Feed consumption, growth and growth efficiency of rainbow trout (*Oncorhynchus mykiss* (Walbaum)) fed on diets containing a bacterial single-cell protein

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The aim of the present study was to compare the nutritive value of bacterial single-cell protein (BSCP) with that of fishmeal in rainbow trout (*Oncorhynchus mykiss* (Walbaum)). Four diets were formulated to contain a total of 458 g crude protein/kg of which 0% was from BSCP in diet 1 (BSCP-0), 25% in diet 2 (BSCP-25), 62·5% in diet 3 (BSCP-62·5) and 100% in diet 4 (BSCP-100); the remainder of the protein was from fishmeal. There were two studies: in study 1, duplicate groups of twenty-five fish were fed on one of the four experimental diets at the rate of 20 g/kg body weight per d for 132 d. Feed consumption rates of individual fish were measured using radiography and the overall apparent absorption efficiency for N in each group was measured over a 2-week period. In study 2, N intake, consumption, absorption and accretion were measured for each fish under controlled environmental conditions (12 h:12 h light–dark regime; 14°). Higher dietary levels of BSCP resulted in significantly higher feed consumption rates but reduced N absorption efficiency and growth rates. However, a diet containing 25% BSCP (75% fishmeal) did not significantly influence growth rates, feed consumption and absorption efficiency compared with a 100% fishmeal diet. The N growth efficiencies were highest in fish fed on the diet containing the highest level of fishmeal and significantly decreased with increasing BSCP content. Construction of N budgets demonstrated that the reduction in growth in fish eating an increasingly larger proportion of BSCP was due to a decrease in N absorption and an increase in the excretion of urea.

Bacterial single-cell protein: Growth: N balance: Rainbow trout

Fishmeal is the main protein source in rainbow trout (*Oncorhynchus mykiss* (Walbaum)) feeds and usually accounts for more than 50% of the total protein (Kaushik & Luquet, 1980; Grant, 1989; Pike *et al.* 1990; Kiessling & Askbrandt, 1993). In recent years there have been many attempts to replace the fishmeal component of fish feeds with alternative protein sources, for example single-cell proteins (SCP) from yeasts, algae and bacteria have been added to fish feeds with varying degrees of success (reviewed by Tacon & Jackson, 1985). Some studies have reported poor growth of fish fed on diets containing SCP and this has been attributed to high levels of nucleic acids (Tacon & Jackson, 1985; Rumsey *et al.* 1992), deficiency in one or more essential amino acids (Tacon & Jackson, 1985) or poor absorption efficiency (Murray & Marchant, 1986; Rumsey *et al.* 1991*a*). A further reason for the poor growth of fish fed on diets containing SCP may be a reduction in feed intake with increasing dietary SCP content (Tacon & Jackson, 1985; Rumsey *et al.* 1990, 1991*b*). Until recently the consumption rates of fish held in groups during nutrition trials have rarely been measured. However, this has now been made possible by the use of radiography

(Talbot & Higgins, 1983; Carter *et al.* 1992, 1994*a*). The measurement of feed consumption rates of individual fish in nutritional trials is important because effects on feed consumption as a result of manipulation of dietary composition can be separated from the effects of the quantity of diet consumed.

The primary aim of the present study was to evaluate the effects of diets containing bacterial SCP (BSCP) on feed consumption, absorption efficiency, growth and growth efficiency of rainbow trout. To achieve this, four diets containing different proportions of BSCP and fishmeal were fed to juvenile rainbow trout. Consumption rates were measured using radiography. Apparent absorption efficiency was measured after faecal stripping using Cr_2O_3 as an inert marker (Austreng, 1978). As the study developed it became clear that information on N balances would be needed to explain the results. Therefore, in addition to the above variables excreted N (ammonia and urea) was measured in individual fish and the effects of the different diets described in terms of N budgets.

MATERIALS AND METHODS

Four diets were formulated to be isonitrogenous and isoenergetic (Table 1). The BSCP content of the protein in diet 1 (BSCP-0) was 0%, 25% in diet 2 (BSCP-25), 62·5% in diet 3 (BSCP-62·5) and 100% in diet 4 (BSCP-100) and the remaining protein was from fishmeal (Table 1). To balance the different oil contents of fishmeal and BSCP, different quantities of fish oil were added to the diets (Table 1). Low temperature fishmeal and fish oil were supplied by United Fish Products, Aberdeen. The BSCP was supplied by Sigma Biotechnology Limited, London. Carboxymethylcellulose (CMC) and α-cellulose were supplied by ICN, High Wycombe, Bucks. The vitamin and mineral mixes were formulated as described by the National Research Council (1981). Pellets with a 2·4 mm diameter were prepared with a California pellet mill and air-dried at room temperature for 2 d. All the diets were stored at 4°. Triplicate samples of the freeze-dried diets were analysed for ash (Association of Official Analytical Chemists, 1975), C and N (Carter *et al.* 1992), acid-insoluble ash (AIA; Bowen, 1981) and gross energy content (Gallenkamp Ballistic bomb calorimeter calibrated with benzoic acid).

Study 1

Rainbow trout (*n* 200) were brought to the Department of Zoology from Almondbank trout farm and divided at random into eight groups of twenty-five fish per tank. Outdoor circular tanks (350 litres) were supplied with a continual flow of aerated fresh water at a rate of 225 litres/h. The fish were acclimated to the experimental conditions for 14 d before feeding the experimental diets. During this period they were fed on a commercial diet (Aqualine Trout Starter, North Eastern Farmers, Aberdeen) to satiation twice daily. After feed deprivation for 2 d the fish were anaesthetized (MS 222, 0·1 g/l) blotted dry and weighed (8·5 (SE 0·1) g). To identify individual fish, each was marked with alcian blue dots on the ventral surface applied using a panjet (Hart & Pitcher, 1969). At the same time, twenty fish were placed in another tank and left without feed for 14 d. A further ten fish were selected as an initial control and were killed, weighed and frozen at −70° until analysed. The experiment lasted for 132 d from 20 May to 28 September 1992. The fish were exposed to the natural variation in photoperiod and temperature. Day length increased from 15 h to 19 h and the mean water temperature was 14·1 (SE 1·2)° (*n* 132) with a range of 10·5 to 15·5°.

Duplicate groups of twenty-five fish were fed on one of the four experimental diets. The groups of fish were hand-fed a daily ration of 20 g/kg body weight (BW) per d. This ration

Table 1. *Ingredients (g/kg) and basic composition (g/kg dry weight) of the diets fed to rainbow trout* (Oncorhynchus mykiss)

	Diets							
	BSCP-0		BSCP-25		BSCP-62·5		BSCP-100	
Ingredients								
Fishmeal	695		521		260		—	
BSCP	—		174		435		695	
Fish oil	110		100		85		70	
α-Cellulose	104·5		114·5		129·5		144·5	
CMC	50		50		50		50	
Vitamin mix*	10		10		10		10	
Mineral mix†	30		30		30		30	
BHA‡	0·5		0·5		0·5		0·5	
Composition	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Nitrogen	79	2	77	1	79	2	78	1
Carbon	468	4	462	3	479	4	496	1
Ash	162	1	139	1	100	1	67	1
AIA	112	2	103	1	76	1	38	1
Gross energy (kJ/g dry wt)	208	1	213	1	221	1	216	1

BSCP, bacterial single-cell protein; CMC, carboxymethylcellulose; AIA, acid-insoluble ash.

* Composition of mix (g/kg): retinal 0·5, cholecalciferol 0·48, α-tocopherol 25·0, menadione 1·0, ascorbic acid 100·0, thiamin HCL 1·0, riboflavin 2·0, pyridoxine HCL 1·2, panthothenic acid 4·4, nicotinic acid 15·0, biotin 0·1, pteroylglutamic acid 0·5, cyanocobalamin 4·0, myoinositol 40·0, cellulose 804·82, choline chloride 10 ml (400 g/l).

† Composition of mix (g/kg): $CaCO_3$ 17·75, $CaH_4(PO_4)_2.H_2O$ 416·6, K_2HPO_4 206·0, $NaH_2PO_4.2H_2O$ 130·0, NaCl 66·4, KCl 50·0, $MgCO_3$ 91·0, $FeSO_4.7H_2O$ 30·0, $ZnSO_4.7H_2O$ 4·0, $CuSO_4.5H_2O$ 1·0, $MnSO_4.4H_2O$ 3·6, KI 0·2, $CoSO_4.7H_2O$ 1·0.

‡ 2(3)-*tert*-butyl-4-hydroxyanisole.

was fed at hourly intervals five times between 09.00 and 13.00 hours. Each ration level was calculated as a percentage of the mean wet BW for each group and adjusted after radiography and weighing of the fish (see below).

After the completion of the experiment, the fish were fasted for 2 d. They were then killed by a sharp blow to the head, blotted dry with tissue paper and weighed. The whole bodies were immediately frozen in liquid N_2 and stored at −70° until carcass analysis (see below).

Measurement of individual consumption rates

The feed consumption rates of individual fish were determined on five occasions (days 15, 34, 56, 77 and 98) using radiography (details as in Carter *et al.* 1992). The rate of feed consumption of each fish was expressed as mg 'dry' feed (mean water content, 79 (SE 1·2) g/kg, *n* 12)/g wet weight per d. Fractional rate of N consumption ($k_{c(N)}$, % per d) was calculated as the N consumed as a percentage of the estimated initial N content of the fish (Carter *et al.* 1993).

Carcass analysis

The carcasses of the initial group, experimental and starving fish were individually freeze-dried to a constant weight. The dried carcasses were then ground to fine particles using a Virtis grinder, sieved (mesh 1·0 mm) and triplicate samples analysed for N after being redried (Carter *et al.* 1992).

Analysis of growth and growth efficiency

The specific growth rates of fish were calculated in terms of wet weight and N content. Specific growth rate (SGR) for individual fish was calculated as:

$$\text{SGR } (\%/\text{d}) = 100(\log_e W_f - \log_e W_o)/t$$

(Ricker, 1979), where W_o and W_f are initial and final wet weight (g) and t is the length of the experiment (132 d). The initial N content of each experimental fish was calculated using its initial wet weight and the mean N composition of the initial group. The final N content of each experimental fish was measured directly and the protein content calculated from the N content using a factor of 5·85 (Gnaiger & Bitterlich, 1984). SGR for N (SGR_N) was calculated as above, using these measurements (Wootton, 1990).

The relationships between rate of feed consumption and SGR and between fractional rate of N consumption and SGR_N were described by:

$$\log_e(\text{SGR}+1) = a + b\log_e(\text{Cm}+1)$$

(Christiansen & Jobling, 1990), where SGR is the specific growth rate (wet weight) and Cm the mean weight-specific feed consumption for each fish, and

$$\text{SGR}_N = a + b\,k_{c(N)},$$

where SGR_N is the specific growth rate for N and $k_{c(N)}$ the fractional rate of N consumption for each fish. Data for the first model were transformed by the addition of 1 in order to include fish with zero consumption rates and negative growth rates. Unfed fish were included in order to weight the regressions for all diets so that the intercept values were below zero.

N growth efficiency (GE_N) was calculated as:

$$\text{GE}_N(\%) = (\text{SGR}_N/k_{c(N)})100$$

(Carter *et al.* 1992), where SGR_N (%/d) is the SGR for N and $k_{c(N)}$ (%/d) is the fractional rate of N consumption.

Study 1: nitrogen losses

Beginning on the morning before the initial feeding, the water flow was stopped, duplicate water samples were taken from each tank and the NH_3 concentration measured (see below). Aeration was maintained in all tanks; it was found that aeration had no effect on the NH_3 concentration in separate tanks spiked with NH_4Cl. At the end of 12 h, duplicate water samples were taken and NH_3 concentration determined. Day-time measurements were made between 08.00 and 20.00 hours and night-time measurements between 20.00 and 08.00 hours. Measurements of NH_3 concentration were made on six occasions, three days and three nights, and day-time and night-time values were then used to estimate the daily rates of NH_3 excretion. At the end of the determination the fish were removed from the tank, anaesthetized and weighed. Rates of the NH_3-N excretion were calculated as μmol N/g wet weight per d and as a percentage of the consumed N (C_N).

Apparent N absorption efficiency was measured during the final 3 weeks of the study. Three days before measurement (Lied *et al.* 1982) the fish were fed with diets containing Cr_2O_3 (10 g/kg; Cr_2O_3 diets were prepared from each of the experimental diets). At 3 h after the daily feeding, fish were anaesthetized and faecal samples collected by stripping (Austreng, 1978). The samples from each group were pooled, freeze-dried to a constant weight, ground for analysis and sub-samples of the dried faecal material analysed for total N using an elemental analyser (Carter *et al.* 1992) and for Cr_2O_3 using the method of

Furukawa & Tsukahara (1966). The apparent N absorption efficiency (AE_N) was calculated as:

$$AE_N(\%) = 100 - (100 \times ((\%\ M_{feed}/\%\ M_{faeces}) \times (\%\ N_{faeces}/\%\ N_{feed})))$$

(Maynard & Loosli, 1969), where M is the Cr_2O_3 in the feed and faeces and N is the nitrogen concentration in the feed and faeces.

Study 2: individual fish

Four rainbow trout (56 (SE 2·26) g) were used in this experiment. They were placed individually in plastic aquaria (15 litres) supplied with a continuous flow of aerated fresh water at a rate of 7 litres/h. Fish were exposed to a 12 h light–12 h dark photoperiod and the water temperature was maintained at 14°. The fish were acclimated to the experimental conditions for 14 d before feeding the experimental diets. During this period fish were fed on the same commercial diet in the same way as the fish in study 1. After feed deprivation for 2 d, fish were fed on one experimental diet at a daily ration of 10 g/kg body weight per d given at two feedings each day. On the seventh day, water samples were collected and analysed as described below.

Study 2: nitrogen losses of individual fish

Beginning in the morning before the initial feeding, the water flow was stopped and duplicate water samples (50 ml) were taken from each tank. Aeration was maintained in all tanks over this period. After feeding the appropriate diet for a 24 h period, the water was stirred thoroughly and duplicate 50 ml samples were taken from each tank. All the samples were stored at −20° until analysis.

Measurements of NH_3 and urea concentrations were made (see below) on two separate days and determinations were continued in the same manner for the other dietary groups. At the termination of the determination for one diet, before commencement of the next diet, fish were adjusted to normal conditions while feeding on commercial diet for 1 week (Beamish & Thomas, 1984; Jayaram & Beamish, 1992). Rates of the excretion of N were measured as NH_3, urea and total N expressed as μmol N/g wet weight per d; urea excretion was expressed as a percentage of total N excretion and total N excretion expressed as a percentage of C_N.

Analysis of ammonia and urea

NH_3 was determined with an NH_3 electrode (Unicam, Cambridge) (Carter & Brafield, 1991; Carter *et al.* 1994*b*). Urea was determined as described by Carter & Brafield (1991) except that the urease solution was 100 units urease (*EC* 3 . 5 . 1 . 5; Sigma U-4002)/ml 0·5 M-sodium citrate (pH 7·0). Rates of NH_3-N and urea-N excretion were expressed as μmol N/g wet weight per d and total N excretion was defined as the sum of N excreted as NH_3 and urea (Carter & Brafield, 1991).

Nitrogen budget

Using the results from the two studies, N budgets were constructed for each diet as

$$100\%\ C_N = GE_N\% + F_N\% + U_N\%$$

(Carter & Brafield, 1992), where C_N is consumed N, GE_N is retained N (%), F_N is faecal N (%) from study 1 and U_N is excreted NH_3 and urea N (%) from study 2.

Statistical analysis

All means are given with their standard errors (SE). Statistical comparisons between the groups were made using analysis of variance (ANOVA). Significance was accepted at a

probability of 5% or less. Where ANOVA indicated a significant difference, Scheffe's multiple comparison was used to identify the means which were significantly different from each other (Zar, 1984). Analysis of covariance (ANCOVA) was used to compare the slope (b) and elevation; a is the intercept and the elevation relates to mean x and mean y coefficients in the consumption–growth regression lines (Zar, 1984). The coefficient of variation (CV, %) was calculated for tank data to examine the inter-individual variability around the tank mean. The CV expresses variability relative to the group mean and its size does not depend on the size of the mean (Zar, 1984). The feed consumption rates of individual fish varied from day to day and the CV of feed consumption (mg/g per d), was used to measure the intra-individual variation in feed intake and termed the coefficient of variation of consumption, CV_c (McCarthy *et al.* 1992).

Some deaths occured. These were at the start of the experiment. There were no significant differences in mortality between the tanks. All data presented refer only to fish which survived the length of the experiment.

RESULTS

Consumption and growth

There were no significant differences (two sample t test) in the mean values for W_o, W_f, Cm and SGR for the fish in duplicate tanks and the data were pooled for each diet. The final mean weight of the BSCP-0 group was the highest and final mean weights decreased with increasing content of BSCP in the diet (Table 2). The CV of the final weights was highest in the BSCP-100 group (Table 2).

Mean feed consumption rates (mg/g per d) were not significantly different between BSCP-0 and BSCP-25, BSCP-25 and BSCP-100 or BSCP-62·5 and BSCP-100 but higher consumption rates were found in fish fed with high levels of BSCP compared with the 100% fishmeal diet (Table 2). Variability in feed consumption (mg/g per d) was found between fish in every group (results not shown). The mean CV_c was significantly higher in fish fed on the BSCP-100 diet compared with the fish fed on the other diets (Table 2).

All the fish in different dietary groups showed positive growth over the experimental period; the BSCP-0 group grew fastest whilst the BSCP-100 group grew slowest and BSCP-25 and BSCP-62·5 were intermediate (Table 2). The mean SGR and SGR_N of the fish fed on the BSCP-0 and BSCP-25 diets were significantly higher compared with those of the fish fed on the BSCP-62·5 and BSCP-100 diets (Table 2). The greatest variation was found in the BSCP-100 diet group where the inter-fish CV for SGR was 42·37% compared with 8·94, 12·27 and 15·02% for the BSCP-0, BSCP-25 and BSCP-62·5 groups respectively. The N concentrations of the whole bodies (mg N/g dry weight) were not significantly different between the different dietary groups at the end of the experiment but the values (Table 2) were lower than the initial N concentrations (106·8 (SE 1·08) mg N/g dry weight, n 6).

The results of regression analyses of the relationships between SGR and Cm, SGR_N and $k_{c(N)}$ for each of the four diets are presented in Tables 3 and 4. The log-linear and linear models explained between 74 to 82% and 62 to 82% of the variation in SGR respectively. ANCOVA demonstrated that the slope of the SGR-Cm regression was significantly lower for fish fed on diet BSCP-100 compared with the slope from the BSCP-0 diet and that the elevation of the BSCP-100 line was significantly lower than the elevations of the lines from the other three dietary groups. The slopes of the SGR_N–$k_{c(N)}$ regressions were significantly lower for the BSCP-62·5 and BSCP-100 diets compared with the BSCP-0 diet (Table 4), and the elevation of the regression line from the BSCP-100 diet was significantly lower than those from the BSCP-0 and BSCP-25 diets (results not shown).

Table 2. *Initial and final number, initial and final wet weight (g) of experimental fish, feed consumption (mg/g per d), specific growth rate for wet weight (% per d) and nitrogen (% per d) and carcass nitrogen (mg N/g dry weight) of rainbow trout* (Oncorhynchus mykiss) *fed for 132 d on four different diets**

(Mean values with their standard errors)

	Diet							
	BSCP-0		BSCP-25		BSCP-62·5		BSCP-100	
Variables	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Number of fish								
Initial	50		50		50		50	
Final	37		43		43		40	
Wet weight								
Initial	8·85[a]	0·31	8·23[a]	0·22	8·12[a]	0·23	9·02[a]	0·27
(CV)	(21·1)		(17·6)		(18·7)		(18·6)	
Final	50·21[a]	1·39	44·14[b]	1·14	33·43[c]	1·10	17·23[d]	0·89
(CV)	(16·8)		(16·9)		(21·6)		(32·6)	
Feed consumption	13·24[c]	0·45	14·90[cb]	0·61	17·38[a]	0·59	16·75[ab]	0·69
(CV)	(40·1[b])	(2·4)	(42·3[b])	(2·9)	(42·8[b])	(3·5)	(55·1[a])	(3·0)
Specific growth rate								
Wet weight	1·32[a]	0·02	1·27[a]	0·02	1·06[b]	0·02	0·51[c]	0·03
Nitrogen	1·41[a]	0·04	1·26[a]	0·05	1·06[b]	0·05	0·48[c]	0·05
Carcass nitrogen	89·37[a]	1·53	86·97[a]	0·86	86·26[a]	1·36	90·22[a]	1·88

CV, coefficient of variation; BSCP, bacterial single-cell protein.
[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and pp. 592–594.

Table 3. *The relationship between specific growth rate (SGR, % per d) and feed consumption (Cm, mg/g per d) in four groups of rainbow trout* (Oncorhynchus mykiss) *fed on diets containing different levels of bacterial single-cell protein (BSCP)*†

Diet	*a*	(SE)	*b*	(SE)	R^2	*F*	*n*	*P*
BSCP-0	−1·339	(0·120)	0·822	(0·054)	0·820	233·16	52	< 0·001
BSCP-25	−1·312	(0·118)	0·772	(0·050)	0·806	237·56	58	< 0·001
BSCP-62·5	−1·334	(0·115)	0·707	(0·046)	0·804	234·53	58	< 0·001
BSCP-100	−1·335	(0·120)	0·610	(0·050)	0·738	150·62	54	< 0·001

* For details of diets, see Table 1.
† Model in the form $\ln(SGR+1) = a + b \ln(Cm+1)$.

Nitrogen absorption, growth efficiency and excretion

The absorption efficiency of N decreased with increasing dietary BSCP content of the diets but no significant differences occurred between the BSCP-0 and BSCP-25 or BSCP-62·5 and BSCP-25 groups (Table 5). However, N absorption efficiency was 13% lower in the 100% BSCP diet compared with the 100% fishmeal diet and was significantly lower in the BSCP-100 diet compared with the other three diets. Although the N growth efficiency was positive in fish fed on the different diets, it was significantly decreased with increasing BSCP content (Table 5).

Table 4. *The relationship between specific growth rate for nitrogen (SGR_N, % per d) and fractional rate of nitrogen consumption ($k_{c(N)}$, % per d) in four groups of rainbow trout* (Oncorhynchus mykiss) *fed on diets containing different levels of bacterial single-cell protein (BSCP)**†

Diets	*a*	(SE)	*b*	(SE)	R^2	*F*	*n*	*P*
BSCP-0	−0·931	(0·196)	0·565	(0·053)	0·815	111·02	26	< 0·001
BSCP-25	−0·879	(0·229)	0·451	(0·056)	0·736	65·12	24	< 0·001
BSCP-62·5	−0·893	(0·236)	0·325	(0·046)	0·683	50·51	24	< 0·001
BSCP-100	−0·910	(0·184)	0·240	(0·037)	0·625	42·76	26	< 0·001

* For details of diets, see Table 1.
† Model in the form $SGR_N = a + bk_{c(N)}$.

Table 5. *Study 1. Nitrogen absorption efficiency* (%), *nitrogen growth efficiency* (%), *excretion of nitrogen as ammonia* (*μmol N/g per d*), *and ammonia excretion expressed as a percentage of the consumed nitrogen in four groups of rainbow trout* (Oncorhynchus mykiss) *fed on diets containing different levels of bacterial single-cell protein (BSCP)**

(Mean values with their standard errors)

	Diets							
	BSCP-0		BSCP-25		BSCP-62·5		BSCP-100	
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE
N-absorption efficiency	85·04[a]	0·71	79·85[ab]	0·83	78·29[b]	1·91	72·20[c]	0·99
N-growth efficiency	38·97[a]	2·68	28·08[b]	3·47	16·11[c]	1·45	7·81[c]	1·44
Ammonia-N	21·24[a]	1·23	22·96[a]	1·39	24·88[a]	1·79	25·48[a]	2·00
Ammonia-N (% consumed N)	23·91[a]	1·39	27·01[a]	1·63	27·53[a]	1·98	24·01[a]	1·89

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 1 and pp. 592–594.

Most of the N excreted was in the form of NH_3. In the first study, diet had no effect on the rate of NH_3-N excretion (Table 5). In the second study the BSCP-25 dietary group excreted significantly less NH_3 compared with the BSCP-0 and BSCP-100 groups, but not compared with the BSCP-62·5 group. Due to the higher amount of urea-N excretion in the BSCP-100 group, urea-N excretion expressed as a percentage of total N was twice as high in the BSCP-100 group compared with the other three groups. The total N excretion and N excretion as a percentage of consumed N were not significantly correlated with the BSCP content of the diets (Table 6).

Nitrogen budgets

Table 7 gives the N budgets for fish fed on the different diets with the different components of the budget expressed as a percentage of the consumed N ($C_N = GE_N + F_N + U_N$). In the case of the BSCP-0 diet the independent measurements from faeces, urine and growth resulted in 98% accountability of the consumed N. In the diets BSCP-25, BSCP-62·5 and BSCP-100, accountability ranged from 76 to 88%.

Table 6. *Study 2. Excretion of nitrogen as ammonia (μmol N/g per d), urea (μmol N/g per d) and ammonia+urea (total; μmol N/g per d), together with urea excretion expressed as a percentage of total nitrogen and total nitrogen excretion expressed as a percentage of consumed nitrogen for individual rainbow trout* (Oncorhynchus mykiss) *fed on four diets containing different levels of bacterial single-cell protein (BSCP)**

(Mean values with their standard errors)

	Diet							
	BSCP-0		BSCP-25		BSCP-62·5		BSCP-100	
Variables	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ammonia-N	21·61[a]	0·64	15·46[b]	1·08	19·00[ab]	1·68	21·63[a]	1·45
Urea-N	3·43[b]	0·31	2·44[b]	0·18	2·20[b]	0·23	7·67[a]	0·85
Total N	25·04[ab]	0·83	17·90[c]	1·00	21·20[bc]	1·73	29·30[a]	1·51
%Urea-N	13·57[b]	1·02	13·97[b]	1·53	10·61[b]	1·22	26·28[a]	3·00
Total N (% consumed N)	44·37[ab]	1·48	32·54[c]	1·81	37·57[bc]	3·07	52·60[a]	2·71

[a, b, c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 1 and p. 595.

Table 7. *Partitioning of nitrogen between retained nitrogen (%), faecal nitrogen (%) and excreted ammonia and urea nitrogen (%) in rainbow trout* (Oncorhynchus mykiss) *fed on diets containing different levels of bacterial single-cell protein (BSCP)**

Diets	Retained N	Faecal N	Excreted N (NH_3, urea)	Total
BSCP-0	39·0	15·0	44·4	98·4
BSCP-25	28·1	20·2	32·5	80·8
BSCP-62·5	16·1	22·0	37·6	75·7
BSCP-100	7·8	27·8	52·6	88·2

* For details of diets and procedures, see Table 1 and p. 595.

DISCUSSION

Several studies involving different fish species and types of SCP have recommended acceptable dietary levels of BSCP for trout. At present these range from 0 to 50% of the diet (reviewed by Tacon & Jackson, 1985; Murray & Marchant, 1986; Steffens *et al.* 1988; Bayourthe & Vellas, 1990; Kiessling & Askbrandt, 1993). The present study suggests that up to 25% of the fishmeal can be replaced by BSCP without deleterious effect on feed consumption, absorption efficiency and growth rate. However, this diet did have a detrimental effect on the final weight, growth efficiency and N excretion of the fish. The 100% BSCP diet adversely affected all the variables tested while maintaining the health of the fish over the duration of the experiment.

Some diets with high concentrations of SCP have been reported to contain concentrations of some essential amino acids (e.g. histidine, methionine) below the requirement level (Windell *et al.* 1974). In the present experiments, analysis of the amino acid composition of the diets indicated that none of the essential amino acids was at a level below the requirements given by Wilson (1989) (W. M. K. Perera, C. G. Carter and D. F. Houlihan,

unpublished results). These results are in general agreement with the studies of Kaushik & Luquet (1980) who reported that there were no deficiencies in essential amino acids when fish were fed with diets containing 350 g bacterial protein/kg (100% substitution).

The measurement of individual consumption rates of fish held in groups using the radiographic method has been used for nutritional studies in order to compare different diets fed to salmonids (Carter *et al.* 1992, 1994*a*). For example, Carter *et al.* (1994*a*) demonstrated that although mean consumption rates of salmon (*Salmo salar*) fed on three diets were similar, growth rates were different due to the nutritional quality of the diets. In the present study the trout may have attempted to compensate for the BSCP in the diet and consumption rates tended to increase with the increasing proportion of the BSCP. However, the marked decrease in growth of fish eating the BSCP-100 diet relative to their high consumption rates clearly points to the poor nutritional characteristics of BSCP. The higher consumption rates found in the group fed on 100% BSCP contrast with the findings of Tacon & Cooke (1980), Kaushik & Luquet (1980) and Rumsey *et al.* (1991*b*), but agree with Kiessling & Askbrandt (1993) who reported an increase in feed intake of trout as dietary BSCP content increased up to 16%. A second explanation for the increased consumption of the diets containing higher levels of BSCP may be the presence of betaine in BSCP. Kiessling & Askbrandt (1993) reported that single-cell bacterial protein sources contained a high level of betaine, a known appetite stimulant for rainbow trout (Hara, 1973; Adron & Mackie, 1978).

Variation in feed intake appears to be a natural feature of feeding fish (Smagula & Adelman, 1982; Cui & Wootton, 1988; McCarthy *et al.* 1992, 1993). The increase in the CV in the 100% BSCP diet is an indication that variability in feed consumption may be used as an indicator of the nutritional quality of a diet.

The N absorption efficiencies reported in the present study compare favourably with those of Murray & Marchant (1986) who reported a decrease in N digestibility from 97 to 64% as the dietary level of SCP increased from 0 to 100% in otherwise isonitrogenous and amino acid balanced diets. Rumsey *et al.* (1991*a*) reported that when brewer's dried yeast cells were fully disrupted the absorption of N increased by more than 20%, and was further increased after the removal of all cell-wall material and the separation of N into amino acid and nucleic acid fractions. It is possible that the bacterial cell wall is resistant to enzymic digestion, thereby limiting the availability of intracellular N. However, diet composition does not explain poor absorption efficiency since none of the non-digestible components was higher in the 100% BSCP diet (e.g. ash, AIA (Table 1)). The absorption efficiencies in the present study are in general agreement with the results from Windell *et al.* (1974) who reported that rainbow trout were able to digest 76% of the protein in brewer's yeast SCP. However, our results are in contradiction with those of Kaushik & Luquet (1980) who reported an increase in dietary protein digestibility with high levels of SCP.

In many studies the amount of N excreted is linearly dependent on N absorption (Savitz *et al.* 1977; Jobling, 1981). In the present study it would appear that the type of diet must be taken into consideration when making measurements of faecal and metabolic N excretion (Tables 6 and 7). From the results of the second study, dietary BSCP has generally been shown to have little or no effect on NH_3-N excretion but urea-N increased twofold in BSCP-100-fed fish compared with those fed on BSCP-0. The major end-product of protein catabolism in freshwater fish in NH_3 and less expenditure of energy is required for the conversion of protein N to NH_3 compared with urea (Goldstein & Forster, 1970; Carter & Brafield, 1991). However, if the total N excretion, exclusive of faecal losses, is estimated from the NH_3, urea and unidentified N compounds, it is found to increase with increasing dietary BSCP content. It could be suggested that the nucleic acid N intake and catabolism increased with increasing dietary BSCP due to the high nucleic acid content

(8–16% by dry weight) of BSCP (reviewed by Tacon & Jackson, 1985). This assumption is supported by the findings of Rumsey *et al.* (1991*b*) who found that the liver uricase (*EC* 1 . 7 . 3 . 3) activity was more than doubled when dietary brewer's dried yeast increased from 500–750 g/kg total weight in feed. This enzyme system is responsive to dietary nucleic acid levels. Even though dietary arginine potentially could be a source of urea, it is unlikely that the degradation of an essential amino acid could provide more than a very minor fraction of the N excreted as urea. Nucleic acids (purines/pyrimidines) however, are a likely source of urea in fish (Forster & Goldstein, 1969). Finally, nucleic acids could be funnelled into the purine pathway during formation of the purine ring and then converted to uric acid, eventually being transformed by uricase into urea and glyoxylic acid, both of which are excreted in the urine (Forster & Goldstein, 1969; Rumsey *et al.* 1991*b*).

Poor N absorption efficiency and high levels of nitrogenous excretion (NH_3-N + urea-N + unidentified N loss) explain why SGR, SGR_N and GE_N were depressed in the BSCP-100 dietary group. Fish fed on the BSCP-0 and BSCP-25 diets had significantly better SGR and SGR_N than those fed on the other two diets. These results are in contrast with the findings of Kaushik & Luquet (1980) and Rumsey *et al.* (1991*b*) who found that fish had significantly better weight gains when fed on 21% and 25% dietary SCP than when fed on the control diet which contained no SCP.

Finally, the present study suggests that differences in growth rates were not due to differences in feed consumption but were due to differences in absorption efficiency and the proportion of excretory products.

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REFERENCES

Adron, J. & Mackie, A. M. (1978). Studies on the chemical nature of feeding stimulants for rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* **12**, 303–310.

Association of Official Analytical Chemists (1975). *Official Methods of Analysis*, 12th ed. Washington DC: AOAC.

Austreng, E. (1978). Digestibility determination in fish using chromic oxide marking and analysis of contents from different sections of the gastrointestinal tract. *Aquaculture* **13**, 265–272.

Bayourthe, C. & Vellas, F. (1990). Nutritive value of bacterial proteins (PBOL) in rainbow trout (*Salmo gairdnerii* Rich.) diets. *Ichthyophysiologica Acta* **12**, 61–70.

Beamish, F. W. H. & Thomas, E. (1984). Effects of dietary protein and lipid on nitrogen losses in rainbow trout, *Salmo gairdneri*. *Aquaculture* **41**, 351–371.

Bowen, S. H. (1981). Digestion and assimilation of periphytic detrital aggregate by *Tilapia mossambica*. *Transactions of the American Fisheries Society* **110**, 239–245.

Carter, C. G. & Brafield, A. E. (1991). The bioenergetics of grass carp, *Ctenopharyngodon idella* (Val.): energy allocation at different planes of nutrition. *Journal of Fish Biology* **39**, 873–887.

Carter, C. G. & Brafield, A. E. (1992). The bioenergetics of grass carp, *Ctenopharyngodon idella* (Val.): the influence of body weight, ration and dietary composition on nitrogenous excretion. *Journal of Fish Biology* **41**, 533–543.

Carter, C. G., Houlihan, D. F., Brechin, J. & McCarthy, I. D. (1993). The relationships between protein intake and protein accretion, synthesis, and retention efficiency for individual grass carp, *Ctenopharyngodon idella* (Valenciennes). *Canadian Journal of Zoology* **71**, 392–400.

Carter, C. G., Houlihan, D. F., Buchanan, B. & Mitchell, A. I. (1994*a*). Growth and feed utilization efficiencies of seawater Atlantic salmon, *Salmo salar* L., fed a diet containing supplementary enzymes. *Aquaculture and Fisheries Management* **25**, 37–46.

Carter, C. G., Houlihan, D. F. & McCarthy, I. D. (1992). Feed utilization efficiencies of Atlantic salmon (*Salmo*

salar L.) parr: effect of a single supplementary enzyme. *Comparative Biochemistry and Physiology* **101A**, 369–374.

Carter, C. G., Owen, S. F., He, Z-Y., Watt, P. W., Scrimgeour, C., Houlihan, D. F. & Rennie, M. J. (1994*b*). Determination of protein synthesis in rainbow trout, *Oncorhynchus mykiss* (Walbaum), using a stable isotope. *Journal of Experimental Biology* **189**, 279–284.

Christiansen, J. S. & Jobling, M. (1990). The behaviour and the relationship between food intake and growth of juvenile Arctic charr (*Salvelinus alpinus* L), subjected to sustained exercise. *Canadian Journal of Zoology* **68**, 2185–2191.

Cui, Y. & Wootton, R. J. (1988). Bioenergetics of growth of a cyprinid, *Phoxinus phoxinus* (L.): the effect of ration and temperature on growth rate and efficiency. *Journal of Fish Biology* **33**, 763–773.

Forster, R. P. & Goldstein, L. (1969). Formation of excretory products. In *Fish Physiology*, vol. 1, pp. 313–350 [W. S. Hoar and D. J. Randall, editors]. New York: Academic Press.

Furukawa, A. & Tsukahara, H. (1966). On the digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bulletin of the Japanese Society of Scientific Fisheries* **32**, 502–506.

Gnaiger, E. & Bitterlich, G. (1984). Proximate biochemical composition and caloric content calculated from elemental CHN analysis: stoichiometric concept. *Oecologia* **62**, 289–298.

Goldstein, L. & Forster, R. P. (1970). Nitrogen metabolism of fish. In *Comparative Biochemistry of Nitrogen Metabolism*, vol. 2, pp. 495–517. New York: Academic Press.

Grant, J. (1989). Fishmeal: what is it doing to the price of the feed? *Canadian Aquaculture* **July/August**, 64–68.

Hara, T. J. (1973). Olfactory responses to amino acids in rainbow trout, *Salmo gairdneri*. *Comparative Biochemistry and Physiology* **44A**, 407–416.

Hart, P. J. B. & Pitcher, T. J. (1969). Field trials of fish marking using a jet inoculator. *Journal of Fish Biology* **1**, 383–385.

Jayaram, M. G. & Beamish, F. W. H. (1992). Influence of dietary protein and lipid on nitrogen and energy losses in lake trout, *Salvelinus namaycush*. *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 2267–2272.

Jobling, M. (1981). Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* **18**, 87–96.

Kaushik, S. J. & Luquet, P. (1980). Influence of bacterial protein incorporation and of sulphur amino acid supplementation to such diets on growth of rainbow trout, *Salmo gairdnerii* Richardson. *Aquaculture* **19**, 163–175.

Kiessling, A. & Askbrandt, S. (1993). Nutritive value of two bacterial strains of single-cell protein for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **109**, 119–130.

Lied, E., Julshamn, K. & Braekkan, O. R. (1982). Determination of protein digestibility in Atlantic cod (*Gadus morhua*) with internal and external indicators. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 854–861.

McCarthy, I. D., Carter, C. G. & Houlihan, D. F. (1992). The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology* **41**, 257–263.

McCarthy, I. D., Houlihan, D. F., Carter, C. G. & Moutou, K. (1993). Variation in individual food consumption rates of fish and its implications for the study of fish nutrition and physiology. *Proceedings of the Nutrition Society* **52**, 427–436.

Maynard, L. A. & Loosli, J. K. (1969). *Animal Nutrition*, 6th ed., p. 613. New York: McGraw-Hill.

Murray, A. P. & Marchant, R. (1986). Nitrogen utilization in rainbow trout fingerlings (*Salmo gairdneri* Richardson) fed mixed microbial biomass. *Aquaculture* **54**, 263–275.

National Research Council (1981). *Nutrient Requirements of Coldwater Fishes*, pp. 14–19. Washington DC: National Academy Press.

Pike, I. H., Andorsdottir, G. & Mundheim, H. (1990). The role of fishmeal in diets for salmonids. *IAFFAM Technical Bulletin* **24**, 35.

Ricker, W. E. (1979). Growth rates and models. In *Fish Physiology*, vol. 8, pp. 677–743 [W. S. Hoar, D. J. Randall and J. R. Brett, editors]. New York: Academic Press.

Rumsey, G. L., Hughes, S. G. & Kinsella, J. E. (1990). Use of dietary yeast (*Saccharomyces cerevisiae*) nitrogen by lake trout. *Journal of the World Aquaculture Society* **21**, 205–209.

Rumsey, G. L., Hughes, S. G., Smith, R. R., Kinsella, J. E. & Shetty, K. J. (1991*a*). Digestibility and energy values of intact, disrupted and extracts from brewers dried yeast fed to rainbow trout (*Oncorhynchus mykiss*). *Animal Feed Science and Technology* **33**, 185–193.

Rumsey, G. L., Kinsella, J. E., Shetty, K. J. & Hughes, S. G. (1991*b*). Effect of high dietary concentrations of brewer's dried yeast on growth performance and liver uricase in rainbow trout (*Oncorhynchus mykiss*). *Animal Feed Science and Technology* **33**, 177–183.

Rumsey, G. L., Winfree, R. A. & Hughes, S. G. (1992). Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **108**, 97–110.

Savitz, J., Albanese, E., Evinger, M. J. & Kolasinski, P. (1977). Effects of ration level on nitrogen excretion, nitrogen retention and efficiency of nitrogen utilization for growth in largemouth bass (*Micropterus salmoides*). *Journal of Fish Biology* **11**, 185–192.

Smagula, C. & Adelmon, C. B. (1982). Day to day variation in food consumption by largemouth bass. *Transactions of the American Fisheries Society* **111**, 543–548.

Steffens, W., Richter, H., Golbs, S., Bentz, H., Martin, S. & Schleicher, J. (1988). Utilization and suitability of methanol grown bacteria biomass for raising rainbow trout (*Salmo gairdneri*). *Archives of Animal Nutrition* **38**, 705–712.

Tacon, A. G. J. & Cooke, D. J. (1980). Nutritional value of dietary nucleic acids to trout. *Nutrition Reports International* **22**, 631–640.

Tacon, A. G. J. & Jackson, A. J. (1985). Utilisation of conventional and unconventional protein sources in practical fish feeds. In *Nutrition and Feeding in Fish*, pp. 119–145 [C. B. Cowey, A. M. Mackie and J. G. Bell, editors]. London: Academic Press.

Talbot, C. & Higgins, P. J. (1983). A radiographic method for feeding studies using metallic iron powder as a marker. *Journal of Fish Biology* **23**, 211–220.

Wilson, R. P. (1989). Amino acids and proteins. In *Fish Nutrition*, 2nd ed., pp. 111–151 [J. E. Halver, editor]. London: Academic Press.

Windell, J. T., Armstrong, R. & Clinebell, J. R. (1974). Substitution for brewer's single cell protein into pelleted fish feed. *Feedstuffs* **46**, 22–23.

Wootton, R. J. (1990). *Ecology of Teleost Fishes*. London: Chapman and Hall.

Zar, J. H. (1984). *Biostatistical Analysis*, 2nd ed. London: Prentice-Hall.

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