3 ratio), respectively. The composition and nutrient levels of experimental diets are presented in Table 1 , and the fatty acid composition is shown in Table 2 . According to fish size changes during growth, diets were processed into sinking extruded pellets with diameters of 3.0 mm and 4.0 mm using a twin-screw extruder (Model MY56X2A, Jiangsu Muyang Group). All diets were produced at the National Freshwater Feed Safety Evaluation Base, air-dried, packed in double-layered plastic bags, and stored at -20°C until use.

1.2 Experimental Fish and Culture Conditions

The feeding trial was conducted in a recirculating aquaculture system at Beijing Chaoyang Hatchery and Aquaculture Farm. Experimental fish were same-batch, uniform-sized Siberian hybrid sturgeon (Acipenser baeri Brandt ×A. schrenckii Brandt) produced by the farm. After 24 h of fasting, fish with an initial body weight of (70.8$±0.5)gwererandomlydistributedinto12tanks(700L, 40fishpertank), withthreetanksperdietary
30, 13 : 30, 17 : 30)for12weeks.Duringthetrial, watertemperaturewasmaintainedat(20±$1)°C, dissolved oxygen at approximately 7 mg/L, and ammonia and nitrite concentrations below 100.0 g/L.

1.4 Sample Collection and Analysis

At the end of the 12-week trial, all fish were fasted for 48 h to establish baseline serum lipid levels. Three fish were then randomly selected from each tank for blood collection. Fish were anesthetized with 0.30 ml/L chlorobutanol, weighed, and blood was drawn from the caudal vein using 2 mL syringes. Blood samples were placed in 2.5 mL centrifuge tubes, centrifuged at 1,500 r/min for 5 min, and serum was collected and stored at -80°C for subsequent analysis. After blood collection, muscle samples were taken from the dorsal lateral region and liver samples were collected; both were stored at -80°C for further analysis. Remaining fish in each tank were weighed and counted for growth performance calculations.

1.5 Nutritional Composition Analysis

Feed and muscle samples were analyzed for moisture, crude protein, crude lipid, crude ash, and fatty acid composition, while liver samples were analyzed for crude lipid content. Samples were dried at 105°C for 6 h to constant weight to determine moisture content before biochemical analysis. Crude protein was determined by the Kjeldahl method, crude lipid by Soxhlet extraction, crude ash by muffle furnace incineration, and gross energy by bomb calorimetry. Fatty acid composition was analyzed using an Agilent 6890 gas chromatograph following the method of Caballero et al. All samples were analyzed in triplicate.

Serum samples were analyzed for triglycerides (TG), total cholesterol (TC), non-esterified fatty acids (NEFA), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and ketone bodies. TG was measured by enzymatic colorimetry, TC by colorimetry, and HDL-C and LDL-C by homogeneous enzymatic colorimetry using reagents from Roche Diagnostics (Germany) on a Roche/E601 automatic biochemical analyzer. Serum ketone bodies and NEFA were measured using commercial kits from Nanjing Jiancheng Bioengineering Institute on a Hitachi HITACHI7160 automatic biochemical analyzer.

Serum liver function indices including lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), as well as antioxidant indices including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), myeloperoxidase (MPO), and total antioxidant capacity (T-AOC) were also measured. LDH, ALT, and AST kits were provided by Roche Diagnostics (Germany); SOD, GSH-Px, and T-AOC kits by Nanjing Jiancheng Bioengineering Institute; and MPO kits by Beijing Huaying Bioengineering Institute. All analyses were performed using a HITACHI 7160 biochemical analyzer.

1.6 Calculations

Survival rate (SR, %) = $100 \times \mathrm{Nf}/\mathrm{Ni}$
Weight gain rate (WGR, %) = $100 \times (\mathrm{Wf} - \mathrm{Wi})/\mathrm{Wi}$
Specific growth rate (SGR, %/d) = $100 \times (\ln\mathrm{Wf} - \ln\mathrm{Wi})/t$
Feed intake (FI, %/d) = $100 \times F/[(\mathrm{Wf} + \mathrm{Wi})/2]/t$
Feed efficiency (FE, %) = $100 \times (\mathrm{Wf} - \mathrm{Wi})/F$

Where Wi = initial mean weight, Wf = final mean weight, Ni = initial number of fish per tank, Nf = final number of fish per tank, t = experimental duration in days, and F = dry feed consumption.

1.7 Statistical Analysis

Data were analyzed using Statistica 7.0 software. One-way ANOVA was performed, and when significant differences were detected ($P<0.05$), Duncan' s multiple range test was used for post-hoc comparisons. Data are expressed as means ± standard error. Two-way ANOVA was used to analyze the effects of sampling time and lipid sources on lipid metabolism. Linear regression and Pearson correlation were used to analyze relationships between dietary and muscle fatty acid compositions, with $R^2>0.5$ indicating significant correlation.

Results

2.1 Effects on Growth Performance

Growth performance indices of hybrid sturgeon fed diets with different ratios of linseed and soybean oils replacing fish oil are presented in Table 3 . Fish in group B exhibited significantly higher final body weight, WGR, and SGR compared to groups A and C ($P<0.05$), but showed no significant difference from group D ($P>0.05$). Total replacement of fish oil by mixed oils significantly improved feed efficiency ($P<0.05$), though the ratio of linseed to soybean oil in the mixed oils did not significantly affect FE ($P>0.05$). No mortality occurred during the trial, with survival rate at 100% across all groups.

2.2 Effects on Muscle Composition and Liver Crude Lipid

Table 4 shows the muscle nutritional composition and liver crude lipid content of hybrid sturgeon fed different diets. Muscle moisture content in group A was significantly higher than in other groups ($P<0.05$). Groups B and D had significantly higher muscle crude protein content than group A ($P<0.05$), with no significant differences among groups B, C, and D ($P>0.05$). Muscle and liver crude lipid contents in group A were significantly lower than in other groups ($P<0.05$). Muscle crude ash content in group A was higher than in groups B, C, and D, with significant differences from groups B and D ($P<0.05$), while no significant differences were observed among groups B, C, and D ($P>0.05$).

2.3 Effects on Liver Function and Antioxidant Indices

As shown in Table 5 , total replacement of fish oil by mixed oils did not significantly affect serum liver function indices (LDH, ALT, and AST activities) or some antioxidant indices (SOD, GSH-Px, and MPO activities) ($P>0.05$). However, serum T-AOC in group B was significantly higher than in groups C and D ($P<0.05$), with no significant difference from group A ($P>0.05$).

2.4 Effects on Serum Lipid Metabolism Indices

Table 6 presents serum lipid metabolism indices of hybrid sturgeon fed different diets. Group B had the lowest serum TG content, which was significantly lower than group C ($P<0.05$) but not significantly different from groups A and D ($P>0.05$). Group C exhibited the lowest serum NEFA content, significantly different from groups A and B ($P<0.05$). Serum HDL-C and LDL-C contents in group B were significantly higher than in the other three groups ($P<0.05$).

Total replacement of fish oil did not significantly affect serum TC or ketone body contents ($P>0.05$).

2.5 Effects on Muscle Fatty Acid Composition

Muscle fatty acid composition of hybrid sturgeon fed different diets is shown in Table 7 . Total replacement of fish oil by mixed oils did not significantly affect total saturated fatty acid (SFA) content in muscle ($P>0.05$). Total monounsaturated fatty acid (MUFA) content was lowest in group A, significantly lower than in group C ($P<0.05$). Total PUFA content in group A was significantly lower than in other groups ($P<0.05$), while EPA and DHA contents were significantly higher than in other groups ($P<0.05$). Table 8 demonstrates significant linear correlations between dietary and muscle contents of linoleic acid, linolenic acid, total n-3 PUFA, total n-6 PUFA, and n-3/n-6 PUFA ratio. Fish fed diet A had the lowest muscle linoleic and linolenic acid contents, significantly lower than other groups ($P<0.05$). Muscle linoleic and linolenic acid contents increased significantly with increasing dietary soybean and linseed oil levels, respectively ($P<0.05$). Similarly, muscle total n-6 PUFA content increased significantly with increasing dietary soybean oil content ($P<0.05$).

Discussion

The 100% survival rate and rapid growth observed across all treatments indicate that juvenile hybrid sturgeon exhibited good adaptability to alternative lipid sources. Sturgeon fed fish oil-free diets achieved equivalent (groups C and D) or even superior (group B) growth performance compared to the fish oil control group (group A). Research has shown that different dietary n-3/n-6 PUFA ratios affect fish growth even when EPA and DHA contents are identical. For instance, animals consuming low n-3/n-6 PUFA ratios are more prone to inflammatory responses and atherosclerotic plaque formation. Şener et al. reported that Russian sturgeon utilized soybean and sunflower oils effectively, while Xu et al. found that white sturgeon (Acipenser transmontanus) performed well on vegetable oil-based diets. In the present study, as dietary linseed oil proportion decreased, final body weight, SGR, and FE in groups C and D declined compared to group B. Since fatty acid composition was the sole variable among the four diets, these growth differences likely resulted from varying dietary n-3/n-6 PUFA ratios. Like other vertebrates, sturgeon cannot synthesize C18 PUFA and must obtain n-6 linoleic acid and n-3 linolenic acid from feed for growth and reproduction. Li et al. demonstrated that Russian sturgeon required both linoleic and linolenic acids, with optimal growth achieved at 1.00% supplementation of each, likely because this level better met the species' requirements. The superior growth performance observed in group B may be attributed to a more appropriate ratio and level of linoleic and linolenic acids in the diet.

Significant changes in muscle nutritional composition and liver crude lipid content resulted from linseed and soybean oil supplementation. The three fish oil replacement groups (B, C, and D) exhibited significantly higher muscle and liver crude lipid contents than the fish oil control group (group A). This lipid deposition may be attributed to higher dietary n-6 PUFA content. Turchini et al. observed significantly higher muscle crude lipid content in brown trout fed rapeseed oil rich in n-6 PUFA compared to fish oil controls. In this study, dietary n-6 PUFA content increased progressively with higher soybean oil proportions in the mixed oils (Table 2), which likely contributed to lipid accumulation in muscle and liver.

LDH, ALT, and AST play important roles in hepatic lipid metabolism and serve as key indicators of liver damage, with significantly elevated activities indicating hepatic degeneration and necrosis due to cellular injury. Despite significantly higher liver crude lipid content in groups B, C, and D compared to group A, serum LDH, ALT, and AST activities showed no significant differences among groups (Table 5). Although dietary lipid source alteration induced lipid deposition in muscle and liver, the three-month feeding period did not cause substantial liver damage, though long-term effects require further investigation.

HUFA such as EPA and DHA can inhibit endogenous cholesterol and TG synthesis, increase lipoprotein lipase activity, promote peripheral tissue clearance of very low-density lipoproteins, reduce serum TG and cholesterol levels, and increase HDL content. Antioxidant capacity can be assessed by measuring T-AOC in tissue homogenates without determining changes in each antioxidant component. For fish, T-AOC reflects antioxidant capacity and correlates closely with health status. Liu et al. reported that HUFA inhibited reactive oxygen species production, increased serum SOD activity, GSH content, and T-AOC, and suppressed serum malondialdehyde (MDA) formation. Thus, increased LC-n-3 HUFA content modifies the oxidative environment. In this study, group B exhibited significantly higher serum T-AOC than groups C and D, likely due to appropriate LC-n-3 HUFA content in diet B (Table 2), consistent with previous findings that increased LC-n-3 HUFA reduced reactive oxygen species generation.

Numerous studies have demonstrated that dietary HUFA, particularly DHA and EPA, significantly promote serum cholesterol reduction and inhibit TG synthesis, while linolenic acid, as a precursor of EPA and DHA, effectively lowers TG levels. In this study, serum TG content in group B showed no significant difference from group A but was lower than in groups C and D. This may be attributed to the high linolenic acid content in diet B playing a key role in TG reduction when EPA and DHA contents were comparable across diets. Additionally, elevated LDL-C may increase vascular and tissue burden. Group B exhibited increased serum HDL-C content but also significantly higher LDL-C content, possibly related to high levels of acyl-CoA:cholesterol acyltransferase (ACAT), an important hepatic enzyme that preferentially utilizes unsaturated fatty acids over saturated fatty acids to esterify free cholesterol into cholesterol esters. Therefore, diet B rich in unsaturated fatty acids enhanced ACAT activity, increasing cholesterol ester delivery to LDL particles and consequently raising serum LDL-C levels. Huang et al. similarly reported significantly higher serum LDL-C content in Amur sturgeon fed rapeseed oil compared to fish oil, attributing this to dietary fatty acid types and contents.

Serum NEFA represents the most important lipid metabolic component in vertebrate blood, reflecting lipid importance in energy metabolism, as NEFA transport in blood facilitates fatty acid oxidation in tissues. Studies have shown that fatty acids mobilized from lipid reserves depend on carbon chain length, degree of unsaturation, and positional isomerism. Saturated fatty acid turnover rates are stable at the whole-organism level and accurately reflect total NEFA turnover, whereas long-chain PUFA exhibit different kinetic behaviors. Higher saturated fatty acid content leads to lower total NEFA turnover. In this study, serum NEFA content was highest in group A (Table 6), likely due to higher dietary saturated fatty acid content (Table 2).

Muscle fatty acid composition was clearly influenced by dietary fatty acid composition, particularly linolenic and linoleic acids (Table 7). Previous reports indicate that any dietary lipid source replacement is reflected in fish tissue fatty acid composition to some degree. In this study, dietary lipid sources were linseed and soybean oils, rich in linolenic and linoleic acids respectively (Table 2). Therefore, the positive correlations between dietary and muscle contents of these fatty acids (Table 8) are expected. Compared to group A, EPA and DHA contents in muscle of group B reached 70.3% and 77.3%, respectively, further confirming that hybrid sturgeon can convert linolenic and linoleic acids into corresponding long-chain n-3 and n-6 PUFA. Thus, using linseed oil as an excellent fish oil substitute in aquafeeds without compromising fish growth performance is feasible.

In summary, total replacement of fish oil by mixed linseed and soybean oils at a 3:1 ratio (75% linseed oil + 25% soybean oil) produced optimal growth effects in hybrid sturgeon.

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