Digestible Energy: Methodological Influences and a Mode of Calculation[†]

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The energy values of fish feed could be a suitable indicator for choosing an appropriate diet for a particular species. This study examined the effect of fish size, water temperature and time of feces collection on energy digestibility. In addition, energy digestibilities of practical diets and commercial feeds were determined to find out if digestible energy (DE) values could be calculated from gross energy (GE) values.

The DE determinations should preferably be conducted on carp weighing about 10 g maintained at around 20°C and with rainbow trout weighing less than 100 g at a water temperature between 10–15°C. The digestibility of energy increased in proportion to the lapse of time of fecal collection, until about 18 h in rainbow trout and even beyond that in carp, due to the leaching of nutrients. It was also confirmed that there was no change in the energy digestibility of fish over time.

Comparing the energy digestibility of practical and commercial diets for carp, it was found that the average figure was 80%, proving that the DE of diets could be estimated from the GE values.

Key words: rainbow trout, carp, diets, digestibility, digestible energy, gross energy

Measurement of the energy values of diets is of great importance for practical fish culture especially in deriving the dietary energy cost of fish growth. Among various energy values of diets, digestible energy (DE) is the most appropriate and easy measure of fuel availability when evaluating the potential energy utilization of formulated feeds. However, information on DE values of practical feeds and feedstuffs is very limited and hence there is no official standard for indicating the energy values of practical fish feeds in Japan. The basic problem is the difficulty in feces collection without nutrient leaching.^{1,2)} On the other hand, determination of metabolizable energy (ME) values of diets for fish is still technically difficult because of the need to quantify both gill and urinary losses. Many investigators have attempted to overcome these difficulties and have developed various systems and procedures.^{3–6)}

This series of studies^{7–9)} on energy values of diets aimed to establish a simple method for measuring DE and ME and estimating the energy values of practical fish feeds. The present experiment examined the effects of fish size, water temperature and time of feces collection on energy digestibility. In addition, the energy digestibility of diets with practical feed stuffs and commercial feeds was determined to establish a simple method for estimating DE values [apparent digestibility of energy (%), ADE] from their gross energy (GE) values.

Materials and Methods

Experimental carp *Cyprinus carpio* and rainbow trout *Oncorhynchus mykiss* were obtained from Tokyo Fisheries Experimental Station and its Okutama branch, re-

spectively, and were kept on commercial carp and rainbow trout diets. Later the required sizes were selected and habituated to the experimental diets.

The apparatus used for feces collection in this experiment was essentially the same as that used for measuring the endogenous and metabolic fecal nitrogen excretion in the previous experiment,⁷⁾ but was simplified to suit just the collection of fecal materials.⁸⁾ The glass tank measured 45 × 30 × 30 cm and had a sloping bottom, and was loaded each time with a particular size of fish, depending on the purpose of the experiment. After collection, the feces were centrifuged at 3,000 rpm for 5 min, and the precipitate was freeze-dried and ground for determination of chromic oxide concentration, nutrient content and the GE. The relevant analytical procedures and calculation of DE values were as described in previous papers.^{7,8)}

Experiment I: Effect of Water Temperature and Fish Size On Digestibility of Energy

The water temperature was adjusted at 15, 20 and 25°C for carp and 5, 10, 15 and 20°C for rainbow trout. Both fish species were classified into four sizes by their weight, in ranges of less than 10 g, 10–50 g, 50–100 g and 100–200 g. For the various combinations of water temperature and fish size, 36 experimental lots were arranged for carp and 48 lots for rainbow trout. The stocking density of fish in the tank depended upon the fish size, being 50 or 80 carp and 70 or 80 rainbow trout of below 10 g, 30 carp and 20 rainbow trout weighing 10–50 g, and 5–9 fish weighing above 50 g for both species. The velocity of the water flow was adjusted at 400 ml/min to maximize settling of feces in the trap glass column. The fecal materials were continu-

[†] Nutritional Energetics in Fish-IV.

ously siphoned through the trap column by the water flow.

The composition of the experimental diets is shown in Table 1. Diet 1 was arranged as a high protein diet with 47–50% crude protein and GE of 4.6–4.9 kcal/g diet. The crude protein content of diet 2 was the same as that of diet 1, and the dietary energy level was adjusted to 4.9–5.1 kcal/g by supplementing pollock liver oil, to serve as the medium-energy diet. Diet 3 was formulated to be a low protein-high energy diet with crude protein content of 36–40% and GE of 5.1–5.3 kcal/g, elevated by oil and starch supplementation.

Experiment II: Effect of Feces Collection Timing on Digestibility of Energy

The water temperature and size of fish used for feces collection were 20°C and 110–130 g (10–13 fish/tank) for carp, and 17°C and 40–50 g (30 fish/tank) for rainbow trout. The details of feeding and feces collection method were essentially the same as in Experiment I. The composition of the experimental diets is shown in Table 2. A relatively high amount of cellulose and α -starch were included in the diet for carp and rainbow trout, respectively, to facilitate easy accumulation of feces.

The fish were accustomed to the dietary regime in the tank with the usual siphon system⁸⁾ for several days before starting fecal collection. The fish were fed three times daily at 10:00, 12:00 and 16:30, each time to satiation. The feces collection commenced from the 4th day after providing the experimental diet. About thirty minutes after the last meal, the tank-bottoms were cleaned to remove feed residues and feces, and the normal siphon column was replaced by the trap column for each tank at 17:00. Thereafter, the feces were collected from each tank at 12, 17, 18, 41 and 65 h for carp, and at 6, 8, 12, 18, 41 and 65 h for rainbow trout, to determine the effect of the feces collection interval on the digestibility of feedstuffs. In order to verify the nutrient loss due to leaching, the feces accumulated after 18 h were retained in the trap-column until collection either at 41 or at 65 h. To facilitate this, fish were all transferred to a separate tank after 17 h, thereby avoiding contamination with the feces excreted after that time. In addition, in the case of rainbow trout, feces were also collected at intervals of 0–6 h, 6–12 h and 12–18 h after installing the trap column to determine whether digestibility obtained with feces excreted at different times changes with lapse of time after feed intake.

Experiment III: Digestibility of Energy of Practical Feeds for Carp

The energy digestibility of practical diets and commercial carp feeds was measured to estimate the digestibility coefficient of commercial fish diets. Feedstuffs such as white fish meal, local fish meal (mainly sardine) and meat meal as animal protein sources and defatted soybean meal and wheat germ meal as plant protein sources were used for formulation of carp feeds (Table 3). Gelatinized starch (α -starch), wheat flour and rice bran were used as carbohydrate sources, and soybean oil, feed oil and animal tallow were added as lipid sources. In addition, five commercial carp diets were purchased to determine their DE values.

Feces were collected from carp weighing 12–25 g (20 or 25 fish/tank) and 150–220 g (3 fish/tank) maintained at

Table 1. Composition of the diets used for determining the relationship between apparent digestibility of dietary energy and fish size or water temperature

Ingredient	Content (%)		
	Diet 1	Diet 2	Diet 3
White fish meal	70.0	70.0	55.0
α -Starch	15.0	10.0	20.0
Mineral mixture* ¹	5.0	5.0	5.0
Vitamin mixture	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5
Cellulose	2.0	2.0	2.0
Chromic oxide* ²	1.0	1.0	1.0
Pollock liver oil	5.0	10.0	15.0
α -Tocopherol acetate	0.1	0.1	0.1
Nutrient content (%)			
Crude protein	47–50	47–50	36–40
Crude lipid	12–13	18	21
Gross energy (kcal/g)	4.6–4.9	4.9–5.1	5.1–5.3

*¹: Ogino salt mixture.

*²: Cr₂O₃:Cellulose::1:1.

Table 2. Composition of the diets used for determining the effect of fecal collection time on digestibility of dietary energy

Ingredient	Content (%)	
	Trout	Carp
White fish meal	48.0	52.0
Wheat flour	14.4	15.6
α -Starch	21.1	2.1
Cellulose	0.4	14.0
Vitamin mixture	3.5	3.8
Mineral mixture*	1.9	2.1
NaH ₂ PO ₄	2.9	3.1
Pollock liver oil	4.0	0.0
Fancy tallow	0.0	3.1
Soybean oil	3.8	4.2
Chromic oxide	0.5	0.5
Energy content		
Gross energy (kcal/g)	4.2	4.7

*: Ogino salt mixture.

around 20°C in order to examine the effect of fish size on energy digestibility.

Results and Discussion

Experiment I

The effect of water temperature and energy content of the diets on apparent digestibility of GE in carp is shown in Fig. 1 for the different sizes of fish. The effect on energy digestibility of each diet seemed to differ between carp weighing below and above 10 g. Digestibility was not influenced much by water temperature in the fish below 10 g, but for sizes above this, there was an increase corresponding to the rise in temperature. Energy digestibility determined at 20°C was almost similar for the different diets, irrespective of the fish size. Thus, when the values determined at 20°C were conventionally expressed as 1, the relative values obtained at 15 and 25°C are indicated in Table 4. The DE value of the low protein-high energy diet (diet 3) was not significantly different between 15 and 20°C and

Table 3. Composition of the practical and commercial diets fed to carp and the apparent digestibility of gross energy in the fish

Ingredient	Content (%)							
	Diet 1	Diet 2-6	Diet 7	Diet 8* ¹	Diet 9	Diet 10	Diet 11-16	Diet 17-20* ¹
White fish meal	43	50-60	50					
Local fish meal					33	33	30-40	
Meat meal							0-4	
Wheat flour	15	15	15		22	45	25-48	
α -Starch	22	5-22	2		15			
Wheat germ meal* ²							0-10	
Soybean meal* ²					8		5-13	
Rice bran* ²					10		10	
Cellulose	0.4	0.4-2.4	13.4					
Mineral mixture	2	2	2		2	2	2	
Vitamin mixture	3.6	3.6	3.6		1	1	1	
NaH_2PO_4	3	3	3		2	2	2	
Fancy tallow	3	0-3	3					
Yellow grease					6	6	0-6	
Soybean oil	4	2-4	4					
Feed oil	4	0-4	4		1	1	1	
Nutrient content (%)								
Crude protein	33	37-43	38	42	30	30	30-40	32-36
Crude lipid	15	7-16	17	6	12	13	8-13	3-6
Gross energy (kcal/g)	4.6	4.2-4.7	4.7	4.3	4.5	4.6	4.4-4.6	4.1-4.2
ADE* ³ (%) 12-25 g	85	87-88	84	78	79	76	78-83	81-82
150-220 g					82	81	74-84	78-81

*¹: Commercial diets.*²: Deffated products.*³: Apparent digestibility of energy. **Fig. 1.** Effect of ambient water temperature and energy content of diets on apparent digestibility of gross energy in carp.

that of the high protein-high energy diet (diet 2) between 20 and 25°C, but the overall trend was an increment in proportion to the rise in water temperature. These results suggested that DE in carp should be determined on fish

weighing less than 10 g maintained at around 20°C.

Figure 2 shows the results obtained in rainbow trout. Unlike carp there was no difference in energy digestibility in rainbow trout weighing less than 100 g at water tempera-

Table 4. Effect of ambient water temperature and energy content of diets on apparent digestibility of gross energy*¹ in carp

Diet	Size and temperature			
	< 10 g	10–200 g		
	15–25°C	15°C	20°C	25°C
Diet 1 CP: 47–50; GE: 4.6–4.9* ²	1.00	0.91	1.00	1.04
Diet 2 CP: 47–50; GE: 4.9–5.1	1.00	0.91	1.00	1.01
Diet 3 CP: 36–40; GE: 5.1–5.3	1.00	0.97	1.00	1.05

*¹: Values are expressed relative to those obtained at 20°C.*²: CP—Crude protein (%); GE—Gross energy (kcal/g).

tures between 5 to 20°C. However, fish over 100 g exhibited a reduction in energy digestibility at higher tempera-

tures. Generally, the values for DE of each test diet was not markedly different between 5 and 10°C, but was slightly higher at 16°C and 20°C. It is probable that at 10–15°C, the optimum water temperature for rainbow trout, the metabolic activity of the fish was greater, leading to higher digestibility. The relative values of energy digestibility obtained when those at 10°C were expressed as unity are shown in Table 5. The results were not as distinct as those in carp but roughly suggested that DE should preferably be determined with rainbow trout weighing less than 100 g at water temperatures between 10 to 15°C.

Experiment II

As shown in Fig. 3, the digestibility of energy increased in proportion with the lapse of time of feces collection, clearly up to 18 h in both species. However, the values continued to increase, particularly in the case of carp, even up to 65 h, indicating that fecal nutrients leached into the am-

Fig. 2. Effect of ambient water temperature and energy content of diets on apparent digestibility of gross energy in rainbow trout.**Table 5.** Effect of ambient water temperature and the energy content of diets on apparent digestibility of gross energy*¹ in rainbow trout

Diet	10–100 g		< 10–100 g		100–150 g			
	5°C	10°C	16°C	20°C	5°C	10°C	16°C	20°C
Diet 1 CP: 47–50; GE: 4.6–4.9* ²	0.99	1.00	1.06	1.05	1.04	1.00	1.06	1.08
Diet 2 CP: 47–50; GE: 4.9–5.1	0.99	1.00	1.04	1.05	0.91	1.00	1.01	
Diet 3 CP: 36–40; GE: 5.1–5.3	1.02	1.00	1.07	1.07	0.96	1.00	0.95	1.00

*¹: Values are expressed relative to those obtained at 10°C.*²: CP—Crude protein (%); GE—Gross energy (kcal/g).

Fig. 3. Apparent digestibility of energy in carp and rainbow trout determined from feces collected at different hours after food ingestion. The empty triangles in the case of rainbow trout indicate DE values at 6 h intervals. The values at 41 and 65 h for both species indicate the nutrient loss from feces due to leaching.

bient water while the feces remained in the trap column. Further experiments are thus needed on nutrients which are lost relatively easily in the water. Generally in digestibility studies, determinations are made on feces collected 17–18 h after installing the trap column. When the values at 0 h were extrapolated from the curves in Fig. 3 and compared with those obtained at 17–18 h, it was found that the DE at 0 h could be arrived at by multiplying the values with a correction coefficient. This factor termed “fecal-leach coefficient” was calculated as 0.9 for rainbow trout and 0.85 for carp.

Figure 3 also shows that the DE values in rainbow trout determined from feces collected during different 6 h periods were almost the same, and were not affected by the lapse of time up to 18 h after intake of diet. It appears that the digestibility of energy did not vary through the 18 h post-ingestion span. The increase in digestibility over time mentioned earlier could thus be attributed to nutrient leaching from the feces. The form of feces may also influence the degree of leaching, the loss being lesser in the case of rainbow trout which releases excretory materials in a better bound matrix, compared to carp.

Experiment III

The energy digestibility of practical diets determined for carp is shown in Table 3. In the diet with white fish meal as protein source, digestibility was 84–88% regardless of the α -starch levels in the diet. In other diets containing commonly available protein ingredients the digestibility of energy was also high, ranging between 76–83%. The values of 78–82% obtained for the commercial carp diets were close to those of the practical diets. There was also no marked difference based on the size of fish. These results suggest that the value of 80% can be applied to estimate the energy digestibility of ordinary commercial carp diets. In other words, the DE of diets can be estimated from the GE value of the diets.

For simple calculation of the DE value of commercial

fish feeds, information on the DE values of each practical feedstuff employed commercially is needed for each fish species. Another related study would evaluate the mode of calculating the DE content of practical diets based on the DE value of each feed ingredient.

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