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Effects of the Dietary Supplementation of Fermented Cactus Fruit (*Opuntia ficus-indica*) Fluid on the Growth of Red Sea Bream, *Pagrus major*

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Two feeding experiments were conducted to investigate the effects of fermented cactus fruit (*Opuntia ficus-indica*) fluid (FCFF) as a feed additive to a commercial diet on the growth of red sea bream, *Pagrus major,* and to determine an effective dose. FCFF was prepared by mixing crushed cactus fruit with a starch solution and commercially available microorganisms for 2 weeks at room temperature. Three triplicate groups of red sea bream had initial mean weights of 84.1 g (Exp-1) and 5.1 g (Exp-2) and were fed experimental diets containing 0%, 1%, and 5% FCFF for 2 months (Exp-1) and 0%, 0.2%, 0.5%, and 1% FCFF for 3 months (Exp-2), respectively. In experiment 1, the mean body weight of fish fed the diet containing 1% FCFF was significantly higher ($P<0.05$) than that of fish fed the diet without FCFF. The survival rate was highest in fish fed the diet containing 1% FCFF, although the difference was not statistically significant. The feed gain ratio (FGR), specific growth rate (SGR), and condition factor (CF) values of fish fed the diet containing 1% FCFF were higher than those of fish in the other dietary groups that received lower levels of FCFF. The daily feeding rate (DFR) of fish fed the diet containing 1% FCFF was slightly lower but not statistically different than the DFR values of fish in the other dietary groups. In experiment 2, the final mean body weight of fish fed the diet containing 1% FCFF was significantly ($P<0.05$) higher than the mean weight of fish in the control group. The FGR, SGR, and CF values of fish fed the diet containing 1% FCFF were better than the values from fish in the other dietary groups that received lower levels of FCFF, although the differences were not statistically significant. However, the DFR of fish fed the diet containing 1% FCFF was lower than those of fish in the other groups. These results suggest that FCFF could be used as a feed additive in commercial fish food and a preferable level of supplementation is at least 1.0% in fingerling and young red sea bream.

Keywords: Cactus fruit (*Opuntia ficus-indica*), Fermentation, Growth, Red sea bream, *Pagrus major*

Introduction

The red sea bream, *Pagrus major,* is a highly valued marine fish that is used as a food source in Japan and Korea. Recently, the production of red sea bream has increased rapidly (Ikenoue and Kafuku, 1992) and now occupies over 30% of the total fish production (260,000 tons in 2001) in Japan, but the aquacultural production of this species of fish is challenging from an economic perspective (Song, 2003). This species is omnivorous (Nakagawa et al., 1997), and its farming requires either minced whole fish as feed or artificial feeds that contain high levels of fish meal (Forster and Ogata, 1998); as a result, the feed used for red sea bream commands a high price. Therefore, the cost of the feed presents the greatest burden on aquaculture farms. Consequently, many researchers are investigating ways to develop feeds that maximize the feed efficiency, reduce feeding costs, decrease environmental pollution, and promote profitable aquaculture of red sea bream by using herbal plants as feed additives. Seaweed has been used as a feed additive because of its beneficial effects on growth, disease resistance, and physiological condition of red sea bream (Nakagawa and Kasahara, 1986; Nakagawa et al., 1997; Satoh et al., 1987; Yone et al., 1986a; Yone et al., 1986b).

The cactus *Opuntia ficus-indica* is a terrestrial herbal resource that has several useful properties including anti-tumor, cholesterol-lowering, anti-ulcer, anti-oxidant, anti-bacterial, and anti-viral effects in mammals (Ahmad et al., 1996; Chung, 2000; Kuti, 2004; Lee et al., 1998; Palumbo et. al., 2003; Shin et al., 1998, 1999). In an earlier study, we found that fermented cactus fruit fluid (FCFF) significantly influences the growth

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and feed efficiency of the olive flounder, *Paralichthys olivaceus* (unpublished data).

The present study was conducted to investigate whether dietary supplementation with FCFF influences the growth and feed efficiency of young and fingerling red sea bream with reference to the practical use of FCFF as a feed additive.

Table 1. Compositions of the commercial diet, cactus, and fermented cactus fruit fluid (FCFF) used in this study (% dry wt.)

	Commercial diet	Cactus fruit	FCFF
Moisture	3.36	83.53	85.59
Crude protein	52.00	1.08	1.08
Crude lipid	12.62	0.54	0.60
Crude fiber	1.86	2.51	0.67
Crude ash	12.00	1.28	1.34
Ca	2.56	0.24	**
P	1.90	0.15	0.11
Vitamin C	0.01	0.06	0.01
Vitamin E (α-Tocopherol)	0.06	0.01	**
Carotenoid	NA*	0.02	0.01
β-Glucan	NA*	0.42	0.45
Flavonoid	NA*	0.10	0.07

Moisture is expressed in % wet wt.
*Not analyzed.
**Less than 0.01%.

Materials and Methods

Experimental fish and conditions

The red sea bream used for this study were produced in the Jeju Province Fisheries Resources Research Institute in Korea. Two experiments were conducted to determine the effective levels of FCFF in the diet of red sea bream. The first experiment (exp-1) was conducted for 2 months, while the second experiment (exp-2) was conducted for 3 months. The first experiment involved three treatments, and the second experiment used four treatments. All treatments had three replicates. Cylindrical polypropylene (PP) tanks (1.2 m diameter × 1.0 m height) were used as experimental tanks. In exp-1, each tank was stocked with 70 young red sea bream with an initial mean length of 16.79 ± 0.15 cm and weight of 84.10 ± 2.45 g. In exp-2, each tank was stocked with 50 fingerling red sea bream with an initial mean length of 9.97 ± 0.51 cm and weight of 15.86 ± 2.85 g. The rearing water was changed 15-20 times a day. The body lengths and weights of the fish were measured every month.

The water quality parameters in the tank were monitored daily using a YSI system (YSI 556 multi-probe system). The ranges were as follows: temperature, 17.2-20.8°C; salinity, 31-34 ppt; dissolved oxygen (DO), 6.72-7.32 mg/L; and pH, 7.30-8.09 in exp-1 and temperature, 15.2-17.3°C; salinity, 32-33 psu; DO, 7.76-8.24 mg/L; and pH, 7.30-8.09 in exp-2.

Manufacturing of fermented cactus fruit fluid (FCFF)

The cactus fruits used for this study were obtained from a local market on Jeju. For fermentation, we used the effective microorganism (EM) product marketed by EM Research Organization, Korea (EMRO). The fermentation process involved pulverizing the fruits and then adding a starch syrup and the EM at 10% and 5% of the cactus fruit volume, respectively. After incubating for 2 weeks at room temperature, the substance was filtered and used for the experiment.

Diet preparation and feeding

The diet used for the experiment was in the form of a commercial extruded pellet (Nisshin formula feed, Japan). FCFF was added to the diet at levels of 0%, 1%, and 5% in exp-1 and 0%, 0.2%, 0.5%, and 1% in exp-2. To add the FCFF, the pellets were soaked in the respective concentrations of FCFF to allow for absorption of the fluid. The pellets were then hand fed to the bream twice daily (10:00, 16:00).

Analytical methods

The initial compositions of the commercial pellets, cactus fruit, and FCFF used in this study were analyzed according to standard procedures by the Science Lab. Center Co., Korea. The results are shown in Table 1.

The feed gain ratio (FGR), specific growth rate (SGR), daily feeding rate (DFR), and condition factor (CF) were calculated by the following formulas:

$$\text{Weight gain} = (FW - IW) \times 100 / IW$$

$$FGR = TF/TWG$$

$$SGR = \{(\ln FW - \ln IW)/t\} \times 100$$

$$DFR = (TF \times 100)/\{(IW + FW) \times \text{day fed}/2\}$$

$$CF = (BW/TL^3) \times 10^3$$

where BW = body weight, IW = initial body weight, FW = final body weight, TF = total feed, TL = total body length, t = rearing time (days), and TWG = total weight gain.

Statistical analysis

Data were analyzed using SPSS version 7.5 for Windows (SPSS Science, Chicago, IL, USA), and significant differences (at $P < 0.05$) were verified using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests.

Results

Experiment 1

Growth performance measures and survival rate

The growth performance measures of young red sea bream fed experimental diets for 2 months are shown in Table 2. The final mean body weight of fish fed the diet containing 1% FCFF was significantly ($P<0.05$) higher than the mean body weights of fish in the other dietary groups. Weight gain was the highest in fish fed the diet containing 1% FCFF among all dietary groups, but the difference was not statistically significant.

The survival of fish in each dietary group was over 95%, and there were no significant differences among treatments ($P>0.05$). Fish fed the diet containing 1% FCFF showed a slightly higher survival rate than fish fed the control diet. The growth performance measures and survival rate were better in fish fed the diet containing 1% FCFF than in fish fed the other diets.

FGR, SGR, DFR, and CF

Table 3 shows the values of FGR, SGR, DFR, and CF during exp-1. The best value of FGR was obtained in fish fed the diet containing 1% FCFF, although the difference was not statistically significant. Similarly, the SGR was higher in fish fed the diet containing 1% FCFF than in the other groups. The lowest DFR was observed in fish fed the diet containing 1% FCFF.

Fish fed the diet containing 1% FCFF showed significantly ($P<0.05$) higher CF values than fish fed the other diets.

Experiment 2

Growth performance measures and survival rate

The final mean body weight of fish fed the diet containing 1% FCFF was significantly ($P<0.05$) higher than the mean weight of fish fed the diet without FCFF (Table 4). Fish fed 1% FCFF experienced a significantly ($P<0.05$) higher weight gain than fish in the other dietary groups. There were no significant differences between the survival rates of fish in the different treatments, and these rates ranged between 92.0% and 96.7%.

FGR, SGR, DFR, and CF

There was no significant difference in the FGR, SGR, DFR, and CF values of fish in the different treatments. However, the groups fed the diets containing FCFF showed better FGR val-

Table 2. Total length, body weight, weight gain (WG), and survival rate of young red sea bream fed experimental diets containing different levels of fermented cactus fruit fluid (FCFF) for 2 months (exp-1)

Group	Initial		Final		WG (%)	Survival rate (%)
	TL (cm)	BW (g)	TL (cm)	BW (g)		
Control	16.88±0.25	84.46±3.89	18.33±0.11	103.34±0.45^{a}	22.3	95.7
1%	16.70±0.11	83.07±2.65	18.29±0.04	107.42±0.30^{b}	28.8	96.4
5%	16.80±0.16	84.78±3.26	18.17±0.09	103.58±1.48^{a}	22.3	95.7

*Values (mean ± SD of triplicate samples) in the same column not sharing a common superscript are significantly different ($P<0.05$).

Table 3. Effect of different levels of dietary fermented cactus fruit fluid (FCFF) fed for 2 months (exp-1) on the feed gain ratio (FGR), specific growth rate (SGR), daily feeding rate (DFR), and condition factor (CF) of young red sea bream

	FGR	SGR	DFR	CF
Control	1.75±0.36	0.47±0.10	0.66±0.01	16.81±0.39^{a}
1 %	1.28±0.27	0.60±0.08	0.65±0.01	17.56±0.06^{b}
5 %	1.79±0.21	0.47±0.12	0.66±0.01	17.29±0.01^{ab}

*Values (mean ± SD of triplicate samples) in the same column not sharing a common superscript are significantly different ($P<0.05$).

Table 4. Total length, body weight, weight gain (WG), and survival rate of fingerling red sea bream fed experimental diets containing different levels of fermented cactus fruit fluid (FCFF) for 3 months (exp-2)

Group	Initial		Final		WG (%)	Survival rate (%)
	TL (cm)	BW (g)	TL (cm)	BW (g)		
Control	10.17±0.23	16.29±1.51	12.67±0.32^{a}	38.66±2.65^{a}	139.4^{a}	95.3
0.2%	9.88±0.08	16.11±0.85	13.19±0.19^{bc}	42.97±1.96^{ab}	167.5^{ab}	92.0
0.5%	9.87±0.56	16.16±2.55	12.92±0.07^{ab}	40.64±1.58^{a}	160.0^{ab}	96.7
1.0%	9.82±0.26	14.92±1.95	13.41±0.32^{c}	46.80±4.32^{b}	219.1^{b}	94.7

*Values (mean ± SD of triplicate samples) in the same column not sharing a common superscript are significantly different ($P<0.05$).

Table 5. Effect of different levels of dietary fermented cactus fruit fluid (FCFF) fed for 3 months (exp-2) on the feed gain ratio (FGR), specific growth rate (SGR), daily feeding rate (DFR), and condition factor (CF) of fingerling red sea bream

	FCR	SGR	DFR	CF
Control	1.41±0.06	1.23±0.09	5.15±0.25	18.71±0.15
0.2 %	1.14±0.17	1.40±0.07	4.89±0.36	18.69±0.77
0.5 %	1.25±0.08	1.30±0.06	4.99±0.15	18.85±0.40
1.0 %	1.09±0.20	1.53±0.13	4.77±0.30	19.23±0.38

*Values (mean ± SD of triplicate samples) in the same column not sharing a common superscript are significantly different ($P<0.05$).

ues than the control group (Table 5). The SGR values were higher in all experimental groups fed diets containing FCFF compared to the control group. On the other hand, fish fed diets containing FCFF had lower DFR values than the control group.

The FGR value increased in relation to the dietary FCFF level, and the best FGR value was obtained by fish fed the diet containing 1% FCFF.

Discussion

Research aimed at enhancing feed quality by adding herbal ingredients has been undertaken in recent years (Kim et al., 2000; Hwang et al., 1999). In our study, FCFF had a significant effect on the mean body weight of red sea bream. In addition, feeding efficiency was greater in fish fed diets containing FCFF, and the optimal concentrate level was around 1.0%.

Red sea bream are omnivorous fish that ingest algae on occasion; thus, algae serve as an important food source for this species (Nakagawa et al., 1997). The usefulness of algae as a dietary protein source has been investigated in fish (Stanly and Jones, 1976; Montgomery and Gerking, 1980). Recently, the efficacies of macroalgae (Nakagawa and Kasahara, 1986; Nakagawa et al., 1997; Satoh et al., 1987; Yone et al., 1986a, b) such as *Ulva* and brown seaweed, and microalgae (Nakagawa et al., 1984, 1992, 2000) such as *Chlorella* and *Spirulina* for use as feed additives have been confirmed in red sea bream. According to Nakagawa et al. (1997), the growth and feed utilization of the seaweed *Ascophyllum* by red sea bream could not be assessed because of the high mortality, whereas Yone et al. (1986a) found that the mean body weight of red sea bream was higher in the 5% *Ascophyllum*-fed group. In a study on feed mixed with seaweed, the effective additive level was 5%, which was slightly higher with the supplementation of sea lettuce for Israeli carp (Kim and Choi, 1996) and sea mustard additive for young rock fish (Yi and Chang, 1994). However, any effect of seaweed is connected to a physiological function (Amano and Noda, 1985; Nakagawa and Kasahara, 1986; Nakazoe et al., 1986; Yone et al., 1986a; Watanabe et al., 1990) and disease resistance (Satoh et al., 1987) rather than the growth of the fish.

The above results were obtained with powdered additive extract, which would yield much higher doses than the doses used in our study. Although the additive levels applied in the experimental feed and the duration of supplementation were different, minimizing the level of addition is important for reducing the production costs (Lee et al., 2001).

In our study, the 5% FCFF dietary level produced values for growth performance measures and feed efficiency that were similar to those of the control. We previously found that a 1% FCFF dietary level had a significant effect on the growth of olive flounder while 5% and 10% FCFF levels reduced growth (unpublished data). These observations of negative effects of higher supplementation levels in fish agree with the conclusion of Yone et al. (1986b), who reported that replacing fish meal with a high level of algae may eventually depress the digestible protein in the diet. In the present study, the FGR values were higher in exp-1 (young red sea bream) than in exp-2 (fingerling red sea bream), which seems to have been caused by the different fish sizes and rearing water temperatures. Likewise, differences have been observed in fish growth and feed efficiency depending upon the fish species, age, concentrate level, and feeding time span (Nakagawa et al., 1984).

Many useful products have been made from plants and by the action of microorganisms. The FCFF used for this study was made through the fermentation with the use of EM composed of lactic acid bacteria and photosynthetic bacteria as probiotics. The effects of probiotics as growth promoters in fish have been studied. Venkat et al. (2004) reported that the growth of fish fed a diet containing *Lactobacillus*-based probiotics was significantly higher ($P<0.05$) than that of a control group fed post-larval *Macrobrachium rosenbergii.* In addition, the weight gain, SGR, feed efficiency ratio, protein efficiency ratio, and protein gain were also significantly higher than those of the control. Chang and Liu (2002) and Moriarty (1998) suggested that bacilli improve the survival of larvae, increase food absorption by enhancing protease levels, and produce better growth. However, the optimum concentration of probiotics must be standardized in the diet of experimental fish. Further experiments are also needed to elucidate the effect and role of bacilli on the growth and survival of fish.

In our experimental diet, the crude protein and crude lipid levels were 52% and 12.62%, respectively (Table 1). These values differ from those of Jeong (1992), who reported the best growth and feed efficiency with a diet containing 47% crude protein and 20% crude lipid for fingerling red sea bream. However, Takeuchi et al. (1991) reported that the optimum dietary protein and lipid concentrations for juvenile red sea bream were 52% and 15%, respectively. The growth of fish depends on the size, species, and environmental conditions in the rearing tank as well as the optimum dietary protein and lipid levels.

The results of our study suggest that the addition of FCFF to feed positively influences the growth performance and feed efficiency of red sea bream and that the proper supplemental level for commercial feed for red sea bream is at least 1.0%.

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