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Evaluation of Lysine Cell Mass as a Dietary Fishmeal Replacer for Juvenile Korean Rockfish, *Sebastes schlegeli*

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On protein equivalence base, fishmeal (FM) was replaced by lysine cell mass (LCM) in selected different diets in Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Eight experimental diets were formulated to contain 100% FM (LCM_0), 90% FM+10% LCM (LCM_{10}), 80% FM+20% LCM (LCM_{20}), 70% FM+30% LCM (LCM_{30}), 60% FM+ 40% LCM (LCM_{40}), 70% FM+30% LCM+lysine (LCM_{30+Lys}), 60% FM+40% LCM+lysine (LCM_{40+Lys}), and 50% FM+50% LCM+lysine (LCM_{50+Lys}). Experimental individuals of the fish (12.6 g) were randomly fed on one of the experimental diets. After 6 weeks of feeding trial, weight gain (WG) and feed efficiency (FE) of fish fed LCM_0 diet was significantly (P<0.05) higher than those of fish fed LCM_{20}, LCM_{30}, LCM_{40}, LCM_{30+Lys}, LCM_{40+Lys}, and LCM_{50+Lys} diets, however, there was no significant difference in WG of fish fed LCM_0 and LCM_{10} diets. Supplementation of lysine has no effect on WG. There was no significant difference in condition factor (CF) of fish fed LCM_0, LCM_{10} and LCM_{20} diets. Hemoglobin (Hb) of fish fed LCM_0, LCM_{10}, LCM_{20}, LCM_{30}, LCM_{40}, LCM_{30+Lys}, and LCM_{40+Lys} diets were not significantly different from each other. No significant differences were observed in hematocrit (PCV) and hepatosomatic index (HSI) among all dietary treatments. Apparent digestibility of dry matter (ADM) and protein (ADP) of diets significantly decreased with increase in dietary LCM level, though there was no difference in ADM and ADP between LCM_0 and LCM_{10}. These results indicate that LCM could replace up to 10% of fishmeal in Korean rockfish diets.

Keywords: Lysine cell mass, Lysine, Fish meal, Replacement, Korean rockfish

Introduction

Fishmeal is one of the most expensive ingredients in the formulated fish feed. The demand for fishmeal is increasing while its production is decreasing (Rumsey, 1994). Consequently, its availability as an ingredient for fish diet may become a serious problem. (Hardy, 1996; Sargent and Tacon, 1999). The idea of utilization of single cell protein (SCP) in fish started from the late of 1970s (Beck et al., 1979; Bergstrom, 1979; Dabrowski et al., 1980; Davies and Wareham, 1988). SCP mostly includes micro algae, bacteria and yeast. It is a non-conventional protein source, frequently used as feed ingredient for fish due to its nutritional value for proteins, B-vitamins, pigments and complex carbohydrates like glucans (Sanderson and Jolly, 1994; Tacon, 1994; Oliva-Teles and Goncalves, 2001). Some experiments have indicated the optimum inclusion level of different types of SCP. For instance, Kaushik and Luquet (1980) reported that in the experimental diet of rainbow trout *Salmo gairdneri* up to 80% of fishmeal could be replaced by *methanophillic* bacteria with no adverse effect on overall performance. Davies and Wareham (1988) evaluated a similar SCP obtained from the bacterium *Micrococcus glutamicus* that is used in the industrial manufacture of L-lysineÞHCl and suggested the potential use of this SCP as a replacement of fishmeal in a complete tilapia ration. Oliva-Teles and Goncalves (2001) found that brewers yeast could replace up to 50% of fishmeal protein with no negative effects in European sea bass. These results are encouraging and confirm the use of SCP in fish diets as a protein source.

Lysine cell mass (LCM) is byproduct from lysine producing bacteria cell mass-*Corynebacterim* ; it is a short-rod type, gram positive, aerobic and typical amino acid producing bacterium. The LCM from lysine fermentation is rich in amino acids but deficient in lysine and thus has a great potential for use as a high protein feed ingredient.

Korean rockfish *Sebastes schlegeli* is one of the commer-

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cially important fish species in Korea. This species has desirable characteristics for culture including the high tolerance to water temperature change, the easiness of seedling production because of a viviparous reproductive style, and the ability to withstand high stocking density. Commercial culture systems of this species have been rapidly developed since the late of 1980s, and its production is followed by the flounder production in Korean mariculture (Bai and Kim, 2001). Therefore, an evaluation of the effect of LCM as a dietary protein source on growth performance of Korean rockfish has become a necessity.

Materials and methods

Experimental diets

Composition of the experimental diets is shown in Table 1. Eight experimental diets were formulated to contain 100% FM (LCM_0), 90% FM+10% LCM (LCM_{10}), 80% FM+20% LCM (LCM_{20}). 70% FM+30% LCM (LCM_{30}), 60% FM+40% LCM (LCM_{40}). 70% FM+30% LCM+lysine (LCM_{30+Lys}), 60% FM+40% LCM+lysine (LCM_{40+Lys}), and 50% FM+50% LCM+lysine (LCM_{50+Lys}). Lysine was supplemented into experimental diets according to the lysine content in LCM_0 diet. The proximate composition and essential amino acids contents of the protein sources used in the experimental diets are shown in Table 2. The experimental diets were formulated to contain 48 % crude protein (Table 1) and 16.8 kJ/g diet available energy (Kim et al., 2001). Available energy was calculated using values of 16.7, 37.6 and 16.7 kJ/g for protein, lipid and carbohydrate, respectively (Garling and Wilson, 1976). All ingredients were mixed and pelleted by a laboratory pellet machine without heating using a 2-mm diameter module (Baokyong Commercial Co., Busan, Korea). After processing, all the diets were kept at -20°C refrigerator until use.

Experimental fish and feeding trial

Juvenile Korean rockfish were transferred from Seboo Fisheries Hatchery (Tongyoung, Korea) to the Institute of Fisheries Science, Pukyong National University, Korea. Prior to the start of the feeding trial, fish were fed LCM_0 diet for 1 week for acclimatization to the experimental diet and conditions. The feeding trial was conducted in a flow-through system with 60 L aquaria receiving filtered seawater at a rate of 1.2l/min. Supplemental aeration was provided to maintain dissolved oxygen near saturation. Water temperature was 16°C at the beginning of the feeding trial and 13°C at the end of the feeding trial according to the changes of natural water temperature, the mean temperature was 14.8±1°C (meanSD). Fish averaging 12.6±0.2 g were randomly distributed to each aquarium as groups of 15 individuals and fed the experimen-

Table 1. Composition of the experimental diets (% of dry weight)

Ingredient	Diet							
	LCM_0	LCM_{10}	LCM_{20}	LCM_{30}	LCM_{40}	LCM_{30+Lys}	LCM_{40+Lys}	LCM_{50+Lys}
LCM[1]	0	5.47	10.95	16.42	21.90	16.42	21.90	27.37
Gelatin[2]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
WFM[3]	52.00	46.80	41.60	36.40	31.20	36.40	31.19	25.99
Wheat flour	24.45	24.65	24.45	24.45	24.80	22.85	22.25	21.75
Squid liver oil[4]	9.25	9.00	8.79	8.47	8.10	9.06	8.98	8.85
EPA+DHA[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[5]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[6]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
CMC[2, 7]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Cellulose[2]	0.30	0.08	0.21	0.26	0.00	0.99	1.30	1.57
Lysine	0	0	0	0	0	0.28	0.38	0.47
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Proximate analysis								
Crude protein	47.6	48.5	48.3	49.3	48.6	49.9	48.6	48.1
Crude fat	14.4	14.3	14.0	14.5	13.9	14.8	14.2	14.1
Crude ash	9.70	9.26	9.00	8.63	8.34	8.62	8.22	8.07

[1]LCM: lysine cell mass, BASF Company Ltd.
[2]United States Biochemical, Cleveland, Ohio 44122.
[3]WFM: white fish meal, Fisheries Co-operative of Korea Feeds Co., Busan, Korea.
[4]E-hwa oil & Fat Industry Company, Ltd., Busan, Korea.
[5] [6]As described by Kim et al. (2001).
[7]CMC: Carboxymethyl cellulose.

Table 2. Proximate composition and essential amino acid (EAA) contents of the protein sources used in the experimental diets (% of dry matter basis)[1]

	Lysine cell mass	White fish meal	Gelatin	Wheat
Proximate analysis (DM basis)				
Crude protein	70.4	74.2	99.1	13.1
Crude lipid	6.0	7.7	0.3	1.3
Crude ash	4.1	14.2	0.3	1.5
Essential amino acids				
Arginine	0.45	4.20	6.97	0.64
Histidine	0.77	1.41	0.71	0.30
Lysine	0.07	4.53	3.55	0.36
Leucine	9.09	4.50	2.74	0.89
Isoleucine	1.63	3.10	1.38	0.51
Methionine	2.73	1.65	0.73	0.21
Phenylalanine	2.01	2.80	1.71	0.63
Threonine	1.10	2.60	1.81	0.37
Valine	1.51	3.25	2.09	0.59

[1]Values are means from duplicate groups of feed stuffs.

tal diets in triplicate at a rate of 3% of wet body weight per day for 6 weeks. Total fish weight per aquarium was determined at week 3 and the end of the feeding trial, and the amount of diet fed was adjusted accordingly.

Sample collection and analysis

Percent weight gain (WG), feed efficiency (FE), hepatosomatic index (HSI), hematocrit (PCV), hemoglobin (Hb) and percent survival were determined at the end of the feeding trial. After the final weighing, 6 fish were randomly removed from each aquarium; blood samples were obtained from the caudal vein using a heparinized syringe and pooled for hematocrit and hemoglobin determination (Brown, 1980). Crude protein, moisture and ash of whole-body samples were analyzed by AOAC's method (1995). Crude fat was determined by using an ether extraction procedure (Soxtec System 1046, Foss, Hoganas, Sweden) after freeze-drying the samples for 12 h.

Digestibility determination

The digestibility trial was made for two weeks after the growth performance trial. Feeding rate was maintained at 3% of wet body weight per day as same as in the growth performance trial. During digestibility experiment, fish were reared in sloped-bottom tank attached with collection columns (5 cm in diameter×30 cm high), which was modified from TUF Fecal Collection Systems (Satoh et al., 1992). Water flow was adjusted to 0.6 l/min in order to maximize the recovery of faeces in the settling column. One hour after the daily meal, the TUF column was brushed out to remove feed residues and feces from the system. At 9 hours in the following day, the settled feces and surrounding water were gently withdrawn from the base of the settling column. Both feces and supernatant were collected from the triplicate groups, and freeze-dried without centrifugation in order to avoid possible loss of nutrients from the feces. It was later finely ground for analysis.

The apparent digestibility of dry matter and protein of eight diets were determined by the indirect digestibility method using chromic oxide (Cr_2O_3) as an external marker (Cho and Slinger, 1979). Cr_2O_3 concentration was determined by flame atomic absorption spectrophotometers following combustion of the sample in a muffler furnace, before and after digestion in nitric acid (AOAC, 1995). Apparent digestibility coefficients were calculated as previously described by Cho and Slinger (1979).

Statistical analysis

All data were analyzed by One-way ANOVA (Statistix 3.1, Analytical Software, St. Paul, MN, USA) to test for the effects of the dietary treatments. When a significant treatment effect was observed, a least significant difference (LSD) test was used to compare means. Treatment effects were considered with the significant level at $P<0.05$.

Results

Growth performance, hemoglobin (Hb) and hematocrit (PCV) were shown in Table 3. After 6 weeks of feeding trial,

Table 3. Percent weight gain, feed efficiency, hepatosomatic index, condition factor, hemoglobin and hematocrit of Korean rockfish fed eight different experimental diets for 6 weeks[1]

	Diets								Pooled SEM[2]
	LCM_0	LCM_{10}	LCM_{20}	LCM_{30}	LCM_{40}	LCM_{30+Lys}	LCM_{40+Lys}	LCM_{50+Lys}	
WG (%)[3]	171.6^{a}	166.8^{ab}	158.6^{b}	144.9^{c}	129.8^{d}	141.7^{c}	125.2^{de}	114.0^{e}	4.17
FE (%)[4]	81.2^{a}	79.0^{ab}	77.3^{b}	71.4^{c}	63.3^{d}	70.7^{c}	62.1^{d}	57.9^{e}	1.71
PER[5]	1.71^{a}	1.63^{ab}	1.60^{b}	1.45^{c}	1.30^{d}	1.42^{c}	1.28^{de}	1.20^{e}	0.04
HSI (%)[6]	3.43	3.12	3.52	3.20	3.14	3.29	3.14	3.13	0.05
CF[7]	1.97^{a}	1.96^{ab}	1.91^{abc}	1.81^{c}	1.82^{c}	1.84^{c}	1.81^{c}	1.85^{bc}	0.02
SGR[8]	2.38^{a}	2.34^{ab}	2.26^{b}	2.13^{c}	1.98^{d}	2.10^{c}	1.93^{d}	1.81^{e}	0.04
PCV (%)[9]	37.8	36.0	37.5	38.0	37.5	39.5	38.1	38.3	0.69
Hb (g/dl)[10]	6.55^{ab}	6.35^{ab}	6.47^{ab}	6.96^{a}	6.29^{ab}	6.85^{ab}	6.24^{ab}	6.09^{b}	0.50

[1]Values are means from triplicate groups of fish where the means in each row with a different superscript are significantly different ($P<0.05$).
[2]Pooled standard error of mean.
[3]WG %(Weight gain %): (final weight initial weight)x100/initial weight.
[4]FE % (Feed efficiency %): increase in biomass of fishx100/dry feed intake.
[5]PER (Protein efficiency ratio): wet weight gain/protein intake.
[6]HSI (Hepatosomatic index): (liver weight/body weight)x100.
[7]CF (Condition factor): [fish wt. (g)/fish length $(cm)^3$]x100.
[8]SGR (Specific growth rate %): (ln final wt. ln initial wt.)x100/time (days).
[9]PCV (hematocrit).
[10]Hb (hemoglobin).

weight gain (WG) and feed efficiency (FE) of the fish fed LCM_0 diet was significantly ($P<0.05$) higher than those from fish fed LCM_{20}, LCM_{30}, LCM_{40}, LCM_{30+Lys}, LCM_{40+Lys}, and LCM_{50+Lys} diets, however, there were no significant differences in WG and FE of the fish fed LCM_0 and LCM_{10} diets, or LCM_{10} and LCM_{20} diets. Supplementation of the amino acid, Lysine, did not improve growth performance of Korean rockfish in the present experiment. Protein efficiency ratio (PER), condition factor (CF) and specific growth rate (SGR) showed similar trends as WG; the values of these parameters decreased with an increase of LCM supplementation. There were no significant differences in hepatosomatic index (HSI) and PCV among experimental groups. The fish fed LCM_{30} showed the highest Hb value, and those fed LCM_{50+Lys} showed the lowest Hb value among the dietary treatments. The survival of Korean rockfish in the present experiment was 100% in all dietary groups.

There was no significant difference in crude protein contents of whole body in Korean rockfish fed the diets containing different levels of LCM (Table 4). Crude fat of whole body decreased with the increase of LCM supplementation; there was no significant difference in crude fat in fish fed LCM_0 and LCM_{10} diets, and fish fed LCM_{10} and LCM_{20} diets. There was no significant difference in whole body crude ash contents in fish fed LCM_0, LCM_{10}, LCM_{20} and LCM_{30} diets. However, whole body crude ash content in fish fed LCM_{40}, LCM_{30+Lys}, LCM_{40+Lys}, and LCM_{50+Lys} diets were significantly ($P<0.05$) higher than that in fish fed LCM_0, LCM_{10}, LCM_{20} and LCM_{30} diets.

Apparent digestibility of dry matter (ADM) and protein (ADP) significantly decreased with the increase of LCM in the diets (Table 5). There was no significant difference in ADM and ADP of LCM_0 and LCM_{10} diets, and LCM_{10} and LCM_{20} diets. ADM and ADP of LCM_{50+Lys} diet were significantly ($P<0.05$) lower than in the other diets.

Table 4. Proximate composition of whole body in Korean rockfish fed eight different experimental diets for 6 weeks (as is basis)[1]

	Diets								Pooled SEM[2]
	LCM_0	LCM_{10}	LCM_{20}	LCM_{30}	LCM_{40}	LCM_{30+Lys}	LCM_{40+Lys}	LCM_{50+Lys}	
Moisture	75.6^{ab}	74.7^{b}	75.4^{ab}	75.7^{ab}	75.5^{ab}	76.2^{a}	75.1^{ab}	75.1^{ab}	0.16
Crude protein	16.5	17.2	16.9	16.7	16.7	16.5	17.3	17.2	0.11
Crude fat	3.40^{a}	3.06^{ab}	2.79^{bc}	2.59^{c}	1.90^{d}	1.76^{d}	1.68^{d}	1.71^{d}	0.56
Crude ash	3.06^{c}	3.09^{c}	3.14^{c}	3.25^{c}	4.68^{ab}	4.06^{b}	4.68^{ab}	4.73^{a}	0.17

[1]Values are means from triplicate groups of fish where the means in each row with a different superscript are significantly different ($P<0.05$).

Table 5. Apparent digestibility of dry matter (AD) and protein (ADP) in the experimental diets (% of dry matter basis)[1]

	Diets								Pooled SEM[2]
	LCM_0	LCM_{10}	LCM_{20}	LCM_{30}	LCM_{40}	LCM_{30+Lys}	LCM_{40+Lys}	LCM_{50+Lys}	
AD (%)	77.6^a	75.7^{ab}	73.4^b	69.1^{cd}	65.0^d	68.2^{cd}	64.1^d	59.2^e	1.34
ADP (%)	92.3^a	90.9^{ab}	87.1^b	83.5^{cd}	80.8^d	84.2^{cd}	79.9^d	73.9^e	1.77

[1]Means of duplicate groups, values in the same row with different superscripts are significantly different ($P<0.05$).
[2]Pooled standard error of mean.

Discussion

Weight gain (WG) and feed efficiency (FE) of Korean rockfish decreased with increasing lysine cell mass (LCM) supplementation to the diet. WG and FE of fish fed LCM_{20} diet were significantly ($P<0.05$) lower than those fed LCM_0 diet, although there were no significant differences in WG and FE between fish fed LCM_0 and LCM_{10} diets. However, the optimum inclusion level of LCM estimated from our present experiment was lower than that described by Kaushik and Luquet (1980), who found that up to 80% of fishmeal in rainbow trout diets could be replaced by SCP of *methanophillic* bacterial origin with no adverse effect on overall performance. Alliot et al. (1979) and Oliva-Teles and Goncalves (2001) found no negative effect on growth performance of European sea bass fed diets with 50% dietary fishmeal protein replaced by yeast protein, and the inclusion of 5% to 15% yeast in the diets could even improve FE. In other species, moderate dietary inclusion levels of yeast also had beneficial effects on fish performance (Ohmae et al., 1979; Rumsey et al., 1991). Although LCM has high crude protein content (70.4% on dry matter basis) that is comparable to fish meal and low crude fat content (4.1% on dry matter basis), it is possible to conclude from the present work that substituting fish meal by LCM at levels more than 10% decreased such parameters as WG, FE, protein efficiency ratio (PER), condition factor (CF) and specific growth rate (SGR) significantly. This might be caused by the composition differences between yeast and LCM, or the differences of fish species to utilize single cell protein as dietary protein sources.

De la Huiguera et al. (1981) and Rumsey et al. (1991) reported that due to their very active liver uricase, the fish seemed to be well adapted for high nucleic acids content of the SCP; but other authors considered that high levels of nucleic acids may have a deleterious effect because RNA and purine basis were implicated as potent depressors of feed intake in farm animals (Tacon and Cooke, 1980; Davies and Wareham, 1988; Rumsey et al., 1992). Tacon and Cooke (1980) found that a nucleic acid extract of bacterial origin depressed feed intake of rainbow trout when supplemented in the diet at a level equivalent to a bacterial SCP intake of 50%. Rumsey et al. (1991) reported that more than 25% of brewers yeast in rainbow trout diet could depress its feed intake. However, Oliva-Teles and Goncalves (2001) did not find any adverse effect on diet palatability or feed intake, when 50% brewers yeast was included in sea bass diets. No negative effect on the feed intake was observed, when LCM was included in the diets in the present study, the growth performance was depressed, when high levels of LCM was included in experimental diets. Further experiments should be made to evaluate whether other anti-nutrient elements present in LCM.

LCM was deficient in lysine and the lysine supplementation in the diets containing 30, 40 and 50% LCM did not improve the performance of the Korean rockfish. However, methionine supplementation in diets containing yeast is known to improve fish growth (Mahnken et al., 1980; Murray and Marchant, 1986). Similar reports on rainbow trout and sea bass showing no positive effect of sulphur amino acids supplementation in diets containing SCP are available (Kaushik and Luquet, 1980; Oliva-Teles and Goncalves, 2001). On the contrary, supplementation with sulphur amino acids depressed rainbow trout growth with various extents.

In the present study, ADM and ADP also showed decreasing trend with increasing levels of LCM in the diets and thereby indicated a lower digestibility of LCM than fish meal in the diet.

In conclusion, the results of the present study indicated that lysine cell mass could replace up to 10% of fish meal protein without any adverse effect on juvenile Korean rockfish growth, and the supplementation of lysine in the diets did not improve the growth of Korean rockfish.

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