Effect of Feed Preparation Methods on Dietary Energy Budgets in Carp and Rainbow Trout

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(Received February 17, 1997)

This study was conducted to clarify the energy requirements for maintenance of body weight and activity, and for maximum growth in carp and rainbow trout, specifically examining the effect of feed preparation methods on dietary energy budgets and nitrogen balance. Carp (31 g, 17°C), rainbow trout juvenile (31 g, 9 and 16°C), and rainbow trout fingerlings (5.7 g, 16°C) were fed isocontent ordinary dry pellets (DP) produced by a pellet mill and extruded pellets (EP) by a twin-screw extruder.

Carp fed EP to satiation showed markedly reduced feeding rate, low percent gain and feed efficiency compared to those fed DP to satiation. In rainbow trout EP tended to be generally lower in the feeding rate and higher in the percent gain and feed efficiency than DP at the satiating levels. This tendency was conspicuous for the growth of fingerling and the feed efficiency at a low temperature. The energy digestibility of EP increased from that of DP in rainbow trout, in relation to the gelatinization of starch.

The energy budget in fish fed enough amounts of energy required for maximum growth was determined. The fecal energy loss in DP was relatively large among all the tests, and the digestible and metabolizable energy values of EP were elevated in relation to the fecal energy values. The non-fecal energy losses in rainbow trout were somewhat higher in EP than those in DP. The heat increment of feeding was high in DP and the proportions of productive energy were high in EP in all the experiments.

Key words: energy budget, digestible energy, metabolizable energy, carp, rainbow trout

The total production of formulated feeds for aquaculture in Japan was about 422,000 t in 1995, 68% of which (287,000 t) was meant for yellowtail and red sea bream. The demand grew at an annual rate of 137% in 1994. The percentage of dry pellets for these two species was 42% of the production, the annual demand rate rose by 180% because of a shortage of raw feeds such as sardine and sand eel. On the other hand, the total production of formulated feeds for common carp, trout, ayu, eel, and tilapia, which are the major freshwater species, was about 103,000 t, constituting 24% of total production of fish feed. The annual growth rate was 99% in 1994. (Japan Fish Feed Association, 1996). The spurt in demand for dry pellets in mariculture was mainly due to the development of a porous, highly hydrophilic, high fat soft-dry pellet, produced using a twin extruder, specifically for marine finfish.¹⁾ It is well established in the food industry that the effect of extrusion cooking on quality and nutritive value of food differ from that of an ordinary manufacturing machine: i.e., the former process causes compression, temper, shear, fusion, sterilization, and expansion of raw materials with the help of an extruder, especially a twin-screw extruder.²⁾ Extruded diets meant for fish were first available for ornamental fish (goldfish, colored carp, etc.) and later for shrimp. The former has floating properties and the latter superior water stability. The types of feed currently available for

freshwater species are mash, ordinary dry pellets (DP) produced by a pellet mill, extruded pellets (EP) produced by an extruder, or crumbled diets. In coastal areas, the so-called Oregon type of moist pellets containing mixed raw fish and formulated mash, or the single moist pellets mixed with water, oil, and formulated mash is prepared before feeding.³⁾ However EP, DP, and crumbles are also being offered to marine finfish. Moreover, recently developed expanded pellet (ingredients are processed by an expander and then passed through a common pellet mill) is widely being employed for red sea bream, trout, and ayu, which has an intermediate character between EP and DP.⁴⁾ The effect of extrusion processing of a single feed ingredient or a mixture on dietary value has been investigated by many researchers.^{5–18)} However, there are only few studies on the effect of feed production methods on the nutritive value of diet itself, prepared either by an extruder or a pellet mill.^{19–22)}

In this series of studies on energy values of diets in carp and rainbow trout,^{23,24)} energy requirements for maintenance of body weight and activity, and for maximum growth were determined by feeding fish high quality diets at different feeding levels. At the same time, dietary energy budgets were examined by analyzing whole body composition and measuring metabolizable energy (ME) values of diets with the system developed by Ogino *et al.*²⁵⁾ Nitrogen

balance was also examined in these experiments.

Following these methods, this study was conducted to clarify the energy requirements of carp and rainbow trout by feeding them isocontent EP and DP, specifically examining the effect of feed preparation methods on dietary energy budgets and nitrogen balance. Four experiments (Experiments I–IV) were arranged by changing the feed production methods, species (carp and rainbow trout), and size of fish and water temperature in rainbow trout.

Materials and Methods

Experimental Diets

The diet composition and its analytical data are listed in Table 1. Both EP and DP were formulated identically, a local fish meal (mainly sardine; Shimamori Fish Processing, Shima-meal) being the protein source, pollock liver oil as a lipid source, and wheat flour and gelatinized potato starch as carbohydrate ingredients and binder. The diets were pelleted either by an extruder or a pellet mill as described below and used for all the 4 experiments. The EP and DP contained 6.1–10.7% moisture, 48.2–50.2% crude protein, 11.7–13.1% lipid, 15.6–16.7% starch, and 4.56–4.98 kcal/g gross energy (GE) and the difference between the experimental diets was little. Although the nutrient contents of diets seemed to be practical and to be adequate to meet the requirements of rainbow trout,^{21,26} they were high protein high calorie diets for carp.²⁷ This aspect was overlooked since the experiment aimed to test identical diets for both species. The gelatinized ratio of EP and DP was 100% and 43% respectively, reflecting the feed prepara-

tion methods.

Extrusion pelleting was carried out using a Twin-screw Extruder DNDG-62 (Buhler Ltd.) in this study. The extrusion conditions are summarized as follows: screw length/die diameter ratio (L/D ratio), 16; material temperature, 96°C; material press, 42–51 bars; barrel temperature, 80°C; feed rate, 80 kg/h; steam rate, 4 kg/h; water injection, 13.4 kg/h; screw speed, 70 rpm; die diameter, $\phi 2.7$ m/m $\times 8$; and drying condition, 100°C $\times 10$ min. Akimoto²⁸ has attempted to improve the nutritive value of soybean meal by extrusion processes under various conditions. He found that material temperature, one of the most important conditions in extrusion processing, was greatly affected by screw speed and water injection rather than barrel temperature. Considering the feeding behavior of the species, the EP were superior as a slow sinking type under lower material temperature to that of a floating type (110–130°C). The ordinary DP were prepared by a Laboratory Pellet Mill (California Pellet Mill Co., Ltd.), under the following operating conditions: size of pellet mill, 2 HP; material temperature, 55–65°C; feed rate, 40–50 kg/h; water injection, 5%; die diameter, $\phi 3.2$ m/m; and drying condition, 70–80°C $\times 1$ h. The pellets were machine-ground into a suitable size for the experimental fish in Expt. IV.

Culture Conditions and Collection of Fecal and Urinary Nitrogenous Compounds

The fish were fed a commercial rainbow trout EP and DP alternately every day (three times daily) to acclimate the animals to the physical properties of the feeds in glass aquaria (60 $\times$ 29 $\times$ 36 cm) for 2 weeks before the initiation of the feeding experiment. At the beginning of each experiment, fish were starved for 24 h and weighed before noon as described previously,^{23,24} after which they were fed the experimental diets at 17:00 on the same day. The non-fecal nitrogen excretion through the gills and in the urine was measured according to the procedures reported elsewhere^{24,29} at the end of each feeding experiment. The collection of feces with a TUF column³⁰ started from 18:30 hr on the 4th day of feeding and continued till 8:30 hr the next day (14 h) for all groups, with the exception of those fed at low levels, in all the experiments according to previous methods.³¹ Although feeding frequency was basically arranged as three times daily (09:00, 11:15, and 17:00 hr), it was revised to once (11:15) or twice (09:00, 17:00) daily for the fish with low feeding levels to facilitate equal distribution of diets among the fish. The culture conditions of the four experiments are presented in Table 2, and the highest feeding level corresponded to satiation. In Expt. I 12 carp having a mean weight of 31.5 g, in Expt. II 14 rainbow trout weighing 31.3 g each, in Expt. III 14 rainbow trout weighing 30.5 g each, and in Expt. IV 40 fingerling rainbow trout weighing 5.7 g each were fed EP or DP at five or six levels ranging from 0 to 3.2% of body weight per day for 23 (Expt. I) or 21 (Expts. II–IV) days at 17.5, 16.3, 9.1, and 16.0°C, respectively. Rearing water was continuously cooled by a Chiller Unit RCU1503E (Hitachi, Ltd.) to maintain the low temperature in Expt. III. Other culture conditions were the same as our previous reports.^{23,24,29,31}

All fish samples kept frozen at -50°C were

Table 1. Composition of the experimental diet for carp and rainbow trout

Ingredients	Pellet type	
	EP ^{*2}	DP ^{*3}
Fish meal	65	
Wheat flour	15	
α -Potato starch	5	
Pollock liver oil	5	
Mineral mixture	5	
Vitamin mixture	1	
Vitamin E (50% pure)	0.1	
Choline chloride (50% pure)	0.8	
Cellulose	3.1	
Cr ₂ O ₃ ^{*1}	1	
Moisture	7.4–10.7	6.1–8.9
Crude protein	48.2–49.2	49.6–50.2
Crude lipid	11.7–12.2	11.8–13.1
Crude starch	15.6–16.7	15.9–16.2
Crude ash	12.7–13.5	13.0–14.1
Crude fiber	0.1	0.1
Ca	2.3	2.3
P	2.0	2.0
Gelatinized ratio	100	43
Gross energy (kcal/g)	4.56–4.98	4.63–4.86

^{*1} Cr₂O₃; cellulose = 1:1.

^{*2} Extruded pellets.

^{*3} Ordinary dry pellets.

Table 2. Culture conditions in the four experiments.

	Species	Pellet type	No. of fish	Initial body wt. (g)	Feeding rate (%/day)	Feeding level	Expt. period (days)	Water temp.* ₁ (°C)
Expt. I	Carp	EP* ₂	12	31.5	0-1.9	6	23	17.1-18.1 (17.5)
		DP* ₃	12	31.5	0.5-2.3	5	23	
Expt. II	Rainbow trout	EP	14	31.3	0-2.1	6	21	15.8-17.0 (16.3)
		DP	14	31.3	0.4-2.3	5	21	
Expt. III	Rainbow trout	EP	14	30.5	0-1.6	6	21	8.9-9.4 (9.1)
		DP	14	30.5	0.2-1.7	5	21	
Expt. IV	Rainbow trout	EP	40	5.7	0-3.1	6	21	15.4-16.5 (16.0)
		DP	40	5.7	0.7-3.2	5	21	

*³ Ordinary dry pellets prepared by a pellet mill.

homogenized by an Ultra-centrifugal Mill ZM-1000 (Retsch GmbH & Co.) under cold conditions maintained by dry ice. Chemical analyses and calculations of energy values were carried out according to the methods given elsewhere.^{8,23)}

Results and Discussion

Energy Requirements for Maintenance of Body Weight and Activity, and for Maximum Growth

The results of feeding and apparent digestibility of energy, protein, lipid, and starch in Expts. I-IV are summarized in Tables 3 and 4.

Carp (Experiment 1): Carp fed EP to satiation showed markedly reduced feeding rate and percent gain, and slightly reduced feed efficiency compared to those fed DP to sati-

ation. However, the best feed efficiency was found for EP at the 1.25% feeding level. There were no marked differences in the digestibility of each nutrient between EP and DP, with the exception of starch where it was slightly higher in fish fed EP to satiation. The digestibility values were almost equal corresponding to feeding rates in carp as observed before.²⁴⁾

The relationship between the digestible energy (DE) intake and the percent gain in Expt. I is shown in Fig. 1 together with the results from rainbow trout. In the fish fed at the low feeding rates, measurement of digestibility was difficult because very little amount of feces could be collected and therefore the digestibilities determined at the nearest feeding rate were used for calculation of DE. The energy value intersecting the horizontal axis is considered as the DE requirement for maintenance of body weight

Table 3. Results on feeding carp of 31 g for 23 days at 17°C and rainbow trout of 31 g for 21 days at 16°C in Expts. I and II

	Feeding rate (%/day)	No. of fish	Total body wt. (g)		Percent gain	Diet consumed (g)	Feed efficiency (%)	Apparent digestibility (%)			
			Initial	Final				Energy	Crude protein	Crude lipid	Crude starch
Expt. I: Carp	0	12	373.5	328.6	-12.0	0	—	—	—	—	—
Extruded pellet	0.41	12	383.7	401.4	4.6	36.8	48.0	90.0	—	—	—
	0.84	12	383.9	455.6	18.7	80.8	88.7	89.5	93.4	95.2	92.3
	1.25	12	378.6	496.0	31.0	125.2	93.7	90.5	93.9	94.9	93.1
	1.57	12	382.3	528.0	38.1	164.9	88.3	90.0	93.5	93.2	92.8
	1.90	12	379.8	528.5	39.2	198.5	75.0	89.8	93.1	92.8	93.3
Dry pellet	0.48	12	369.7	391.1	5.8	41.7	51.5	89.2	—	—	—
	0.98	12	384.2	467.1	21.6	95.8	86.6	90.0	93.1	94.0	93.2
	1.46	12	373.7	506.4	35.5	147.4	90.0	89.6	93.4	94.9	92.3
	1.86	12	370.2	547.5	47.9	196.2	90.4	88.0	91.8	89.7	91.0
	2.27	12	375.4	569.8	51.8	247.1	78.7	88.7	92.3	93.5	90.3
Expt. II: Rainbow trout	0	14	438.6	393.8	-10.2	0	—	—	—	—	—
Extruded pellet	0.37	14	437.8	457.9	4.6	35.1	57.2	91.0	—	—	—
	0.76	14	435.6	507.0	16.4	75.3	95.0	89.9	93.7	95.2	90.5
	1.10	14	437.4	578.5	32.3	117.9	119.7	92.3	95.8	97.1	93.3
	1.59	14	439.7	645.5	46.8	181.3	113.5	89.6	93.5	94.6	90.5
	2.06	14	433.1	669.7	54.6	238.0	99.4	84.8	89.6	87.9	85.9
Dry pellet	0.38	14	436.4	450.6	3.3	35.1	40.6	91.9	—	—	—
	0.76	14	441.4	516.4	17.0	76.3	98.4	90.8	95.6	96.7	83.2
	1.12	14	435.3	565.0	29.8	117.4	110.5	89.6	95.3	97.5	79.8
	1.63	14	441.3	635.4	44.0	184.1	105.5	85.6	91.2	93.6	77.2
	2.31	14	436.7	671.4	53.8	268.8	87.3	79.3	85.3	89.0	67.8

Table 4. Results on feeding rainbow trout of 31 g for 21 days at 9°C and rainbow trout fingerlings of 5.7 g for 21 days at 16°C in Expts. III and IV

	Feeding rate (%/day)	No. of fish	Total body wt. (g)		Percent gain	Diet consumed (g)	Feed efficiency (%)	Apparent digestibility (%)			
			Initial	Final				Energy	Crude protein	Crude lipid	Crude starch
Expt. III: Rainbow trout Extruded pellet	0	14	429.7	387.1	-9.9	0	—	—	—	—	—
	0.20	14	429.4	426.4	-0.7	18.0	-17.0	—	—	—	—
	0.48	14	429.2	484.8	13.0	46.4	119.7	90.7	—	—	85.0
	0.79	14	428.3	547.0	27.7	81.0	146.6	90.1	95.1	98.1	87.8
	1.14	14	430.4	612.6	42.3	124.8	146.0	92.0	95.5	97.5	92.3
	1.58	14	422.2	662.9	57.0	179.7	134.0	90.1	94.3	94.7	88.2
Dry pellet	0.20	14	427.4	425.6	-0.4	17.9	-10.5	—	—	—	—
	0.48	14	427.8	485.4	13.4	46.4	124.1	88.1	—	—	70.3
	0.80	14	426.4	536.1	25.7	80.7	135.9	87.0	94.4	96.0	72.2
	1.14	14	428.0	606.8	41.8	124.0	144.2	87.4	94.3	95.9	67.9
	1.69	14	423.2	651.7	54.0	190.3	120.1	82.0	89.4	92.6	61.4
Expt. IV: Rainbow trout fingerlings Extruded pellet	0	40	244.3	211.3	-13.5	0	—	—	—	—	—
	0.67	40	212.3	232.5	9.5	31.3	64.5	—	—	—	—
	1.32	40	225.4	325.7	44.5	76.4	131.2	91.5	95.4	97.9	92.8
	1.87	40	226.3	394.1	74.2	121.7	138.0	91.4	95.0	96.4	92.7
	2.51	40	224.0	485.5	116.7	187.0	139.8	90.9	94.6	96.4	92.3
	3.14	40	237.1	594.7	150.8	273.9	130.5	87.5	91.5	93.5	88.3
Dry pellet	0.67	40	211.6	231.1	9.2	31.1	62.7	—	—	—	—
	1.33	40	226.5	324.8	43.4	76.8	127.9	89.7	95.0	97.2	81.0
	1.86	40	222.1	388.7	75.0	119.5	139.5	89.4	95.0	96.9	79.8
	2.71	40	224.8	469.5	108.8	197.8	123.7	84.7	91.1	93.8	70.5
	3.22	40	244.4	569.4	133.0	257.2	118.1	83.3	89.8	93.1	68.1

Fig. 1. Relationship between digestible energy intake and percent gain in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I, II, and III.

Expt. I: Carp (31 g, 17°C); ●—●, EP; ○—○, DP.

Expt. II: Rainbow trout (31 g, 16°C); ▲—▲, EP; △—△, DP.

Expt. III: Rainbow trout (31 g, 9°C); ■—■, EP; □—□, DP.

and activity, and the value at a nearby inflection point or at the satiation feeding level was close to the DE requirement for maximum growth. These values are given in Table 5. When EP and DP were fed to carp, the DE requirements for maintenance of body weight and activity were 12.1 and 13.0 kcal/kg body weight per day respectively, the corresponding figures in GE being 13.4 and 14.6 kcal/kg body weight per day; these requirements

being met at the feeding rates of 0.30 and 0.32% of body weight per day, being lower in EP. The DE requirements of those for maximum growth were 67 and 82, the corresponding GE values being 75 and 93 kcal/kg body weight/day, at the respective feeding rates of 1.64 and 2.03% of body weight/day, indicating an effect of feed preparation methods. Carp fed EP at approximately 60 kcal/kg body weight/day of DE or more showed poor growth compared

Table 5. Energy requirements for maintenance of body weight and activity, and a maximum growth determined based upon percent gain and energy balance in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP)

Energy requirement	Expt. I Carp (31 g, 17°C)		Expt. II Rainbow trout (31 g, 16°C)		Expt. III Rainbow trout (31 g, 9°C)		Expt. IV Rainbow trout (5.7 g, 16°C)	
	EP	DP	EP	DP	EP	DP	EP	DP
Maintenance of body weight and activity:								
DE (kcal/kg body wt./day)	12.1	13.0	10.9	12.5	8.6	9.0	17.2	18.6
Feeding rate (%/day)	0.30	0.32	0.26	0.30	0.21	0.22	0.42	0.45
Maximum growth:								
DE (kcal/kg body wt./day)	67	82	75	77	65	61	124	124
Feeding rate (%/day)	1.64	2.03	1.94	2.11	1.58	1.60	3.14	3.22
Maintenance of energy content of fish body:								
DE (kcal/kg body wt./day)	12.6	13.9	17.8	18.7	14.8	15.7	21.4	25.0
Feeding rate (%/day)	0.31	0.34	0.43	0.44	0.36	0.39	0.52	0.60
Decrease of energy content during starvation = NEm*: (kcal/kg body wt./day)	10.0		16.5		13.3		8.9	

* NEm, maintenance energy.

to those fed DP (Fig. 1). Jeong *et al.*¹¹⁾ reported that carp fed diets containing different levels of potato starch either with or without extrusion processing to satiation revealed a reduction of daily feed consumption and growth linked to an elevation of gelatinized ratios (more than 20–40%) in the diets. This agreed well with the present observation. Digestion and absorption of diets with high gelatinized ratios dragged on, judging from the passage of time in the alteration of blood triglycerides and cholesterol values, and the delayed gastric emptying time seemed to reduce the feed intake. On the other hand, diets containing extruded potato starch were superior in digestibility of starch in their experiments, and differed from this study. This might have been caused by the difference of carbohydrate sources in diets. Similar results were reported by Furuichi *et al.*³²⁾

Rainbow trout (Experiments II, III, and IV): Since the fish size and water temperature were similar, Expt. II on rainbow trout was compared with Expt. I on carp. At the satiation level, rainbow trout were excellent in growth response and feed performance compared to carp fed both the diets (Table 3). The digestibility of each nutrient in rainbow trout was lower than that in carp at the satiation feeding level, especially the starch digestibility in fish fed DP. Unlike carp in rainbow trout fed EP to satiation, reduction of feeding rate, growth, and feed efficiency were not observed. This might be due to a difference of fish species.¹¹⁾

In Expts. II, III, and IV EP tended to be generally lower in the feeding rate and higher in the percent gain and feed efficiency than DP at the satiating levels (Tables 3 and 4). This tendency was conspicuous for the growth of fingerling (Expt. IV) and for the feed efficiency at a low temperature (Expt. III). Fish fed EP at a low temperature showed very good growth response and feed performance. The digestibilities of EP were comparable to those of DP at the satiation feeding levels. The protein digestibility of EP was slightly higher than that of DP (Expt. II: 4.3%, Expt. III:

4.9%, and Expt. IV: 1.7%). The digestibility of lipid revealed hardly any difference between EP and DP. The starch digestibility of EP, similar to several other studies,^{8,10,11,14,17,18,21,28,32)} was distinctly higher from that of DP by 18, 27, and 20% in Expts. II, III, and IV, respectively. This was probably due to the gelatinization of starch in the diets by the extrusion process, leading to increased digestibility.⁸⁾ Although the energy digestibility of EP increased by values of 6, 8, and 4% from that of DP in Expts. II–IV, in relation to the digestibility of starch the differences were not so large because only low levels of carbohydrates were supplemented. These results generally agreed with those reported by Akimoto *et al.*²¹⁾ except for the protein digestibility, which did not differ in the case of identically formulated EP and DP, but differed due to fish meal manufacture methods.

Next, the digestibilities were compared between the three experiments. The digestibility of the different nutrients was higher by 2.3–5.3% in Expt. III (low temperature) and 0.3–5.2% in Expt. IV (fingerlings) than those in Expt. II with the exception of the starch digestibility of DP in Expt. III. However, it is technically difficult to collect feces without any leaching of soluble compounds in fish.³³⁾ Therefore, it may be difficult to assure that the difference in digestibility of 3–5% was significant. The digestibility of each nutrient in rainbow trout fed to satiation was reduced in Expts. II to IV, and hence differed from carp. The decrease of digestibility with the increase of feed intake has been observed in carnivorous fish such as rainbow trout,^{12,34)} brown trout,³⁵⁾ and African catfish.³⁶⁾ This might have been caused by the structural difference in the digestive tract.

Briefly reviewing some of the feeding studies on EP and DP, Hilton *et al.*¹⁹⁾ demonstrated in rainbow trout fed isocontent EP and DP that the former was superior in feed efficiency but inferior in growth. Seymour and Johnsen²⁰⁾ reared Atlantic salmon on EP and DP which contained different materials, and observed that EP was high both in

growth and feed efficiency compared to DP. Akimoto *et al.*²¹⁾ in studies with rainbow trout on the same formulated EP and DP achieved elevated feed efficiency and comparable growth with EP. They noted that the growth and feed efficiency were affected by the quality of fish meal in EP. Wilson and Poe²²⁾ reported that digestibilities were hardly influenced by extrusion processing, when channel catfish received nine kinds of feed ingredients in the form of EP and DP. Pongmaneerat and Watanabe^{13,14)} investigated the effect of extrusion processing on the nutritive value of defatted soybean meal in rainbow trout, and reported that the meal was not affected by extrusion processes, although extrusion pelleting of a feed ingredient mixture containing defatted soybean meal was beneficial. Thus we find that the advantages of EP varied depending on fish species, feed composition, and feed ingredients. Further detailed experiments on EP, examining the extrusion conditions seem to be necessary.

The relationship between DE intake and the percent gain in rainbow trout is shown in Figs. 1 and 2. The DE requirements for maintenance of body weight and activity determined with EP in Expts. II, III, and IV were respectively 10.9, 8.6, and 17.2 kcal/kg body weight/day, corresponding to the GE values of 12.0, 9.5, and 18.8 kcal/kg body weight/day and those determined with DP were 12.5, 9.0, and 18.6 kcal/kg body weight/day, corresponding to the GE values of 13.6, 10.2, and 20.7 kcal/kg body weight/day (Table 5), influenced by fish size as well as water temperature and being a little lower for EP than DP like carp. These values obtained in Expt. II were nearly equal to those of carp (Expt. I) as observed in an earlier experiment.²⁴⁾ In Expts. II, III, and IV the respective DE requirements for maximum growth of fish fed EP were 75, 65, and 124 kcal/kg body weight/day (88, 72, and 141 kcal/kg body weight/day in GE) and those on DP were 77, 61, and 124 kcal/kg body weight/day (97, 74, and 149 kcal/kg body weight/day in GE), depending on the fish size and temperature. These DE values were not affected by the feed preparation methods, although the GE values were slightly higher for DP under the influence of the energy digestibility.

Figure 3 indicates the relation between the DE intake and the feed efficiency in Expts. I–IV. The DE values of EP required to obtain a maximum feed efficiency were approximately 50, 40, and 100 kcal/kg body weight/day and those of DP were 48, 38, and 78 kcal/kg body weight/day from Expts. II to IV, respectively, being lower than those for the maximum growth, similar to carp (Expt. I; EP, 48 kcal; DP, 60 kcal/kg body weight/day) and also as in a former experiment.²⁴⁾ In the case of the high feeding levels in rainbow trout, EP showed higher feed efficiency than DP. This suggested that the gelatinized starch was fully utilized as an energy source, leading to efficient utilization of protein and lipid. The relationship between the DE intake and the PER in Expts. I–IV is shown in Fig. 4. The values of PER tended to be the same as the feed efficiency, especially the dietary protein being efficiently available at low water temperatures in Expt. III.

Energy Requirement for Maintenance of Fish Body Energy Content and Net Energy Value for Maintenance and Activity

The proximate composition of whole body of carp in Expt. I and that of rainbow trout in Expts. II, III, and IV are shown in Tables 6 and 7. The rainbow trout raised at 9°C in Expt. III were lower in body lipid content and higher in body moisture content than those raised at 16°C in Expt. II. These values were not recognized as obvious changes linked to feed production methods, and agreed with the study of Jeong *et al.*¹⁰⁾ in rainbow trout, but different from that in carp.¹¹⁾ Even in the fish accepting enough amounts of energy to satisfy the requirement for maintenance of body weight and activity, the body lipid content decreased with increase of the moisture content in the same way as for rainbow trout²³⁾ and carp²⁴⁾ in the preceding papers. Therefore, Figs. 5 and 6 were made by plotting the energy retention in fish body on the vertical axis and the ME intake on the horizontal axis in all the experiments. The energy value intersecting the horizontal axis (zero point in energy balance of fish body) is equal to the ME requirement for maintenance of energy content of fish body. Furthermore, the energy cost at the zero point on

Fig. 2. Relationship between digestible energy intake and percent gain in rainbow trout fingerlings (5.7 g) fed on the extruded pellet (●—●, EP) or the dry pellet (▲—▲, DP) at 16°C in Expt. IV.

Fig. 3. Relationship between digestible energy intake and feed efficiency in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I, II, III, and IV.

Expt. I: Carp (31 g, 17°C); ●—●, EP; ○—○, DP.
 Expt. II: Rainbow trout (31 g, 16°C); ▲—▲, EP; △—△, DP.
 Expt. III: Rainbow trout (31 g, 9°C); ■—■, EP; □—□, DP.
 Expt. IV: Rainbow trout fingerlings (5.7 g, 16°C); ◆—◆, EP; ◇—◇, DP.

Fig. 4. Relationship between digestible energy intake and PER in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I, II, III, and IV.

Expt. I: Carp; ●—●, EP; ○—○, DP.
 Expt. II: Rainbow trout; ▲—▲, EP; △—△, DP.
 Expt. III: Rainbow trout (9°C); ■—■, EP; □—□, DP.
 Expt. IV: Rainbow trout fingerlings; ◆—◆, EP; ◇—◇, DP.

the vertical axis, which indicates the decrease of fish body energy content during fasting, is estimated to be approximately equal to the net energy value for maintenance and activity (NEM).²³⁾

The DE value for maintenance of energy content of fish body was also obtained graphically on the basis of the relation between the DE intake and the energy retained in fish body, and is indicated in Table 5 together with the NEM value. In Expts. I (carp), II, III, and IV, the DE values of

EP for maintenance of energy content of body weight were 12.6, 17.8, 14.8, and 21.4 kcal/kg body weight/day, the GE values being 14.0, 19.6, 16.3, and 23.4 kcal/kg body weight/day, at the corresponding feeding rates of 0.31, 0.43, 0.36, and 0.52% of body weight/day; the DE values for DP were 13.9, 18.7, 15.7, and 25.0 kcal/kg body weight/day, being 15.6, 20.4, 17.8, and 27.9 kcal/kg body weight/day in GE, at the feeding rates of 0.34, 0.44, 0.39, and 0.60% of body weight/day, respectively, being

higher in rainbow trout than those in carp and indicating a decrease with decrease of water temperature and increase of fish body weight. These values were affected by feed

Table 6. Proximate composition of carp fed on the extruded pellet (EP) or the dry pellet (DP) in Expt. I

Pellet type	Feeding rate (%/day)	Moisture (%)	Crude protein (%)	Crude lipid (%)	Crude ash (%)	Gross energy (kcal/g)
EP	Initial	73.4	15.6	8.1	3.1	1.61
	0	74.0	15.7	7.8	3.6	1.59
	0.41	74.2	15.9	7.9	3.3	1.61
	0.84	73.6	16.3	7.8	3.2	1.73
	1.25	71.2	16.3	9.6	3.1	1.87
	1.57	70.9	15.8	10.2	3.3	1.96
	1.90	73.9	15.6	8.6	3.2	1.96
DP	0.48	74.6	16.4	7.0	3.4	1.57
	0.98	75.2	16.0	8.5	3.8	1.75
	1.46	75.7	15.9	8.5	3.9	1.80
	1.86	72.5	15.9	9.5	3.1	1.83
	2.27	74.4	15.6	8.2	3.1	1.86

production methods, the same way as the energy values for maintenance of body weight and activity (low in EP). The relative NEm values were 10.0, 16.5, 13.3, and 8.9 kcal/kg body weight/day and seemed to be affected by species, temperature, and fish size. However, the results in rainbow trout were contrary to our previous description,²³⁾ the NEm values decreasing in the small size of fish. Takeuchi and Watanabe³⁷⁾ found that starving rainbow trout weighing 28 g at 10–15°C reduced the hepatosomatic index by half during 14 days, but after that the index did not change significantly during the period of 14 to 42 days. This might have been due to the influence of the prolonged experimental period (from 14 to 21 days) on the NEm values by any fish size. This needs further investigation.

Dietary Energy Budgets

Dietary energy budgets at each feeding rate in the four experiments were determined as established previously.^{23,24)} Namely, the energy retention in fish body calculated by the proximate analysis is equal to the productive energy (NEp) value, a part of the net energy (NE) value. The heat

Table 7. Proximate composition of rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. II, III, and IV

	Feeding rate (%/day)	Moisture (%)	Crude protein (%)	Crude lipid (%)	Crude ash (%)	Gross energy (kcal/g)
Expt. II (31 g, 16°C) EP	Initial	74.5	16.9	7.6	2.6	1.60
	0	75.6	17.4	5.8	3.0	1.41
	0.37	75.5	16.8	6.6	2.7	1.52
	0.76	74.8	16.6	7.4	3.3	1.60
	1.10	76.1	16.4	6.7	2.6	1.65
	1.59	74.9	16.5	7.6	2.6	1.67
	2.06	74.9	16.2	7.0	2.7	1.63
DP	0.38	74.4	16.8	6.7	3.1	1.50
	0.76	75.5	16.9	7.1	2.6	1.56
	1.12	75.2	16.4	7.4	3.0	1.55
	1.63	75.5	15.9	7.3	2.7	1.58
	2.31	75.5	15.5	7.9	2.7	1.59
Expt. III (31 g, 9°C) EP	Initial	77.4	16.0	4.8	2.7	1.41
	0	78.2	16.5	4.2	2.7	1.27
	0.20	77.8	16.5	4.3	2.8	1.30
	0.48	78.8	16.4	4.2	2.6	1.34
	0.79	78.8	16.2	4.4	2.5	1.37
	1.14	77.9	16.0	5.7	2.5	1.43
	1.58	78.2	15.9	5.5	2.4	1.48
DP	0.20	78.1	16.6	4.5	2.8	1.26
	0.48	78.8	16.3	4.5	2.6	1.31
	0.80	77.9	16.4	4.6	2.6	1.35
	1.14	79.0	15.7	5.2	2.4	1.34
	1.69	79.0	15.1	4.8	2.4	1.34
Expt. IV (5.7 g, 16°C) EP	Initial	82.0	15.2	3.1	2.9	1.10
	0	80.6	15.6	2.6	3.5	1.07
	0.67	80.8	15.9	3.0	3.1	1.07
	1.32	81.1	15.5	3.8	2.9	1.16
	1.87	80.8	15.5	4.6	2.6	1.22
	2.51	79.4	15.5	5.6	2.7	1.33
	3.14	81.7	15.2	6.5	2.3	1.41
DP	0.67	81.5	15.5	2.7	3.2	1.03
	1.33	80.8	15.4	4.1	3.0	1.08
	1.86	80.2	15.4	4.4	2.7	1.12
	2.71	82.3	14.9	5.0	2.7	1.28
	3.22	79.9	15.1	5.9	2.7	1.36

Fig. 5. Relationship between metabolizable energy intake and energy retention in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I, II, and III.

Expt. I: Carp; ●—●, EP; ○—○, DP.

Expt. II: Rainbow trout; ▲—▲, EP; △—△, DP.

Expt. III: Rainbow trout (9°C); ■—■, EP; □—□, DP.

Fig. 6. Relationship between metabolizable energy intake and energy retention in rainbow trout fingerlings fed on the extruded pellet (●—●, EP) or the dry pellet (▲—▲, DP) in Expt. IV.

increment (HI) can be determined by the following equation:

$$HI = (ME - NEm) - NEp = ME - NE(NEm + NEp)$$

The HI, NE, NEm, and NEp values obtained by this method and the percentage of measured fecal energy (FE) and non-fecal energy values in the GE or ME values are summarized in Tables 8 (Expts. I and II) and 9 (Expts. III and IV). The amount of energy supply which results in negative energy retention can not satisfy the NEm requirement. Thus, these tables show the energy balance only in those fish which increased energy retention (ex., including the fish fed EP at the level of 0.37% of body weight/day in Expt. II). On comparing the ratio of each energy value in ME, the increase of ME involved the decrease of NE value and the increase of HI value in Expts. I, II, and III.

However, in Expt. IV with rainbow trout fingerlings, the pattern contradicted, the NE value increasing while the HI of feeding value decreased. The increase of GE or ME was linked to the decrease of NEm value in all the experiments as reported previously.^{23,24} The proportion of NEp/ME was slightly higher at the satiation feeding level than that at the restricted feeding levels in rainbow trout fed EP at 9°C (Expt. III), which showed good growth response, and in rainbow trout fingerlings fed EP and DP (Expt. IV), which showed high feed performance.

The energy budgets in fish fed enough amounts of energy required for maximum growth (percent gain) are illustrated in Fig. 7. In Expts. I, II, III, and IV the energy partitioning of EP and DP at the GE intake (100%) level required for maximum growth were as follows: i) EP FE: 10.0, 15.2, 9.9, and 12.5%, non-fecal energy: 1.9, 2.7, 2.7,

Table 8. Energy budgets in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I and II

		Feeding rate (% /day)	GE (kcal/kg body wt. /day)	FE/GE (%)	Non-fecal energy /GE (%)	HI/GE (%)	NE/GE (%)	NEm/GE (%)	NEp/GE (%)	HI/ME (%)	NE/ME (%)	NEm/ME (%)	NEp/ME (%)
Expt. I Carp (31 g, 17°C)	EP	0.41	18.5	10.0	4.3	15.7	70.0	53.7	16.3	18.4	81.6	62.6	19.0
		0.84	38.1	10.5	2.5	14.6	72.4	26.1	46.2	16.8	83.2	30.1	53.1
		1.25	56.7	9.5	2.1	15.5	72.9	17.6	55.4	17.5	82.5	19.9	62.6
		1.57	71.7	10.0	1.8	18.7	69.5	13.9	55.6	21.2	78.8	15.8	63.1
		1.90	86.5	10.2	1.9	29.7	58.2	11.5	46.7	33.8	66.2	13.1	53.1
	DP	0.48	21.9	10.8	4.1	30.5	54.6	45.6	9.0	35.9	64.1	53.5	10.6
		0.98	44.9	10.0	2.4	20.5	67.1	22.2	44.9	23.4	76.6	25.3	51.3
		1.46	66.8	10.4	2.2	26.5	60.8	14.9	45.9	30.4	69.6	17.1	52.6
		1.86	85.3	12.0	1.9	29.2	56.9	11.7	45.2	33.9	66.1	13.6	52.5
		2.27	104.3	11.3	2.0	36.9	49.8	9.5	40.3	42.5	57.5	11.0	46.5
Expt. II Rainbow trout (31 g, 16°C)	EP	0.76	34.6	10.1	2.8	6.2	80.9	47.6	33.2	7.2	92.8	54.7	38.2
		1.10	50.3	7.7	2.7	9.1	80.5	32.8	47.7	10.1	89.9	36.6	53.3
		1.59	72.4	10.4	2.8	18.5	68.3	22.8	45.5	21.4	78.6	26.2	52.4
		2.06	93.5	15.2	2.7	27.2	54.8	17.6	37.2	33.2	66.8	21.5	45.4
	DP	0.76	34.8	9.2	2.5	12.3	76.0	47.4	28.6	13.9	86.1	53.7	32.4
		1.12	51.3	10.4	2.4	21.8	65.4	32.1	33.3	25.0	75.0	36.8	38.2
		1.63	74.7	14.4	2.4	25.8	57.3	22.1	35.3	31.0	69.0	26.5	42.4
		2.31	106.0	20.7	1.9	31.5	45.9	15.5	30.3	40.7	59.3	20.1	39.2

Abbreviations: GE, gross energy; FE, fecal energy; HI, heat increment; NE, net energy; NEm, maintenance energy; NEp, productive energy; ME, metabolizable energy.

Table 9. Energy budgets in rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. III and IV

		Feeding rate (% /day)	GE (kcal/kg body wt. /day)	FE/GE (%)	Non-fecal energy /GE (%)	HI/GE (%)	NE/GE (%)	NEm/GE (%)	NEp/GE (%)	HI/ME (%)	NE/ME (%)	NEm/ME (%)	NEp/ME (%)
Expt. III Rainbow trout (31 g, 9°C)	EP	0.48	22.1	9.3	1.4	7.4	81.9	60.1	21.7	8.3	91.7	67.4	24.4
		0.79	36.1	9.9	1.8	12.8	75.6	36.8	38.8	14.4	85.6	41.6	43.9
		1.14	52.0	8.0	2.1	16.9	73.0	25.5	47.4	18.8	81.2	28.4	52.8
		1.58	72.0	9.9	2.7	22.0	65.4	18.4	47.0	25.1	74.9	21.1	53.8
	DP	0.48	22.4	11.9	2.1	11.9	74.1	59.4	14.7	13.8	86.2	69.0	17.1
		0.80	37.0	13.0	2.1	16.5	68.4	35.9	32.4	19.4	80.6	42.3	38.2
		1.14	52.8	12.6	2.2	23.9	61.3	25.1	36.2	28.0	72.0	29.5	42.5
		1.69	78.0	18.0	2.3	31.1	48.6	17.0	31.6	39.0	61.0	21.4	39.6
Expt. IV Rainbow trout fingerlings (5.7 g, 16°C)	EP	0.67	30.2	8.5	4.1	46.8	40.6	29.7	11.0	53.5	46.5	33.9	12.5
		1.32	59.5	8.5	2.6	35.8	53.1	15.0	38.1	40.2	59.8	16.9	42.9
		1.87	84.1	8.6	2.7	35.9	52.8	10.6	42.2	40.5	59.5	12.0	47.5
		2.51	113.0	9.1	2.7	32.7	55.5	7.9	47.6	37.1	62.9	9.0	54.0
		3.14	141.2	12.5	3.3	31.0	53.2	6.3	46.9	36.8	63.2	7.5	55.7
	DP	0.67	31.0	10.3	4.3	53.2	32.3	28.9	3.4	62.3	37.7	33.8	3.9
		1.33	61.4	10.3	2.6	44.2	42.9	14.6	28.3	50.8	49.2	16.7	32.5
		1.86	86.2	10.6	2.6	41.7	45.2	10.4	34.8	48.0	52.0	12.0	40.1
		2.71	125.6	15.3	2.4	36.4	45.9	7.1	38.8	44.2	55.8	8.7	47.1
		3.22	149.1	16.7	3.0	34.5	45.8	6.0	39.8	43.0	57.0	7.5	49.6

Abbreviations: GE, gross energy; FE, fecal energy; HI, heat increment; NE, net energy; NEm, maintenance energy; NEp, productive energy; ME, metabolizable energy.

and 3.3%, HI: 20.5, 24.4, 22.0, and 31.0%, and NE: 67.6, 57.7, 65.4, and 53.2% (NEm: 13.4, 18.7, 18.4, and 6.3%, NEp: 54.2, 39.1, 47.0, and 46.9%); ii) DP FE: 12.0, 20.7, 18.0, and 16.7%, non-fecal energy: 2.0, 2.1, 2.3, and 3.0%, HI: 32.0, 28.8, 29.5, and 34.5%, and NE: 54.0, 48.4, 50.2, and 45.8% (NEm: 10.7, 17.1, 18.0, and 6.0%, NEp: 43.3, 31.4, 32.3, and 39.8%), respectively. When the energy budgets were compared between EP and DP, the FE loss in DP was relatively large among all the tests; the DE and ME values in EP were high in relation to the FE

values. The non-fecal energy losses in rainbow trout were somewhat higher in EP than those in DP in the three experiments. The HI of feeding were high in DP and the proportions of NE, NEm, and NEp increased in EP in the four experiments. These results have proved that, in all the experiments, the energy requirements for maintenance of body weight and activity, and for maintenance of energy content of body weight were lower in EP than those in DP and that, in rainbow trout, the growth and feed efficiency were better in EP. Hilton *et al.*¹⁹⁾ noted that DP passed

Fig. 7. Energy balance pattern at a level required for maximum growth in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I, II, III, and IV.

Expt. I: Carp (31 g, 17°C); ●, EP; ○, DP.

Expt. II: Rainbow trout (31 g, 16°C); ▲, EP; △, DP.

Expt. III: Rainbow trout (31 g, 9°C); ■, EP; □, DP.

Expt. IV: Rainbow trout fingerlings (5.7 g, 16°C); ◆, EP; ◇, DP.

through the stomach in a much shorter period of time than did EP in rainbow trout (13 h to empty half vs. more than 22 h), and the delayed gastric emptying time might be responsible for the reduction in daily feed consumption and the improved feed efficiency in EP. The emptying time of digestive organs has been observed to be influenced by the composition of diet in rainbow trout^{38,39,40} and tilapia.⁴¹ Studies hereafter should examine the digestive velocity of diets too.

Furthermore, comparing the energy budgets from the four experiments, the FE loss in rainbow trout fed EP at 9°C (Expt. III) was the lowest and was comparable to that of those fed EP at 16°C (Expt. II). The HI of feeding was more than 30% in rainbow trout fingerlings fed EP and DP and carp fed DP. On the other hand, the percentage of NEp was elevated to more than 45% in carp fed EP, and for trout fed EP at 9°C or their fingerlings fed EP at 16°C. The proportion of non-fecal energy/GE was not affected by water temperature, as noted in a previous study.²⁹ The HI of feeding constituted a relatively large portion, 20–35%, although values were relatively lower compared to earlier observations (32–39%).^{23,24} On comparing our data with those reported by Brett and Groves⁴² and Cho,⁴³ generally, the ratio of non-fecal energy losses was higher and that of HI was lower than in the present experiment.

Non-fecal Energy Loss

The non-fecal energy losses through the gills and in the urine together with the ME contents of diets are given in Tables 10 (Expts. I and II) and 11 (Expts. III and IV), which were measured at 18.0, 16.5, 10.5, and 17.2°C from Expts. I to IV, respectively. The non-fecal energy loss in fasting fish was higher in carp than that in rainbow trout, and for the latter it decreased proportionally with temperature and inversely with fish body weight. The ratio of non-fecal nitrogen (N)/N intake was identical in carp fed EP or DP to satiation. But, the ratio was higher in EP than that in DP in the three experiments with rainbow trout, which is probably related to the difference in protein utilization between EP and DP. This tendency was the same as that of the non-fecal energy/DE rate, although the differ-

ence was small. In Expts. II and IV the non-fecal energy/DE rate at the satiation feeding level did not depend on fish size in rainbow trout, unlike in carp.^{24,31} Consequently, the proportion of ME/DE at the satiating level ranged between 97.0 to 97.7% (97.4% on average) in all the tests, being independent of the feed preparation methods, fish species, water temperature, and fish size.

Figures 8 and 9 indicate the relationship between the DE intake and the non-fecal energy losses in Expts. I and II, and III and IV, respectively. These energy losses are shown as total non-fecal energy losses, without considering the endogenous nitrogen excretion (ENE) value. The non-fecal energy losses rose proportionally to the increase of DE intake because of the increase of N intake as described previously,^{23,24} it being quite significant in Expt. IV with fingerlings at high feeding rates (Fig. 9). Figure 10 presents the relationship between the non-fecal energy/DE rate and the DE intake. The non-fecal energy losses were calculated using the value of Ogino *et al.*²⁵ in carp and that obtained in a former experiment²⁹ for rainbow trout, as the ENE value. The tendency of the non-fecal energy/DE rate was almost the same as in the preceding report,²⁴ suggesting that tissue proteins were utilized to yield energy at the low feeding levels. The DE intake at which the non-fecal energy/DE ratio stabilized differed from the preceding report, the value being higher than that for maintenance of energy content of body weight in all the experiments, and seemed to be affected by the diets.²³ In the case of rainbow trout fed EP at 9°C (Expt. III), the non-fecal energy/DE rate showed a unique pattern,^{23,24} the rate increasing inevitably corresponding to the DE values.

Protein Requirement for Maintenance of Fish Body Protein

In Expts. I–IV the relationship between the nitrogen balance and the amount of nitrogen absorbed determined by the methods given elsewhere²³ is shown in Fig. 11. The amount of nitrogen absorption where the nitrogen balance becomes zero is equal to the nitrogen requirement for maintenance of nitrogen content of fish body. At the zero point of nitrogen absorption, the sum of metabolic fecal

Table 10. Non-fecal energy loss and metabolizable energy contents in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I and II

		Total body weight (g)	Feeding rate (%/day)	Non-fecal energy (kcal/kg body wt./day)	Non-fecal N /N intake (%)	Non-fecal energy/DE (%)	ME (kcal/g diet)	ME/GE (%)	ME/DE (%)
Expt. I	EP Carp (31 g, 17°C)	328.6	0	1.13	—	—	—	—	—
		401.4	0.38	0.80* ¹	44.8	5.1	3.89	85.4	94.9
		455.6	0.80	0.97	25.9	3.0	3.95	86.8	97.0
		496.0	1.17	1.21	22.2	2.5	4.02	88.3	97.5
		528.0	1.26	1.28	21.8	2.5	3.99	87.8	97.5
		528.5	1.67	1.68	21.6	2.5	3.99	87.6	97.5
	DP	391.1	0.48	0.90	39.3	4.6	3.91	85.2	95.4
		467.1	1.00	1.06	22.2	2.6	4.02	87.7	97.4
		506.4	1.47	1.47	20.9	2.4	4.01	87.4	97.6
		547.5	1.77	1.64	19.4	2.3	3.95	86.0	97.7
		569.8	2.01	2.08	21.7	2.5	3.97	86.4	97.5
Expt. II	EP Rainbow trout (31 g, 16°C)	393.8	0	0.53	—	—	—	—	—
		457.9	0.38	0.52* ²	29.4	3.3	4.00	88.0	96.7
		507.0	0.80	0.98	26.2	3.0	3.97	87.2	97.0
		578.5	1.14	1.34	25.2	2.8	4.08	89.7	97.2
		645.5	1.70	2.00	25.2	2.9	3.96	87.0	97.1
		669.7	2.35	2.55	23.3	2.8	3.75	82.4	97.2
	DP	450.6	0.39	0.57	30.6	3.5	4.07	88.7	96.5
		516.4	0.79	0.88	23.1	2.6	4.06	88.4	97.4
		565.0	1.16	1.23	22.2	2.6	4.01	87.3	97.4
		635.4	1.74	1.81	21.8	2.7	3.82	83.3	97.3
		671.4	2.31	2.05	18.7	2.4	3.55	77.3	97.6

*¹ The amount of endogenous nitrogen excretion was calculated as 6.8 mg/100 g body weight/day at 18.0°C.²⁹⁾*² The amount of endogenous nitrogen excretion was calculated as 8.7 mg/100 g body weight/day at 16.5°C.²⁹⁾**Table 11.** Non-fecal energy loss and metabolizable energy contents in rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. III and IV

		Total body weight (g)	Feeding rate (%/day)	Non-fecal energy (kcal/kg body wt./day)	Non-fecal N /N intake (%)	Non-fecal energy/DE (%)	ME (kcal/g diet)	ME/GE (%)	ME/DE (%)
Expt. III	EP Rainbow trout (31 g, 9°C)	387.1	0	0.36	—	—	—	—	—
		426.4	0.20	0.11* ¹	11.7	1.3	4.08	89.5	98.7
		484.8	0.49	0.31	13.7	1.6	4.07	89.3	98.5
		547.0	0.83	0.64	16.5	1.9	4.03	88.4	98.1
		612.6	1.16	1.10	20.3	2.3	4.10	90.0	97.7
		662.9	1.71	1.94	24.3	2.8	4.00	87.6	97.2
	DP	425.6	0.20	0.23	24.5	2.8	3.96	85.6	97.2
		485.4	0.49	0.46	20.1	2.3	3.98	86.0	97.7
		536.1	0.84	0.79	19.8	2.3	3.93	85.0	97.7
		606.8	1.17	1.14	20.9	2.4	3.94	85.2	97.6
		651.7	2.00	1.77	18.8	2.3	3.70	80.1	97.7
Expt. IV	EP Rainbow trout fingerlings (5.7 g, 16°C)	211.3	0	1.29	—	—	—	—	—
		232.5	0.64	1.23* ²	41.8	4.7	3.93	87.2	95.3
		325.7	1.28	1.54	26.1	2.9	4.00	88.8	97.1
		394.1	1.84	2.24	26.5	3.0	3.99	88.7	97.0
		485.5	2.50	3.01	26.2	2.9	3.97	88.2	97.1
		594.7	3.88	4.63	26.1	3.0	3.82	84.9	97.0
	DP	231.1	0.64	1.33	44.0	5.0	3.95	85.2	95.0
		324.8	1.29	1.61	26.6	3.0	4.03	87.0	97.0
		388.7	1.84	2.26	26.1	3.0	4.02	86.8	97.0
		469.5	2.67	2.99	23.8	2.9	3.81	82.3	97.1
		569.4	4.32	4.49	22.1	2.7	3.75	81.1	97.3

*¹ The amount of endogenous nitrogen excretion was calculated as 4.0 mg/100 g body weight/day at 10.5°C.²⁹⁾*² The amount of endogenous nitrogen excretion was calculated as 9.3 mg/100 g body weight/day at 17.2°C.²⁹⁾

Fig. 8. Relationship between digestible energy intake and non-fecal energy loss in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I and II.

Expt. I: Carp (31 g, 17°C); ●—●, EP; ○—○, DP.

Expt. II: Rainbow trout (31 g, 16°C); ▲—▲, EP; △—△, DP.

Fig. 9. Relationship between digestible energy intake and non-fecal energy loss in rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. III and IV.

Expt. III: Rainbow trout (31 g, 9°C); ■—■, EP; □—□, DP.

Expt. IV: Rainbow trout fingerlings (5.7 g, 16°C); ◆—◆, EP; ◇—◇, DP.

and endogenous nitrogen (carp: 14 mgN, rainbow trout: 9.5 mgN/100 g body weight/day) in fish fed a protein-free diet obtained by Ogino *et al.*⁴⁴⁾ was used for the starved fish. The absorbed nitrogen values for maintenance of nitrogen content of fish body were not different between EP and DP. These values were 17.5, 14.4, 8.3, and 19.3 mg, (109, 90, 52, and 121 mg protein absorption), corresponding to nitrogen intake of 18.8, 15.2, 8.8, and 20.3 mg/100 g body wt./day in Expts. I, II, III, and IV, respectively. Figure 11 reveals that although the difference related to fish species and size was little, the effect of temperature in rainbow trout was the most profound. Ogino⁴⁵⁾ measured the nitrogen balance in carp weighing 87–419 g fed a casein diet at 20 and 23°C, and reported that the nitrogen intake for the maintenance was 16–17 mg/100 g

body weight/day. Ohta and Watanabe²⁴⁾ also measured the nitrogen balance in carp weighing 4–243 g fed a casein diet and a fish meal diet at 22–27°C, the values being 19–23 mg/100 g body weight/day. The present data in carp is an intermediate value (18.8 mg) between those two studies. The nitrogen intake for maintenance of nitrogen content of fish body in rainbow trout was relatively higher than that in an earlier experiment (8.6 mg/100 g body weight/day).²³⁾ The nitrogen absorption value for a maximum nitrogen balance was obtained at satiation, greatly affected by water temperature and fish size. The present study has also demonstrated that the nitrogen balance at the satiation levels of DP was higher than that of EP, affected by the nitrogen intake and the non-fecal nitrogen excretion values.

Fig. 10. Effect of digestible energy intake on non-fecal energy loss in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I, II, III, and IV.

The amount of endogenous nitrogen excretion was calculated as 6.8, 8.7, 4.0, and 9.3 mg/100 g body wt./day in Expts. I-IV, respectively.

Expt. I: Carp; ●—●, EP; ○—○, DP. Expt. II: Rainbow trout; ▲—▲, EP; △—△, DP.

Expt. III: Rainbow trout (9°C); ■—■, EP; □—□, DP.

Expt. IV: Rainbow trout fingerlings; ◆—◆, EP; ◇—◇, DP.

Fig. 11. Relationship between nitrogen absorbed and nitrogen balance in carp and rainbow trout fed on the extruded pellets (EP) or the dry pellets (DP) in Expts. I, II, III, and IV.

Expt. I: Carp (31 g, 17°C); ●, EP; ○, DP.

Expt. II: Rainbow trout (31 g, 16°C); ▲, EP; △, DP.

Expt. III: Rainbow trout (31 g, 9°C); ■, EP; □, DP.

Expt. IV: Rainbow trout fingerlings (5.7 g, 16°C); ◆, EP; ◇, DP.

Conclusion

Four experiments were arranged by changing the feed production methods, species (carp and rainbow trout), size of fish and water temperature in rainbow trout. Fish received the same formulated ordinary DP produced by a pellet mill and EP by a twin-screw extruder. The DE requirement for maximum growth was lower in carp fed EP, indicating an effect of feed preparation methods. The DE requirements for maintenance of body weight and activity in rainbow trout were influenced by fish size as well as

water temperature, being slightly lower for EP than DP like in carp. However, although the DE requirements for maximum growth of rainbow trout depended on the fish size and temperature, these DE values were not affected by the feed preparation methods. In carp, rainbow trout, rainbow trout reared at low water temperatures, and rainbow trout fingerlings, the energy partitioning of EP and DP at the GE intake level required for maximum growth was determined. The proportion of ME/DE at the satiating level ranged between 97.0 to 97.7% (97.4% on average) in all

the tests, being independent of the feed preparation methods, fish species, water temperature, and fish size.

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