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Effects of *Shochu* Distillery By-products on the Energy Budget of Common Carp

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Abstract

The effects of *Shochu* distillery by-products (SDBP) were investigated by supplementary different levels of SDBP to the basal diets for Japanese Common carp. To clarify the growth performances, the energy budget equation was applied in this study. The feeding trial was conducted for 20 days by feeding the test diets containing SDBP at 0, 2,1, and 4.2%, respectively. Based on the energy budget equation, the digestible energy (DE) requirements for the maximum growth ranged from 49.0 – 55.8 kcal/ind/d. The 4.2% SDBP diet yielded 6.5% higher growth energy but 7.1% lesser metabolic energy than the control. The energy budget on a 4.2% SDBP diet accounted for 10.6% of feces (2.42 kcal/ind/d), 4.2% of non-fecal (0.93 kcal/ind/d), 39.4% of metabolic loss (9.03 kcal/ind/d) and 45.9% of growth (10.5 kcal/ind/d), respectively. Correspond to the control, non-fecal and metabolic energies were lower, in 4.2% SDBP resulting in higher product energy. The above results indicate that SDBP can be utilized as a supplement ingredient for the growth promotion of carp feed since it could provide a superior property for the growth energy of fish by reducing the energy of metabolic loss and non-fecal losses.

Key words: *Shochu* distillery by-products; Energy requirements; Metabolic loss; Growth; Carp

PENDAHULUAN

The growth in aquaculture production in the last decade, the prognosis for expected growth, and the need for cost-efficient feed resources in addition to traditional fishmeal have resulted in increased demand for alternative feed resources of similar qualities to wholly or partly replace conventional ingredients. Such alternative resources must ensure the same production results, in terms of fish growth, health, and product quality as obtained when using high-quality fishmeal as feed ingredients.

Although fishmeal has been used extensively in feeds because of its unique nutrient specifications (Watanabe et al., 1997), the high inclusion of fishmeal is problematic in aquaculture cost (Tacon and Jackson, 1985; Tacon, 1994; Higgs *et al*., 1995; Carter and Hauler, 2000) and competition between aquaculture and other animal production sectors for its use (Hardy, 1995) as well as an environmental concern. Thus many factors will limit that fish meal constitutes a high proportion of the feed (Carter and Hauler, 2000)

Shochu distillery by-products (SDBP) are primary fermentative products from sweet potato, and they it was found to be suitable ingredients as supplements in aquafeeds containing plant proteins for the common carp *Cyprinus Carpio* L. (Mokolensang, *et al*., 2003a,b). SDBP was also found to have a growth-promoting effect in cows, pigs, and chickens (Mahfudz *et al*., 1996; Mahfudz et *al*., 1997; Ohtsuka *et al*., 1998). However, the finding of dietary SDBP has not been fully understood for aquatic animals.

Determining energy requirements in cultured fish is very important to obtain maximum growth and formulate the diet with an appropriate nutrient composition. The metabolic energy costs of animals can be derived either by measuring heat production or by a calorimetric technique (Smith *et al*., 1978).

Therefore, it will be very important to clarify the energy portioning of fish when fed diets containing fundament ingredients such as SDBP.

The aim of this study is thus to determine the effects of SDBP on the energy budget and develop an economical and less pollutive fish diet by effective use of SDBP as supplement nutrition and of common carp.

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MATERIALS AND METHODS

A juvenile carp *Cyprinus Carpio* L. which was obtained from a hatchery in Kitakyushu, Fukuoka Prefecture, Japan, was transported to the Azuma Station, the Education and Research Center for Marine Resources and Environment, Faculty of Fisheries, Kagoshima University, and kept in indoor tanks filled with 200 L of aerated dechlorinated freshwater.

Shochu distillery by-products (SDBP) were obtained from Yamamoto *shochu* production company in Kagoshima, Japan. The pH of SDBP was from 4.0 to 7.0 with 4 *N* sodium hydroxide and then stored in a freezer at –30 °C.

For preparation of a carp diet, SDBP (2.1 g on dry basis or equivalent to 37.5 mL and 4.2 g on dry basis equivalent to 75 mL of the upper limit to form a firm pellet) was added to a commercial fish feed, casein, CMC (Carboxylmethyl cellulose) and cooking oil (corn oil) (Table 1). The CMC was used for binding, thus making the pellets unbreakable in the tank. Commercial diet and other ingredients were mixed thoroughly as moist pellets with a diameter of 3.2 mm through a Meiko 10 pelletizer (Meiko, Japan). The pellets were placed in plastic bags and frozen at –30 ºC before used.

The growth trial was carried out in a system consisting of nine 200-L fiberglass tanks (diameter 75cm) containing filtered dechlorinated fresh water with a flow rate of 140 mL/min. Prior a the feeding trial, all fish were acclimated to the pellet diets and standardized environmental indoor conditions for a week in a one-meter cubic water tank. After acclimation, fish were weighed, divided into three groups (SDBP 0%, 2%, and 4%) with three replicates. Methylene blue was used to prevent the fish diseases.

The fish were fed test diets to satiation (ad libitum) once a day (at 08:00) for three weeks. The amount of feed supplied was recorded, and the uneaten feeds were removed 30 min after feeding, dried at 60 ºC for 48 hours, and stored in a desiccator. The uneaten feed was placed in water for 30 min, then collected, dried and weighed to estimate the recovery ratio. The recovery ratio was used to adjust the amount of feed intake. The weights of fish were measured at the beginning and at the end of experiment; they were anesthetized with phenoxyethanol (5 mL in 10 L of water), and weighted after wiped with a paper towel to eliminate errors resulting from surface adhered moisture.

Table 1. Formulations of SDBP diets used for feeding experiment of common carp

Ingredients	SDBP diet (%)		
	0	2.1	4.2
Fish feed[1]	82.8	81.7	80.7
SDBP[2]	0.0	2.3	4.5
Casein[3]	6.7	5.8	5.0
CMC[4]	5.0	5.0	5.0
Corn oil[5]	5.5	5.2	4.8
Proximate			
Crude protein	42.2	41.7	41.3
Crude lipid	9.6	9.6	9.5
Carbohydrate	39.1	39.6	40.2
Ash	9.1	9.1	9.0
Energy (kcal/g)	4.9	4.9	4.9

[1]Crude protein 42.9%, Crude oil 5.0%, Carbohydrate 33.5%, Ash 11.0%, Moisture 7.6%; Energy 269 kcal/g (Nippon Formula Feed Company, Tokyo, Japan).
[2]Protein 37.8%, Lipid 15.2%, Ash 3.6%, Fiber 22.6%, Nitrogen-free extract (NFE) 19.7%, Moisture 1.1%; Energy 44.3kcal/g.
[3]Protein 100.0%
[4]Carboxylmethyl cellulose
[5]Corn oil

The non-fecal nitrogen excretion through the gills and in the urine was measured according to the procedure of NRC (NRC, 1993) after the termination of feeding experiment. During the experiment feces were collected every day from the bottom and outlet of the tank and dried at 60 ºC for 48 hours on further analysis. For the initial whole-body sample, 5 fish were collected from the stock tank on the day when the feed trial was started, and preserved at –30 C. Similarly, at the end of the trial, all fish from each tank were taken, pooled and preserved for the final whole-body analysis.

The aerated fish tank had dissolved oxygen more than 6.1 mg L-1, water temperature 23.8 – 25.7 C, pH about 7.5, and ammonia-nitrogen less than 0.23 mg L-1. Proximate compositions of the test diets are shown in Table 2. It has determined of the test diets were: ash by use of a muffle furnace at 550 C for 12h; moisture by drying at 105 C for 12h; crude protein (Nx25) by a semi-micro Kjeldahl method (AOAC, 1990) and crude lipids by a soxhlet extraction method (Bligh and Dyer, 1959). Calorie values of body components, feed and feces were measured with a YM Nenken digital calorimeter (Yoshida, Japan).

A model was constructed to stimulate growth of carp based on the standard bioenergetic equation that has been used in the similar studies for variety species (Kitchell et al., 1977; Kadowaki, 1994; Watanabe and Ohta, 1995; Bevelhimer, 2002). In its simplest form the bioenergetic approach provides an accounting mechanism for the utilization and assimilation of consumed energy. The basic bioenergetic model equates the amount of consumed food with the ultimate fate of that food:

$$GE = FE + NFE + ME + PE$$
$$DE = GE – FE$$

where GE is gross energy, DE is digestible energy, FE is fecal energy, NFE is non-fecal energy, ME is metabolic energy and PE is productive energy.

A value of 5.94 kcal/g ammonia N (Elliott and Davison, 1975) was used to calculate the non-fecal energy value. The excretion rate was calculated for starved or fed fish; the excretory loss was expressed as a percentage of energy intakes during the period when nitrogen excretion was measured. The excretory loss was extrapolated to the whole experimental period based on the overall mean weight or daily mean energy intake. Daily variations of ammonial concentration in rearing water were collected at 8:00 am.

Bioenergetic Calculations.

Calculation of energy feed intake:

$$GE = \sum_{n=1}^{20} (X_n \times \alpha) / (\overline{BW} \times T_{20})$$

where GE is gross energy of feed intake (kcal/kg body weight/day), n is n th day, Xn is dry g of feed intake per fish, α is kilocalorie per dry g of feed, BW is mean body weight in kg wet and T20 is culture period in 20 days.

Calculation of fecal energy:

$$FE = \sum_{n=1}^{20} (Y_n \times \beta_n) / (\overline{BW} \times T_{20})$$

where FE is fecal energy (kcal/kg body weight/day), n is n th day, Yn is dry g of fecal per fish, βn is kilocalorie per dry g of fecal on the n th day.

Calculation of non-fecal excretion:

$$F_{5,12,20} = \frac{\text{Average ammonia concentration on the 5, 12 or 20 th day}}{\text{Ammonia concentration at 8 am}}$$

$$Z_n = F_{5,12,20} \times a_n \times b / 5$$

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where Zn is ammonia excretion on an nth day (mg N/ind/day), n is n the day, F5,12,20 is a factor on the 5, 12 or 20 th day, a is ammonia concentration on the n th day at 8 am (mg N/L), b is water flow (200 L/day) and value of 5 ia s number of fish cultured in each tank.

Calculation of non-fecal energy:

$$NFE = \sum_{n=1} Z_n \times 5.94 / (\overline{BW} \times T_{20})$$

where NFE is non-fecal energy (kcal/kg body weight/day), n is n th day, Zn is ammonia excretion on the n th day (mg N/ind/day) and a value 5.94 is kcal per one g of ammonia nitrogen (Elliot and Davison, 1975).

Calculation of energy of growth:

$$PE = (W_f - W_i) \times \gamma) / (\overline{BW} \times T_{20})$$

where PE is productive energy (kcal/kg body weight/day), Wi is initial body weight (g, dry), Wf is final body weight (g, dry) and γ is calorie content per dry g of the whole body.

The free ammonia-nitrogen, dissolved oxygen (DO), water temperature, and pH were monitored periodically. Dissolved oxygen was measured with a YSI Model 57 DO meter Yellow Spring Instrument Company, Yellow Springs, Ohio, USA). Water temperature and pH were measured with a Yokogawa Model 81 pH meter (Yokogawa Electric Company, Tokyo, Japan and) were monitored daily before the water was changed.
Ammonia-nitrogen in the tanks was determined once a week according to the method of Strickland and Parsons (1972).

RESULTS

The growth performance and diet utilization of the common carp Cyprinus carpio L. are presented in Table 2. All fish survived with no abnormal signs during the feeding experiment. The body weight gain (WG) of fish fed with diet content shochu distillery by-products (SDBP) showed a higher than the control. A statistically fish-fed diet content of 4.2% of SDBP showed significandifferencesnt from the control. When fed to satiation for three weeks, they grew from 50 to 97 g on 4.2% SDBP, 50.6 to 92.4 g on 2.1% SDBP, and 51.9 to 88.8 g on the control, respectively. The growth ratio was 82 or 93% for the 2.1 or 4.2% SDBP diet whereas the control diet gave 71%ahe value which was much lower than those fed with SDBP supplementation in diets.

Feed intake (FI) of fish in the control group showed less variation than in the treatment groups. The fish fed witthe h content of SDBP in the diets had significantly higher FI than the control. This is consistent with the findings for a specific growth rate (SGR). The growth was promoted with an increase of containing SDBP levels. However, a no significant difference ($P < 0.05$) was noted on food conversion ratio (FCR) among the control and contained SDBP groups. In each group SGR was highest for the fish fed with 4.2% content of SDBP in diet and decreased with lesser inclusion of SDBP in diets. The results showed that there was a positive correlation between FI and SGR.

Three feeding trials were conducted to determine the energy content of carp diets needed for the maintenance of body weight and activity for maximum growth. The energy budget of Japanese common carp fed with different levels of content SDBP is shown in Table 3.

The results were signed higher in gross energy (GE), fecal energy (FE), and digestible energy (DE) in fish fed with h content of 2.1 or 4.2% SDBP on the diets than the control. Non-fecal energy (NFE) and metabolic energy (ME) were found not different between 0% and 4% SDBP diets, but it signed different between 0% and 2%. A remarkable difference was found between 0%, 2%, and 4% SDBP diets of productive energy (PE) analysis.

The digestible energy (DE) requirements for maintenance of body weight and activity were 245.1, 279.3, and 279.0 kcal/kg body weight/day, and the corresponding feeding rates were 5.8, 6.8, and 6.9 %. The values of gross energy (GE) 310.1 and 309.5 kcal/kg body weight/day

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and feces energy (FE) 30.8 and 32.7 kcal/kg body weight/day between 2.1% and 4.2% SDBP diets were determined very close to each other. The DE requirement of assimilation energy for the maximum growth ranged from 49.0 – 55.8 kcal/ind/d. The highest value feed efficiency was found at the fish fed with 4% SDBP.

The percentage energy budget of common carp fed different levels of SDBP diets for 20 days is shown in Tables 4 and 5. The energy partitioning of the experimental diets, at the GE (100%) level required for maximum growth was as follows: FE, 9.0-10.6%; DE, 90.1–91.0%; NFE, 12.6–13.7%; ME, 39.4–46.5% and PE, 106.1–142.2%, respectively.

Table 2. Effect of dietary treatments on growth performance of common carp

Growth performance	Dietary treatments (% SDBP)		
	0	2.1	4.2
Initial body weight (W_i) (g)	51.9±0.1^{a}	50.6±1.4^{a}	50.9±1.1^{a}
Final body weight (W_f) (g)	88.8±1.0^{a}	92.4±9.3^{b}	97.4±3.6^{c}
Weight gain (WG) (g)	36.8±4.8^{a}	41.7±7.9^{b}	46.5±3.5^{c}
Feed Intake (FI) (g)	81.0±0.3^{a}	96.5±0.6^{b}	102.1±0.8^{c}
Feed conversion ratio (FCR)	2.2±0.2^{a}	2.22±0.5^{a}	2.2±0.3^{b}
Specific growth rate (SGR)(% d^{-1})	2.6±0.4^{a}	2.98±0.6^{a,b}	3.26±0.3^{c}

WG: final weight (W_f) – initial weight (W_i)
FI: cumulative feed consumption (g $fish^{-1}d^{-1}$)
FCR: feed intake (FI) in dry basis (g) / weight gain (g)
SGR: 100 x [(ln final weight] – ln initial weight) / days]
These values are means ± SD for each dietary treatment. Values in the same column with different superscripts (a, b and c) are significantly different (*P*<0.05) when analyzed using ANOVA with a Tuckey test.

Table 3. Energy budget of common carp with different levels of SDBP diets for 20 days.

SDBP Diet (%)	GE (*)	FE (*)	DE (*)	NFE (*)	ME (*)	PE (*)
0	269.4	24.3	245.1	13.7	125.3	106.1
2.1	310.1	30.8	279.3	15.4	143.8	120.1
4.2	309.5	32.7	279.0	12.6	124.2	142.2

(*) : kcal/kg ind/day

Table 4. Percentaged budget of common carp with dietary treatments.

Diets	Feed intake (I)	Feces (F)	Non-fecal (U)	Excretion (E = F + U)	Metabolic loss (M=I-F-U-G)	Growth (G)
Control	100.0	9.0	5.1	14.1	46.5	39.4
2.1% SDBP	100.0	9.9	5.0	14.9	46.3	38.7
4.2% SDBP	100.0	10.6	4.0	14.6	39.4	45.9

Table 5. Percentaged energy budget of common carp fed with different levels of SDBP diets for 20 days

SDBP (%)	ME/DE (%)	PE/ME (%)
0	51.1	84.7
2.1	51.5	83.5
4.2	44.5	114.5

Abbreviations: GE, gross energy; FE, fecal energy; DE, digestible energy; NFE, non-fecal energy; ME, metabolic energy and PE, productive energy

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DISCUSSION

In the feeding experiment using the common carp Cyprinus carpio L., it was shown that the indicators of growth performance, such as FI, FCR and SGR could be improved when the dietary level of SDBP as a supplement of feedstuff was increased. In the present studies, it appears that SDBP can be as supplemented nutrition without adverse effects on growth. Growth of some fish species has been getting slow when fed diets in which all the fishmeal without content of SDBP in the diets. Similar result (Webster et al., 1992; Pongmaneerat and Watanabe, 1993; Rumsey, 1993; and Kim et al., 1997) were the fish fed by substitution fish meal with soybean meal. However, (Webster et al., 1992) suggested that feeding diets containing a combination of protein sources may allow the use of a high percentage of soybean meal without growth reduction(Baskar et al., 2011). SDBP does not have any known anti-nutritional factors, such as gossypol; however, published data on the use of SDBP in aquaculture diets are limited. The requirement for maintenance of body weight was higher in the fish fed with 4.2% SDBP diet because the fish might have consumed more energy with increased feed intake on the addition of SDBP (Mokolensang, et al., 2003a,b, Chakraborty, 1999; Chakraborty et al., 1995). The energy required for fish with 4.2% SDBP diet was 20.5 kcal/ind/d (Ohta and Watanabe, 1996) suggested that the energy requirement for carp to maintain the body weight is 18-35 kcal/ind/d. In the fish fed with a lower content of SDBP, the growth was lowered.

In these studies, the food or energy intake of carp increased with growth. There was observed a similar energy intake in diet groups, suggesting that no marked difference might exist in palatability. The difference in feed consumption among them seems to be associated with size, even though the feed consumption will be affected by environmental conditions, culture regimens, design of feeding experimental, methods of computation for ingestion rates, and so on. Ammonia is a potent neurotoxin, and it cannot be allowed to accumulate to any substantial levels in fish. For most fishes, ammonial excretion is easily accomplished by direct diffusion from the plasma across the gill tissue to the large sink of surrounding water. This diffusion is probably enhanced by the acidification of the gill boundary layer that acts to convert NH_3 to NH_4^+. According to Kaushik (1980) and Watanabe et al., (1987) excretion of ammonia is related to the intake of protein. The increase of ammonial excretion after feeding is the resultant energy loss associated with assimilation of dietary protein (Jobling, 1981). In the present experiment, a higher ammonial excretion of the carp fed with lower SDBP diet could be ascribed to the catabolism of SDBP. There was found a difference in ammonial production among the dietary treatments. Two factors may be involved in this. First, a small proportion of dissolved nitrogen waste is excreted as urea (Kaushik, 1980; Brett and Zala, 1975). Secondly, some of the excreted ammonia is likely to be transformed into nitrite/nitrate by bacterial oxidation in the water (Horne and Goldman, 1994).

A higher dietary concentration of SDBP might also improve the digestive enzyme activity of fish. Routine metabolic rates are often higher in well-fed animals than in those subjected to a long period of malnutrition or starvation. This metabolic difference has been attributed to the biochemical composition of the body tissue, the relative sizes of different organ systems, and increasing rates of synthesis and turnover of various tissue components. The increase in body weight at the end of the experiment was used to gauge the growth of the carp.

CONCLUSION

In the conclusion of this experiment, we found that the DE intake was positively correlated to energy requirement. The feeding rate was calculated based on this, such that the energy requirements of the

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fish are satisfied. Although, the experimental diet within the limited time span, this study has succeeded in establishing the protein and energy requirement in Japanese carp.

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