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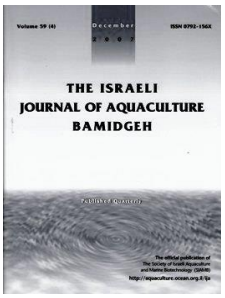

Substitution of Krill meal for Fish Meal in Feed for Russian Sturgeon, *Acipenser gueldenstaedtii*

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Abstract

Krill meal (KM) is a potential feed ingredient to partially replace fish meal (FM) in aquaculture. To better understand the efficacy of krill meal, a 200-day feeding trial was conducted with Russian sturgeon (*Acipenser gueldenstaedtii*). Four extruded diets in which KM replaced 0%, 10%, 20%, or 30% of FM in the formulation were fed to sturgeons (weight 481 g) for 200 days. Growth of Russian sturgeon fed diets containing KM was as good, or even better, than fish fed a FM control diet. No differences were observed in terms of dorsal muscle composition between the fish fed diets which contained KM and those fed the FM control diet. Fluoride concentrations in the dorsal muscle, liver, and kidney, as well as swim bladder in all dietary groups were below a detectable limit. In the gills, skin, vertebral bone and dorsal scutes, the fluoride content from the KM groups progressively increased with dietary fluoride concentration. In conclusion, KM can be a partial substitute for FM in the diets of Russian sturgeon without affecting normal growth performance.

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Introduction

Russian sturgeon, *Acipenser gueldenstaedtii*, are amongst the most valued freshwater fish cultured in many countries for caviar, and they have recently received considerable attention in China as well (Wei et al., 2011). Production of cultivated sturgeon species for luxury caviar has risen steadily over the past 10 years. Commercial diets for this species have traditionally been based on fish meal (FM) and fish oil. However, rapid development in intensive aquaculture has resulted in limited supply and higher prices of FM. A significant challenge in the aquaculture feed industry is reduction of FM levels while at the same time increasing the amount of alternative protein sources in feeds, without compromising growth, health, and quality of cultured fish.

Antarctic krill (*Euphausia superba*) is receiving increased attention as a viable source of proteins, lipids, and omega-3 fatty acids (Nicol et al., 2012). The biomass of Antarctic krill has been estimated to be around 379 million tons (Atkinson et al., 2009). The 316,408 ton annual capture in 2014 (FAO 2016) is merely 5% of the precautionary catch quota (6.2 million tons annually) set by the Convention on the Conservation of Atlantic Marine Living Resources (Nicol et al., 2012). The availability of Antarctic krill as a feed source in the form of meal for several finfish species has recently been investigated (Olsen et al., 2006; Hansen et al., 2010). It was concluded that krill meal (KM) could be a good candidate for partial replacement of FM in diets without negative growth or health effects. However, information is limited regarding KM inclusion in diets of commercially cultured freshwater fish (Gaber 2005).

A limiting factor for further application of KM is concern about the high fluoride content and its potential accumulation in organs especially bone. Studies have been conducted on KM inclusion and fluoride accumulation on many fish species such as Atlantic salmon *Salmo salar,* (Julshamn et al., 2004), Atlantic cod *Gadus morhua*, Atlantic halibut *Hippoglossus hippoglossus* (Moren et al., 2007), and yellowtail *Seriola quinqueradiata,* (Yoshitomi and Nagano 2012). Results showed that no fluoride accumulation was evident in the muscle or bone of fish reared in seawater, but increased retention in the bones of rainbow trout reared in freshwater was observed. Studies on the effect of KM on the retention of fluoride in tissues of other commercial freshwater fish species are limited.

The aim of the present experiment was to evaluate the effect on growth performance, feed utilization, body composition, and fluoride accumulation in tissues of freshwater Russian sturgeon fed practical diets where FM was partially replaced by commercial KM.

Materials and Methods

Experimental diets. The krill used in this experiment were harvested from Antarctic waters and immediately frozen whole in large blocks onboard the ship. The frozen blocks were then freeze-dried and finely ground on land at the Keruier Biological Products Co., Ltd., Jinan, China. Diets were formulated to satisfy the nutritional requirements of sturgeon and industrially produced as dry pellets in a feed mill (TECH-BANK Co., Ltd., Ningbo, China). A total of four extruded diets for sturgeon were prepared, each containing KM to replace 0%, 10%, 20%, or 30% of FM (denoted as K0, K10, K20, and K30). The pellets were stored in sealed bags prior to shipping to a fish farm. Formulation of the experimental diets is presented in Table 1, and proximate composition, amino acid composition and fluoride content are listed in Table 2.

Table 1. Formulation of four experimental diets

Ingredients (g/kg)	*K0*	*K10*	*K20*	*K30*
Fishmeal	500	400	300	200
Soybean meal	99	99	99	99
Full fat soybean	100	100	100	100
Krill meal	0	100	200	300
Wheat flour	120	120	120	120
Fish oil	90	90	90	90
Mineral premix	20	20	20	20
Vitamin premix	4	4	4	4
Mono-dicalcium phosphate	10	10	10	10
Lecithin powder	40	40	40	40
Soybean lecithin	10	10	10	10
Vitamin E	1	1	1	1
Choline chloride	6	6	6	6

Table 2. Proximate, amino acid composition and fluoride content of experimental diets

	K0	*K10*	*K20*	*K30*
Dry matter (DM), g/kg	936.5	933.4	910.4	892.5
In DM,				
Crude protein (CP), g	483.4	478.2	474.7	465.9
Crude lipid, g	162.3	185.7	171.4	168.6
Ash, g	154.4	149.6	145.3	146.2
Amino acids, g/kg				
Arginine	26.7	28.2	26.6	26.7
Histidine	11.6	11.5	10.8	10.9
Isoleucine	16.6	17.6	17.0	17.4
Leucine	33.0	34.9	32.8	32.7
Lysine	32.3	32.9	30.3	30.0
Methionine	8.4	5.9	10.3	8.8
Phenylalanine	18.7	20.1	19.0	19.4
Threonine	17.9	18.6	17.2	17.2
Tryptophan	4.2	4.2	3.7	3.9
Valine	19.9	20.7	9.3	19.4
Aspartic acid	40.0	42.4	40.1	41.0
Glutamic acid	72.0	75.8	71.0	71.3
Serine	20.1	20.1	18.7	18.7
Proline	22.2	23.4	22.1	21.9
Glycine	24.6	24.8	22.6	21.4
Alanine	24.4	25.3	23.2	22.6
Tyrosine	14.8	16.1	15.3	15.8
Cysteine	22.8	23.2	18.3	17.2
Taurine	3.0	3.0	2.9	2.7
Fluoride, mg/kg	55.11	185.15	300.76	390.51

Fish and facilities. The growth trial was conducted in net cages in a commercial sturgeon farm at Hangzhou, China, where Russian sturgeon were fed the experimental diets for a period of 200 d under natural photoperiod. Sturgeons were collected from a commercial sturgeon company (Kaluga Queen, Hangzhou, China). At the start of feeding trial, the fish were bulk weighed. Twelve groups of 481 g Russian sturgeon were randomly allocated to 540 m^3 net cages in lakes (1000 fish/cage). Triplicate groups of fish were distributed to each of the experimental diets. The sturgeons were hand-fed to apparent satiation at 08:30 and 16:30 h daily.

Sampling procedure. Before the onset of the experiment, samples from the four diets were collected for chemical analyses. At the end of the growth trial, fish from each net cage were bulk weighted to calculate the growth parameters. At the end of the experiment three fish from each net cage were randomly selected, killed, cleaned, and dissected to study the gills, skin, dorsal muscle, vertebral bone, dorsal scutes, liver, and kidney, as well as swim bladder. All samples were dried and ground with a small coffee grinder for fluoride analysis. Sampled dorsal muscles were also used to determine their main chemical composition. All samples were stored frozen at -80°C until analysis.

Chemical analysis. Proximate composition of homogenized diet and dorsal muscle samples were evaluated according to the standard AOAC procedures (AOAC, 2005). Dry matter was determined by drying samples at 105°C to a constant weight. Crude protein content was measured by determining nitrogen content (N×6.25) using automated Kjeldahl analysis (Tecator Kjeltec Auto 2030 analyzer, Foss, Warrington, U.K). Fat was determined after acid hydrolysis, followed by petroleum ether extraction (Tecator 148 Soxtec system 2050 Auto Extraction apparatus, Foss, Warrington, U.K.). Ash was determined by combustion to a constant weight in a muffle furnace at 550°C. Fluoride content in different tissues was determined in accordance with the methods specified in the Chinese National Standard: GB/T 5009.18-2003 (Determination of fluoride in feeds or foods). Specifically, a fluoride ion selective combination electrode (PF-1, Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China) was used.

Calculations and statistical analyses. Fish growth performance was calculated using the following equations:

Weight gain (WG) = Final body weight (FBW, g) - Initial body weight (IBW, g)

Specific growth rate (SGR) = 100× [(Ln (FBW, g) - Ln (IBW, g)]/ day fed

Feed conversion ratio (FCR) = Feed fed (g, dry weight)/ weight gain (g, wet weight)

Survival rate =100 × Final fish number/ Initial fish number

All statistical analysis was carried out using IBM SPSS 21.0 software package for Windows. Normality and equal variance of the data were tested before performing one-way ANOVA. Tukey's multiple comparisons test was employed to detect the significant differences. Differences were regarded as significant when $P<0.05$. All results are presented as means and standard error of mean (S.E.M).

Results

Growth performance and feed utilization. Fish growth performance and feed utilization are shown in Table 3. Growth in all KM groups was as good as, or significantly better than that of the FM control diet. Final body weight (FBW), weight gain (WG) and specific growth rate (SGR) increased while feed conversion ratio (FCR) decreased as the proportion of krill meal replacement in the diet increased ($P<0.05$). The fish fed KM at 20% and 30% replacement levels exhibited significantly higher FBW, WG and SGR values and lower FCR than fish fed the FM control diet.

Table 3. Growth performance and feed utilization of sturgeon fed with the experimental diets

	IBW(g)[1]	*FBW (g)*[2]	*WG (g)*[3]	*SGR (%/d)*[4]	*FCR*[5]	*Survival rate (%)*[6]
K0	492.5±2.5	1353.6±13.6^{b}	861.1±11.1^{c}	0.50±0.00^{b}	1.34±0.01^{c}	87.0±1.4
K10	479.0±2.4	1373.5±36.1^{ab}	894.5±12.1^{bc}	0.53±0.01^{ab}	1.29±0.02^{bc}	82.7±1.5
K20	471.0±1.0	1417.4±15.3^{a}	946.4±16.3^{ab}	0.55±0.01^{a}	1.19±0.02^{ab}	84.3±1.6
K30	483.0±3.0	1489.2±23.6^{a}	1006.2±29.1^{a}	0.56±0.01^{a}	1.10±0.02^{a}	83.0±1.0

Within the same column, values with different superscripts are significantly different ($P < 0.05$)
[1] IBW: Initial body weight (g)
[2] FBW: Final body weight (g)
[3] Weight gain (WG) = Final body weight (FBW, g) - Initial body weight (IBW, g)
[4] Specific growth rate (SGR) = 100× [(Ln (FBW, g) - Ln (IBW, g)]/ day fed
[5] Feed conversion ratio (FCR) = Feed fed (g, dry weight)/ weight gain (g, wet weight)
[6] Survival rate =100 × Final fish number/ Initial fish number

Nutritional composition. The proximate dorsal muscle composition of sturgeon at the end of the feeding trial can be seen in Table 4. No differences between treatments were observed in terms of moisture, crude protein, crude lipid, and ash contents in fish fed with the test diets or the control FM diet.

Table 4. Nutritional composition of sturgeon fed with the experimental diets

	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
K0	78.73±1.35	16.12±0.42	2.59±0.24	1.04±0.13
K10	78.58±0.52	16.94±0.16	2.55±0.09	0.94±0.05
K20	78.10±0.85	16.96±0.21	2.54±0.21	1.11±0.09
K30	79.74±0.38	16.22±0.26	2.60±0.03	1.07±0.01

Fluoride concentrations. At the end of the experiment fluoride concentrations in different tissues were analyzed (Table 5). After 200 d of feeding, fluoride concentrations in the dorsal muscle, liver, kidney, as well as swim bladder fish in all dietary groups were below the detectable limit (1 mg/kg). Fluoride level of gills, skin, and dorsal scutes in sturgeon ranged from 56.58 mg/kg to 336.36 mg/kg, 276.17 mg/kg to 844.10 mg/kg, and 552.73 mg/kg to 2591.39 mg/kg on a dry weight basis, respectively. Fluoride content in the gills, skin, and dorsal scutes evidently increased with dietary fluoride concentration. The fluoride level in vertebral bone increased with the inclusion of KM at the 20% and 30% levels, but was not as high as recorded from the gills, skin, and dorsal scutes.

Table 5. Fluoride concentrations (mg/kg, dry matter basis) in tissues of sturgeon fed with the experimental diets.

	Gills	Skin	Vertebral bone	Dorsal scutes
K0	56.58±3.50^{d}	276.17±10.18^{d}	48.19±0.29^{c}	552.73±10.68^{d}
K10	166.75±6.75^{c}	519.15±11.63^{c}	49.43±1.12^{c}	1124.34±23.80^{c}
K20	257.20±3.51^{b}	682.93±18.62^{b}	65.13±1.31^{b}	1644.35±81.67^{b}
K30	336.36±8.11^{a}	844.10±25.86^{a}	73.31±2.18^{a}	2591.39±137.45^{a}

Within the same column, values with different superscripts are significantly different ($P<0.05$)

Discussion

Antarctic krill has been used as a feed source for cultured fish. The effects of KM on growth and feed utilization in some fish species have been well documented (Olsen et al., 2006; Tibbetts et al., 2011; Yoshitomi and Nagano 2012). Partial replacement of FM with KM generally had either no negative effect or increased growth in marine fish, such as Atlantic salmon (Julshamn et al., 2004), Atlantic cod, rainbow trout, and Atlantic halibut (Moren et al., 2007). These results showed that KM could be included in marine fish diets substituting up to 40% of FM without any negative effect on fish growth performance. However, such replacement reduced the growth rate of rainbow trout (Yoshitomi et al., 2006).

By contrast, in the present experiment, the partial replacement of FM with KM had no negative effect on the growth of freshwater Russian sturgeon reared in net cages. The significant growth enhancement of fish fed KM at 20% and 30% replacement levels is in line with previous results that showed increased growth rate and nutrient utilization in Atlantic cod and Atlantic halibut fed FM-based diets with partial replacement of FM with freeze-dried KM (Tibbetts et al., 2011). Our results are the first to show that growth of Russian sturgeon fed diets containing freeze-dried KM in place of FM is as rapid, or even better, than fish fed FM control diet, even without amino acid supplementation. A likely explanation for the positive results found in the present study could be an increase in feed consumption owing to the presence of unidentified growth factor(s) and/or increased feed palatability from soluble fraction(s) of freeze-dried KM during the feeding procedure for Russian sturgeon. Growth stimulatory benefit and/or increased palatability may also explain growth enhancement found in the feeding trial in some cultured species (Williams et al., 2005; Querol et al., 2014). However, further research is required to identify the factor(s) responsible for the higher growth rate found in sturgeon fed a diet which included KM.

The use of Antarctic krill in fish feeds is limited by its high fluoride level (Storebakken 1988). Fluoride in krill is located mainly in the exoskeleton, and the fluoride level in whole KM has been shown to be 1000 to 6000 mg/kg dry-weight (Moren et al., 2007). Results have indicated that fluoride concentration in muscle and bone of Atlantic salmon was unaffected by the dietary fluoride level, and Atlantic salmon are highly tolerant of dietary fluoride up to 350 mg/kg (Julshamn et al., 2004). Similar results were found in the bones or muscles of rainbow trout, Atlantic halibut, and Atlantic cod when fed KM with high levels of fluoride in diets (Moren et al., 2007). However in freshwater rainbow trout, fluoride concentration in vertebral bones increased as dietary fluoride content increased (Yoshitomi et al., 2007). In the present experiment, the fluoride levels in the muscle of sturgeon concurred with previous results reported for other fish species. Meanwhile, the fluoride concentrations in other soft tissues, such as liver, kidneys, as well as swim bladder of sturgeon in all dietary groups, were below the detectable limit (1 mg/kg). Fluoride content in the gills, skin, vertebral bone, and dorsal scutes increased with dietary fluoride concentration. Siberian sturgeon (*Acipenser baerii*) also showed increased fluoride concentration in the skin and gills with dietary fluoride concentration, whereas the muscles and liver were was not affected by fluoride levels in the diet (Shi et al., 2013). The differences between seawater and freshwater fish may be attributed to the effect of water salinity. Fluoride toxicity decreases with increasing water levels of chloride and calcium and thus with increasing salinity. A similar effect of salinity on reducing the toxicity of fluoride from fish diets may be present as well. This explanation was confirmed by Hansen et al., (2011), who found that including CaCl2 in diets can efficiently reduce the fluoride uptake of rainbow trout reared in freshwater. However, it was found that fluoride accumulated in bones (2150 mg/kg) of marine yellowtail fed a high content (580 mg/kg) of dietary fluoride (Yoshitomi and Nagano 2012). From the literature cited here, fluoride accumulation in tissues appears to depend on the fish species, and not only on salinity, as such fish may differ in their sensitivity to fluoride toxicity (Camargo 2003). Our experiment was conducted using freshwater sturgeon, and fish fed a high content of dietary fluoride accumulated fluoride in dorsal scutes and skin, rather than in vertebral bones. The lower rate of fluoride accumulation in the vertebral bone of Russian sturgeon compared with other species might be attributed to the fact that the hardest bone tissue in sturgeons is located in their dorsal scutes and skin, whereas their vertebral bones are made of a type of soft tissue. The reason for the high fluoride concentration in gills is unclear, but is most likely a defense mechanism against fluoride toxicity as it may prevent dietary fluoride from travelling to other

organs. The ingested fluoride may also be excreted through the gills into the water. However, this hypothesis requires further research.

In conclusion, KM can partially substitute FM in practical diets for Russian sturgeon without negatively affecting growth, feed utilization, and body composition. In addition fluoride accumulation in fish soft tissues is low. Russian sturgeon appear able to tolerate dietary fluoride levels at 390 mg/kg without compromising growth throughout a long-term feeding experiment, although accumulation of fluoride may high in hard tissues. This experiment demonstrated the potential of KM inclusion in extruded diets for Russian sturgeon.

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