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Effects of cottonseed oil in diets on growth, feed efficiency and health parameters of chum salmon *Oncorhynchus keta* fry

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Abstract: We evaluated the effects of replacing fish oil in diets with cottonseed oil on chum salmon *Oncorhynchus keta* fry. Chum salmon fry were fed commercial diets supplemented with 0.5% or 2.0% fish oil (0.5 FO, 2.0 FO) or cottonseed oil (0.5 CO, 2.0 CO) for two to three months in aquariums in 2005 and 2006 to compare parameters between 0.5 FO and 0.5 CO, 2.0 FO and 2.0 CO diet groups, respectively. In 2005, fish fed 0.5 CO diet for three months, 2.0 CO diet for two and three months showed significantly better growths. Feed efficiencies of fish fed 0.5 CO and 2.0 CO diets were almost better both in 2005 and 2006. The ATP contents of fish fed 0.5 CO and 2.0 CO diets for two months were significantly increased both in 2005 and 2006. Burst swimming velocities of fish fed 0.5 CO and 2.0 CO diets for two months were significantly faster in 2006. Tolerances to fasting were significantly higher in fish fed 2.0 CO in 2005, 0.5 CO and 2.0 CO in 2006. These results suggest that supplementing the diet with cottonseed oil is effective for improving the growth, feed efficiency and health parameters of chum salmon fry.

Keywords: Chum salmon, Cottonseed oil, Growth, Health parameter

Chum salmon *Oncorhynchus keta* is the second most abundant salmon in the North Pacific Ocean and one of the most important species for coastal fisheries in northern Japan. Approximately 1 billion fry have been released since the early 1980 s and over 30 million adult fish have returned to the coastal areas of Hokkaido, northern Japan, every year since the mid 1980 s (Watanabe, 1999). Unfortunately, the price of adult fish has declined and the total amount of returned adult fish has not increased in Hokkaido recently (Shimizu, 2002). So the cost of rearing chum salmon fry must be reduced. Also, rearing methods to create healthy fry must be developed in order to attain higher return rates to coastal areas as adult fish.

Chum salmon fry are fed artificial diets for a couple of months after emergence in hatcheries of Hokkaido and the cost of the diet for fry is one of the main expenses in the rearing of chum salmon fry. With the decline in the catches of pelagic fish such as pilchard, demands for fish meal as the ingredient of diets in aquaculture have risen recently in Japan (Tacon, 1996). Fish oil is often supplemented to the diet, however, the supply of fish oil is expected to remain static or dwindle in spite of increasing demand in the future (Higgs and Dong, 2000). So the costs of diets for chum salmon fry are increasing.

Lipids play important roles in the nutrition of fish (Watanabe, 1982) and specifically fatty acids are the favored source of metabolic energy in fish (Sargent *et al.,* 2002). Supplementation of oil to the diets has protein, the main component of fish meal, sparing effect (Lee and Putnum, 1973). One report suggests that supplementation of fish oil to diets is effective for growth in salmonids (Watanabe and Takeuchi, 1976). The replacement of fish oil with vegetable oils in diets has been investigated because vegetable oils are often cheaper than fish oil (Naylor *et al*., 2000). Many reports suggest that vegetable oils have equal effects compared to fish oil on growth in salmonids (Bell *et al*., 2001, 2002, 2003). Moreover, supplementation of vegetable oils to diets improves the health of fish as shown by their swimming performance (McKenzie *et al*., 1998) and resistance to fish pathogens (Bransden *et al*., 2003).

In the present study, we investigated the effects of replacing fish oil (FO) in diets with cottonseed oil (CO) from

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the viewpoint of improving the growth, feed efficiency and health parameters of chum salmon fry.

Materials and Methods

Fish

Experiments were conducted in 2005 and 2006. In 2005, chum salmon eggs were artificially fertilized on September 27 in the previous year and fry were fed from January 12. In 2006, eggs were artificially fertilized on October 10 in the previous year and fry were fed from January 10. They were reared in a 60 *l* volume acrylic tank using running well water until April 1, 2005 and March 10, 2006, respectively. The water temperature ranged from 7.36 to 8.35°C during the experiment.

Design of the experiments

A total of 800 chum salmon fry were divided into 4 experimental groups in each year. Each batch of 200 fish was fed commercial diet supplemented with 0.5% (w/w) or 2.0% fish oil (0.5 FO and 2.0 FO), 0.5% or 2.0% cottonseed oil (0.5 CO and 2.0 CO). Commercial diet and FO were provided by Marubeni Nissin Feed (Tokyo, Japan) and Nihon Chemical Feed (Hakodate, Japan), respectively. CO was purchased from Kanto Chemical (Tokyo, Japan). The fatty acid component of FO, CO and lipid in the commercial diet, and the chemical composition of the commercial diet were analyzed by Japan Frozen Foods Inspection Corporation (Tokyo, Japan). Fish were fed every day and the total amount of diet supplied to each group from January 12 to March 10, and from March 11 to April 1 were each 200 g for each group, respectively in 2005. Those from January 10 to February 10, and from February 11 to March 10 were 85 g and 160 g, respectively in 2006. In each group, the number of dead fish was counted and the amount was weighed every day.

Sampling procedure

Fish were sampled on March 10 and April 1 in 2005, and on February 10 and March 10 in 2006. Thirty-six fish were caught randomly from each experimental group and anesthetized in 2-phenoxyethanol solution on each sampling day. The body weight (BW, g) of the captured fish were measured. Five fish from the group were stored at −80°C immediately after measuring for analysis of adenosine triphosphate (ATP) content as described below.

ATP content analysis

The ATP content of fish was measured using the bioluminescent reaction of luciferin to the existence of luciferase and ATP, according to the method described by Maeda (2004). One part of the body sample homogenized using luminometer buffer (in mmol/*l* $MgCl_2$ 10, HEPES 25, to pH 7.75 with NaOH) was mixed with one part of luciferase -luciferin (18.5 mg/*ml* in distilled water; Wako Pure Chemical. Tokyo, Japan), and the luciferin radiation was measured for 5 min using a luminescence reader (BLR-201, Aloka, Tokyo, Japan). The ATP standard (Kanto Chemical, Tokyo, Japan) ranged from 0.1 fmol/*l* to 10 nmol/*l*. The ATP content was shown as ATP (pmol) per g BW.

Burst swimming velocity

Fifteen fish were caught randomly from each experimental group on April 1, 2005 and on March 10, 2006. Burst swimming velocity (BV) of fish was measured using the water tunnel apparatus for chum salmon, which was first described by Kobayashi and Ohkuma (1983) and modified by Ohkuma *et al*. (1998). The horizontally-placed swimming chamber received a fixed inflow of water from a header tank from which water overflowed. The water current velocity in the chamber could be easily changed by adjusting the height of the drain pipe. The internal diameter and length of the swimming chamber were 20 mm and 30 cm, respectively. Using polynomial regression analysis, the water current velocity in the chamber was proportional to the height difference between the chamber and the drain pipe, as follows:

$$\text{Water current velocity (cm/s)} = 7.32 \times \text{height difference (cm)}^{0.628}$$

After the fish were housed in the swimming chamber, the water current velocity was immediately changed. The BV was defined as the water current velocity in the swimming chamber into which the fish were swept, and was expressed in centimetres per second.

Tolerance to fasting

Thirty fish were caught randomly from each experimental group on April 1, 2005 and March 10, 2006. They were fasted in the same aquariums used in the experiment with running well water. The number of dead fish was counted every day, and changes in survival rate during the fasting experiment were examined. This experiment was continued until all the fish had died.

Feed efficiency

The feed efficiency (FE) of each group from January 12 to March 10, from March 11 to April 1 in 2005 and from January 10 to February 10, from February 11 to March 10 in 2006 was calculated using the following equation:

$$FE(\%) = BWi\ (g) / SD\ (g) \times 100$$

In this equation, BWi and SD mean total increased BW and total amount of supplied diets in each period, respectively. Total increased BW was calculated using the following equation:

$$BWi\ (g) = (BW_{t1} - BW_{t0}) \times Ns + (BW_{d} - BW_{t0}) \times Nd$$

In this equation, BW_{t1} and BW_{t0} mean average BW at the end and at the beginning of each sampling period, respectively. Ns means the number of surviving fish at the end of each sampling period. BW_{d} and Nd mean average BW and the number of deceased fish during each sampling period, respectively.

Statistical analysis

The student *t*-test was used for comparison of BW, ATP content and BV between fish fed 0.5 FO and 0.5 CO, and between fish fed 2.0 FO and 2.0 CO diets, respectively in each year. The Kaplan-Meier method followed by the Logrank test was used to determine the difference in the survival rate in the tolerance test to fasting. Results were considered as significant if $P < 0.05$.

Results

Chemical composition of commercial diet and fatty acid compositions of oils and lipid in commercial diet

The chemical composition of the commercial diet is shown in Table 1. Fatty acid compositions of FO and CO and lipid in the commercial diet are shown in Table 2. Fatty acid compositions of FO and lipid in the commercial diet were similar; palmitic acid, oleic acid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were the main components. Linoleic acid was also one of the main components of lipid fatty acid in the commercial diet. In FO, eicosenic acid and docosenoic acid were also the main components of fatty acid. About half the amount of fatty acid in CO was linoleic acid.

Table 1 Chemical composition of commercial diet fed to chum salmon fry

Composition	Weight (%)
Protein	48.1
Lipid	7.7
Carbohydrate	21.0
Ash	13.4
Moisture	9.8

Growth of experimental fish

The average BWs of fish in each group on the sampling days in 2005 and 2006 are shown in Table 3. Initial BWs (average ± S.D.) were 0.281 ± 0.04 g on January 12 in 2005 and 0.314±0.05 g on January 10 in 2006. In 2005, the average body weight of fish fed 2.0 CO diet on March 10 was significantly larger than that of fish fed 2.0 FO diet (P= 0.002). On April 1, the average BWs of fish fed 0.5 CO and 2.0 CO diets were also significantly larger than those of fish fed 0.5 FO and 2.0 FO diets, respectively ($P < 0.001$). In 2006, no significant differences in the average BWs of fish between these groups were observed both on February 10 and March 10.

Feed efficiency

FEs of each group in 2005 and 2006 are shown in Table 4. There were no leftover diets after feeding in all groups. The FEs of all fish fed CO diets were better than those of fish fed FO diets in 2005. In 2006, FEs of fish fed CO diets were better than those of fish fed FO diets except the fish fed 0.5 CO diet from January 10 to February 10.

ATP content

The ATP content of fish in each group on the sampling days in 2005 and 2006 is shown in Fig. 1. The ATP content of fish fed 0.5 CO and 2.0 CO diets on March 10 was more significantly increased than those of fish fed 0.5 FO and 2.0 FO diets, respectively both in 2005 and 2006.

Burst swimming velocity

The results of BV of fish in each group conducted in 2005 and 2006 are shown in Fig. 2. In 2005, no differences were observed in the values of BV between the fish fed FO and CO diets. In 2006, the values of BV of fish fed 0.5 CO and 2.0 CO diets were significantly higher than those of each fish fed 0.5 FO and 2.0 FO diets, respectively.

Tolerance to fasting

The changes of survival rate in the tolerance test to fasting

Table 2 Fatty acid composition (%) of fish oil, cottonseed oil, and lipid in commercial diet

	Fish oil	Cottonseed oil	Lipid in commercial diet
Lauric acid (12:0)	N.D.(1)	N.D.	0.1
Myristic acid (14:0)	3.9	0.7	5.9
Myristoleic acid (14:1 n-5)	0.1	N.D.	0.4
Pentadecanoic acid(15:0)	0.3	N.D.	0.5
Palmitic acid (16:0)	10.0	19.7	19.0
Palmitoleic acid (16:1 n-7)	6.8	0.7	7.3
Hexadecadienoic acid (16:2 n-4)	0.5	N.D.	0.7
Hexadecatrienoic acid(16:3 n-3)	0.4	N.D.	N.D.
Hexadecatetraenoic acid (16:4 n-1)	0.9	N.D.	N.D.
Heptadecanoic acid (17:0)	0.2	0.1	0.4
Heptadecenoic acid (17:1 n-8)	N.D.	0.3	0.3
Stearic acid (18:0)	1.9	2.8	5.1
Oleic acid (18:1 n-9)	16.0	23.1	12.9
Linoleic acid (18:2 n-6)	1.0	51.1	11.3
Linolenic acid (18:3 n-3)	0.7	0.4	1.9
Octadecatetraenoic acid (18:4 n-3)	2.2	N.D.	N.D.
Arachidic acid (20:0)	0.1	0.5	0.3
Eicosenoic acid (20:1 n-9)	12.7	0.2	1.6
Eicosadienoic acid (20:2 n-6)	0.2	N.D.	0.3
Eicosatrienoic acid (20:3 n-6)	N.D.	N.D.	0.3
Eicosatetraenoic acid (20:4 n-3)	0.8	N.D.	N.D.
Arachidonic acid (20:4 n-6)	0.6	N.D.	1.3
Eicosapentaenoic acid (20:5 n-3)	11.2	N.D.	11.2
Heneicosapentaenoic acid (21:5 n-3)	0.6	N.D.	0.5
Behenic acid (22:0)	0.1	0.2	N.D.
Dococenoic acid (22:1 n-9)	18.3	N.D.	1.4
Docosatetraenoic acid (22:4 n-6)	0.1	N.D.	N.D.
Docosapentaenoic acid (22:5 n-3)	1.4	N.D.	1.6
Docosapentaenoic acid (22:5 n-6)	0.1	N.D.	N.D.
Docosahexaenoic acid (22:6 n-3)	7.9	N.D.	11.9
Lignoceric acid (24:0)	N.D.	0.1	N.D.
Tetracocenoic acid (24:1 n-9)	0.9	N.D.	N.D.
Others	0.1	0.1	3.8

(1): Not detected

Table 3 Effect of diets containing 0.5 or 2.0% (w/w) supplementary dietary lipid as fish oil (FO) and cottonseed oil (CO) on growth of chum salmon fry in 2005 and 2006

A. 2005

	Mar. 10	Apr. 1
0.5FO	1.378±0.39	2.052±0.61 a
0.5CO	1.477±0.36	2.594±0.61 b
2.0FO	1.315±0.32 a	2.062±0.58 a
2.0CO	1.548±0.30 b	2.507±0.41 b

B. 2006

	Feb. 10	Mar. 10
0.5FO	0.728±0.17	1.765±0.43
0.5CO	0.672±0.21	1.768±0.34
2.0FO	0.667±0.16	1.712±0.37
2.0CO	0.701±0.16	1.769±0.40

Body weight expressed as means ± S.D.
Means without a common letter differ between 0.5FO and 0.5CO or 2.0FO and 2.0CO (t-test, $p < 0.05$)

Table 4 Effect of diets containing 0.5 or 2.0% (w/w) supplementary dietary lipid as fish oil (FO) and cottonseed oil (CO) on feed efficiency (%) of chum salmon fry in 2005 and 2006

A. 2005

	Jan. 12 - Mar. 10	Mar. 11 - Apr. 1
0.5FO	94.5	42.1
0.5CO	101.4	68.1
2.0FO	86.8	45.6
2.0CO	107.8	59.4

B. 2006

	Jan. 10 - Feb. 10	Feb. 11 - Mar. 10
0.5FO	95.5	102.4
0.5CO	83.0	108.9
2.0FO	79.7	100.6
2.0CO	89.7	106.8

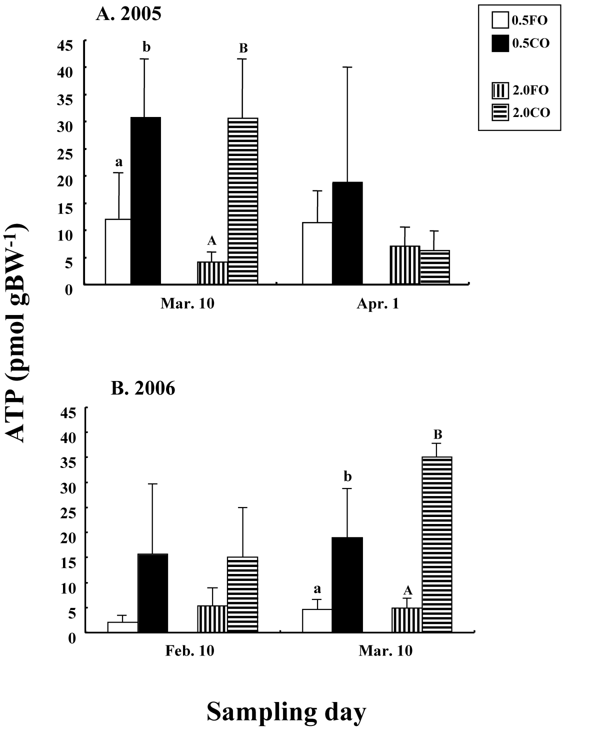

Fig.1 Effects of diets containing 0.5 or 2.0% (w/w) supplementary dietary lipid as fish oil (FO) and cottonseed oil (CO) on ATP content of chum salmon fry in 2005 and 2006. Results expressed as mean ± S. D. Bars without a common letter differ between 0.5 FO and 0.5 CO or 2.0 FO and 2.0 CO ($P < 0.05$).

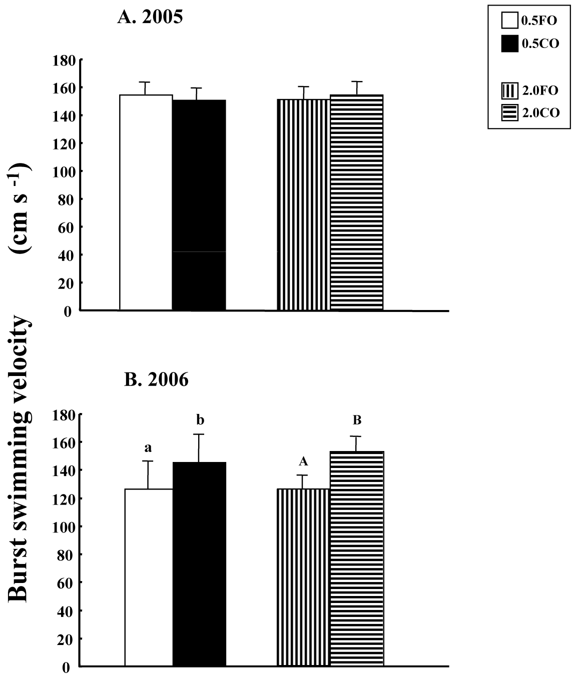

Fig.2 Effects of diets containing 0.5 or 2.0% (w/w) supplementary dietary lipid as fish oil (FO) and cottonseed oil (CO) on burst swimming velocity of chum salmon fry in 2005 and 2006. Results expressed as mean ± S.D. Bars without a common letter differ between 0.5 FO and 0.5 CO or 2.0 FO and 2.0 CO ($P < 0.05$).

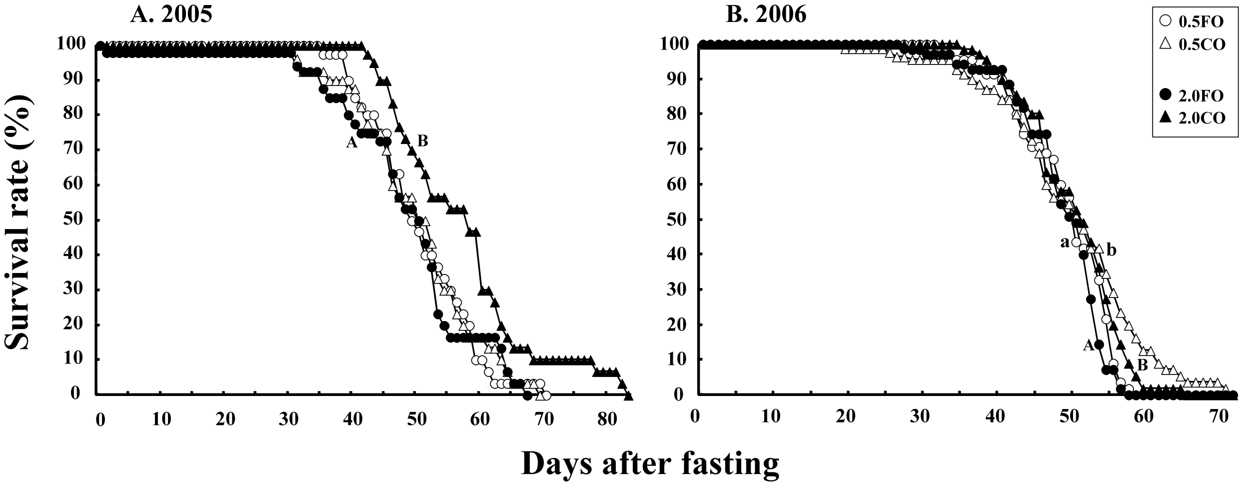

Fig.3 Effects of diets containing 0.5 or 2.0% (w/w) supplementary dietary lipid as fish oil (FO), and cottonseed oil (CO) on survival rate during fasting of chum salmon fry in 2005 and 2006. Lines without a common letter differ between 0.5 FO and 0.5 CO or 2.0 FO and 2.0 CO ($P < 0.05$).

conducted in 2005 and 2006 are shown in Fig. 3. In 2005, the survival rate of fish fed 2.0 CO diet was significantly higher than that of fish fed 2.0 FO diet. In 2006, survival rates of fish fed 0.5 CO and 2.0 CO diets were significantly higher than those of fish fed 0.5 FO and 2.0 FO diets, respectively.

Discussion

The weight of fish fed 2.0 CO diet on March 10 and 0.5 CO, 2.0 CO diets on April 1 in 2005 was significantly better than that of fish fed FO diets on each day. The replacement of fish oil with vegetable oil in the diet is reported to show an equal effect on the growth in salmonids (Bransden *et al*., 2003; Menoyo *et al*., 2005). But CO has a better effect on the growth of chum salmon fry than FO in the present study. Fish do not synthesize n-3 and n-6 polyunsaturated fatty acid (PUFA), so they must be provided with it in the diet for normal growth (Nicolaides and Woodall, 1962; Castell *et al*., 1972; Watanabe *et al*., 1974). In salmonid fish such as rainbow trout *O. mykiss*, n-3 PUFA like linolenic acid, EPA and DHA are more effective in their growth than n-6 PUFA like linoleic acid (Watanabe, 1982). However, Takeuchi *et al*. (1979) suggested chum salmon fry fed the diet supplemented with each of 1% linoleic acid and linolenic acid showed somewhat better growth than those of fish fed the diet supplemented with n-3 PUFA. Although the commercial diet and FO used in this study contain a great amount of n-3 PUFA as EPA and DHA, they contain less n-6 PUFA. Linoleic acid that is highly prevalent in CO might be effective for the growth of chum salmon fry. In contrast, no differences of growth between the CO and FO diet group were observed in 2006. Takeuchi *et al*. (1979) reared chum salmon fry at a water temperature of 15 to 18^{o}C for 7 weeks. In our experiment, the water temperature was relatively low and the rearing period in 2006 was 2 months, shorter than that in 2005. Perhaps the rearing period in 2006 was insufficient in such water temperature to clarify the effect of fatty acid on the growth of chum salmon fry.

Feed efficiencies of the fish fed CO diets were better than those of fish fed FO diets except in fish fed 0.5 CO diet from January 10 to February 10 in 2006. In the supplementation experiment of vegetable oil to diet, the appropriate feeding period of diet or supplemented ratio of oil to diet to gain good feed efficiency have not been thoroughly examined to date; however, replacement of FO with canola oil that contains a lot of linoleic acid had a better effect in feed efficiency in rainbow trout for 140 days feeding (Drew *et al*., 2007). Feeding of 0.5 CO diet for one month might be insufficient from the viewpoint of the feeding period of diet or supplemented ratio of this oil to diet for better feed efficiency in chum salmon fry.

Health parameters such as ATP content, burst swimming velocity and tolerance to fasting of fish fed CO diets showed greater improvement than those of fish fed FO diets. In particular, feeding of 0.5 CO and 2.0 CO diets for two months is most effective because the ATP content, burst swimming velocity and tolerance to fasting in these groups were all improved in this study. Mizuno *et al*. (2007) suggested a positive correlation between the ATP content and burst swimming velocity in masu salmon *O. masou.* The results observed in this study correlate with that result. ATP produces the metabolic energy as its hydrolysis, and is used for various life activities such as swimming. Swimming performance is an integrated assessment of several physiological processes (Plaut, 2001). Larval striped bass *Morone saxatilis* had slower swimming speed were less responsive to simulated predator attacks than larva had faster swimming speed (Chick *et al*., 2000). As the chum salmon fry has high ATP content and burst swimming velocity a high survival rate after release is expected. The reason for the effectiveness in the improvement of ATP content and burst swimming velocity of fish fed CO is unclear in this study; however, McKenzie *et al*. (1998) reported that swimming performance increased significantly with higher levels of oleic, linoleic and linolenic acid in diets. They suggested this fact indicates that these mid chain fatty acids are efficiently oxidized and preferred substrates of exercise performance. Perhaps oleic and linoleic acid, which are contained in high amounts in CO, affect the increase of ATP content and burst swimming velocity. Improvement of tolerance to fasting is important for fish because starvation would directly relate to survival in jack mackerel *Trachurus symmetricus* Ayres (Theilacker, 1986) and indirectly in Atlantic herring *Clupea harengus* (Gamble and Hay, 1989). Therefore, feeding of 0.5 CO and 2.0 CO diets for two months would be effective for improving the survival rate of chum salmon fry after release. The amount of triglyceride, the main component of neutral fat, in fish affects survival during fasting in masu salmon (Misaka *et al*., 2004); however, their amounts did not differ among the fish fed FO and CO diets during fasting in this study (data not shown). Therefore the reason for the effects of CO in the improvement of the tolerance to fasting must be investigated.

Cottonseed contains gossypol causing high mortality in sea lamprey *Petromyzon marinus* by intraperitoneal injection (Rinchard *et al*., 2000). The amount of gossypol is not analyzed in this study, however, the CO used in this study was purified as a chemical reagent to remove impurities including gossypol. The influence of gossypol is therefore considered negligible in this study.

In conclusion, CO supplementation to diet showed more positive effects on growth, feed efficiency and some health parameters in chum salmon fry than FO in this study. Improvement of health parameters such as the ATP content, burst swimming velocity and tolerance to fasting by supplementation of CO suggests that it would be effective for producing healthier chum salmon fry with an expected high survival rate after release. Moreover, vegetable oils are cheaper than fish oil as described above, so replacement of FO with CO might be effective for reducing the cost in rearing chum salmon fry. Therefore, supplementation of 0.5 or 2.0% of CO to the diet for two months is most effective from the viewpoint of cheaper production of healthy chum salmon fry. In future, further investigations are required to clarify the most appropriate feeding period of CO supplemented diet or supplemented ratio of CO to diet in chum salmon fry.

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