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The effect of short-term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morhua*

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Summary. Rates of protein synthesis and oxygen consumption ($\dot{M}O_2$) in cod were compared in both fasted and refed animals. During a 14-day fast both protein synthesis and respiration rates fell to stable values after 6 days. When a meal of whole sandeel at 6% body weight was fed to fish fasted for 6 days, protein synthesis and $\dot{M}O_2$ increased to a maximum at between 12 and 18 h after feeding. Peak $\dot{M}O_2$ was about twice the pre-feeding values, while whole animal protein synthesis increased four-fold. There were differences between tissues in the timing of maximum protein synthesis; the liver and stomach responded faster than the remainder of the body. Maximum protein synthesis rates in the liver and stomach occurred at 6 h after feeding, at which time their calculated contribution to total $\dot{M}O_2$ was 11%. Similar calculations suggested that the integrated increment in whole animal protein synthesis contributed between 23% and 44% of the post-prandial increase in $\dot{M}O_2$. It was concluded that protein synthesis is an important contributor to increased $\dot{M}O_2$ after feeding in cod.

Key words: Fasting – Refeeding – Oxygen consumption – Protein synthesis – Cod, *Gadus morhua*

Introduction

When fish feed there is an increase in their metabolic rate, referred to as the apparent specific dynamic action [ASDA (Beamish 1974; Tandler and Beamish 1979, 1981; Jobling 1981, 1983)]. The basis of this rise in metabolism remains unclear, but several hypotheses have been advanced. The processes most likely to account for the ASDA are: (1) mechanical and/or biochemical processing of the meal (Tandler and Beamish 1979); (2) production of nitrogenous waste products (LeGrow and Beamish 1986); and (3) biosynthesis of new macromolecules from absorbed precursors, particularly proteins (Jobling 1985). If the ASDA is due largely to the cost of amino acid catabolism then the efficiency of dietary protein utilization might be improved if the ASDA were reduced (LeGrow and Beamish 1986; Beamish and MacMahon 1988). However, if the ASDA represents the costs of protein anabolism, and hence growth (Jobling 1985), it should be directly related to protein utilization.

Evidence that protein synthesis may be an important contributor to the ASDA comes from observations that protein synthesis rates increase rapidly after refeeding in both rainbow trout (McMillan and Houlihan 1988) and Atlantic salmon (Fauconneau et al. 1989), while the costs of food processing and nitrogenous excretion are relatively small in fish (Tandler and Beamish 1979; Randall and Wright 1987). Previous work on the crab, *Carcinus*, has shown that post-prandial $\dot{M}O_2$ is closely related to changes in protein synthesis rates in this species (Houlihan et al. 1990), but no equivalent studies have been conducted on fish. This paper reports the changes occurring in both protein synthesis and $\dot{M}O_2$ during short-term fasting and after refeeding in cod.

Materials and methods

Animals. Juvenile cod, *Gadus morhua*, [mean weight = 177.2 ± 6.37 g (SEM)] were obtained from Millport Marine Station, Isle of Cumbrae, and held in running seawater at 8–12°C and a salinity of 33 mg · ml^{-1}. Prior to experimentation they were fed chopped sandeels (*Ammodytes* spp..) ad libitum on alternate days during the working week.

Food composition. A sample of sandeels was analysed for protein (Lowry et al. 1951), RNA (Mejbaum 1939), lipid (Bligh and Dyer 1959) and energy (Philipson 1964) content. Ash and water contents were determined gravimetrically; the results are shown in Table 1.

Abbreviations: A_s, absolute rate of protein synthesis; ASDA, apparent specific dynamic action; ATP, adenosine triphosphate; k_s, fractional rate of protein synthesis; k_s/RNA, amount of protein synthesized per unit RNA; $\dot{M}O_2$, oxygen consumption; PCA, perchloric acid; RNA, ribonucleic acid

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Table 1. Composition of sandeels used to feed cod. All values are mean ± SEM expressed per gram of wet tissue

Constituent	Content	*n*
Protein	173.04 ± 7.64 mg · g^{-1}	20
RNA	1.81 ± 0.06 mg · g^{-1}	20
Lipid	79.01 ± 5.48 mg · g^{-1}	12
Water	708.75 ± 5.58 mg · g^{-1}	12
Ash	22.58 ± 1.12 mg · g^{-1}	12
Energy	7.16 ± 0.21 kJ · g^{-1}	12

Protein synthesis and oxygen consumption during starvation. Cod were transferred to individual 70-l tanks subject to a photoperiod of 11L:13D and a mean water temperature of 10 °C (range: 9.0–11.0 °C). They were fed once at approximately 6% body weight with sandeels before being starved for 6, 10 or 14 days. Two days before the end of the starvation period the fish were anaesthetized with MS222 (100 mg · l^{-1} final concentration in seawater), weighed and their volume determined by displacement of water before transfer to a respirometer. Measurements of $\dot{M}O_2$ were made for up to 2 h immediately before the fish were removed from the respirometers and protein synthesis rates determined as described.

Protein synthesis after refeeding. Twelve days before the experiment, fish were transferred to partitioned tanks in temperature-controlled rooms maintained at a photoperiod of 11L:13D. Two distinguishable fish were placed in each compartment to allow precise measurement of the size of the single meal. The mean water temperature was 9.7 °C (range: 8.9–10.0 °C) and the mean salinity 32.5 mg · ml^{-1} (range: 31.3–32.9 mg · ml^{-1}). The fish were fed ad libitum with sandeels every second day for 6 days, after which food was witheld for a further 6 days. One group was sampled at this point, while the remainder were refed with sandeels at a mean ration of 6.4 ± 1.9 (SD) % body weight and injected with radiolabel at 3, 6, 12, 18, 24 and 48 h post-feeding.

Oxygen consumption after refeeding. Eight cod were transferred to individual 70-l tanks supplied with running seawater (mean temperature: 10.5 °C, range: 8.5–11.5 °C; other conditions as above). They were fed sandeels ad libitum every second day for at least 6 days, followed by 6 days without food. On the fourth day after the last meal each fish was anaesthetized as above its weight and volume determined. It was then transferred to a respirometer and left undisturbed for at least 36 h before measurements of $\dot{M}O_2$ were taken. After 6 days without food the fish were refed inside the respirometer with sandeels at a mean ration of 5.8 ± 0.4 (SD) % body weight. $\dot{M}O_2$ rates were then followed for up to 48 h, although no measurements were made within 2.5 h after feeding to allow the fish to settle.

Measurements

Oxygen consumption. $\dot{M}O_2$ was determined using intermittent flow respirometers in conjunction with Eheim 1018 pumps (2.7 l · min^{-1}). Respirometers were immersed in an aerated seawater jacket and O_2 determinations were made using a Radiometer E4056 polarographic O_2 sensor thermostatted in a water-jacketted cell (Radiometer D616) supplied with water from the respirometer tank. The O_2 sensor was calibrated to zero in a solution of 10 mmol · l^{-1} sodium tetraborate containing 100 mmol · l^{-1} sodium sulphite, and to atmospheric O_2 tension in air-saturated sea water as calculated from the tables of Weiss (1970). The cod usually remained stationary in the respirometer unless disturbed. All fish were observed from behind a screen throughout the measurement period and $\dot{M}O_2$ values were only accepted if the fish's activity was minimal. The O_2 concentration in the respirometers never fell below 85% of the air-saturated value. Background respiration was measured in the closed respirometers after the fish were removed, and was subtracted from the calculated $\dot{M}O_2$.

Protein synthesis. Protein snthesis rates were determined using the methodology of Garlick et al. (1980). Each fish was injected via the caudal blood vessels with a solution containing L-2,6-^{3}H-phenylalanine at 37 · 10^6 Bq · ml^{-1} (100 μCi · ml^{-1}) and 150 nmol L-phenylalanine · ml^{-1} in Cortland saline, as modified by Farrell et al. (1986), adjusted to pH 7.9. The dose was 1 ml of injection solution per 100 g wet body weight. The fish were replaced in aerated seawater where they recovered rapidly and rested quietly before being killed after a mean incubation time of 43.5 min. The liver and stomach were dissected out, weighed and frozen in liquid N_2 with the rest of the body. All dissections were done on ice and took less than 7 min. Frozen tissue samples were stored at −20 °C until analysis. Both tissue samples and whole bodies were analysed using the same methods as Houlihan et al. (1988), although some modifications were required for liver samples rich in lipid. Briefly, after homogenization in PCA, the free-pools and subsequent washes were removed by aspiration from beneath the floating lipid layer. Both the lipid and protein pellet were then incubated together in 0.3 *M* NaOH to redissolve all the proteins, and the sample centrifuged. An aliquot of the dissolved protein solution was removed from beneath the lipid layer and analysis continued on this as described previously below (Houlihan et al. 1988). The frozen whole bodies (everything remaining after tissue removal) were broken up and homogenized in 800 ml 0.5 *M* PCA. The resulting homogenate was throughly mixed and a sub-sample weighed into a tared centrifuge tube for further analysis.

Protein content was determined by the technique of Lowry et al. (1951), as modified by Schachterle and Pollack (1973). RNA was extracted by the Schmidt-Thannhauser procedure (Munro and Fleck 1966) and its concentration measured using the orcinol method of Mejbaum (1939), with calf liver RNA as standard.

Calculations. Fractional rates of protein synthesis (k_s) were calculated as follows:

$$k_s = \frac{S_a}{S_b} \cdot \frac{24}{t} \cdot 100\% \cdot \text{day}^{-1}$$

where S_b and S_a are the protein-bound and free-pool specific radioactivities respectively, after the incubation time t (in hours). For each fish, k_s was multiplied by the protein content of the tissue or whole body to give the absolute protein synthesis rate (A_s) in milligrams per hour.

All protein synthesis and $\dot{M}O_2$ data were adjusted to correspond to a standard 180-g animal according to the equation described by Soofiani and Priede (1985). An exponent of 0.8 was considered appropriate for both $\dot{M}O_2$ (Rao 1968; Edwards et al. 1972) and protein synthesis (Houlihan et al. 1986). Whole body protein synthesis rates were corrected to include protein synthesized by the liver and the stomach.

Theoretical costs for observed protein synthesis rates were derived on the assumptions that 4 mol of ATP equivalents were required per mole of peptide bonds synthesized (36 mmol ATP · g protein^{-1}), and that 1 mol of oxygen led to the formation of 6 mol of ATP (Reeds et al. 1985). No allowance was made for costs of transport. In addition, costs of protein synthesis were also obtained from the cycloheximide inhibition studies of Aoyagi et al. (1988), which gave a value of 71 mmol ATP · g protein^{-1}. These values were used to calculate $\dot{M}O_2$ from the observed protein synthesis rates. Integration of the areas under the plots of oxygen consumption versus time allowed comparison with the observed ASDA, giving two estimates for the proportion of the ASDA attributable to protein synthesis.

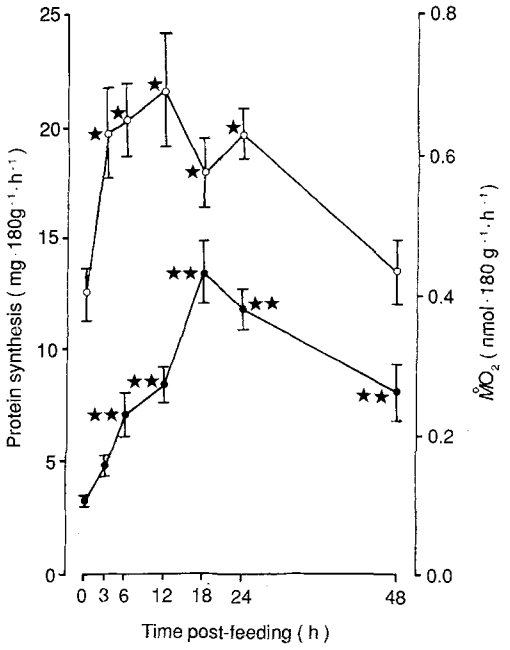

Fig. 1. Absolute protein synthesis rates *(A_s; closed symbols)* and $\dot{M}O_2$ *(open symbols)* in cod after refeeding a single meal of sandeel at a ration of 6.4% body weight. Values are means ± SEM corrected to a standard-sized fish of 180 g weight. Protein synthesis rates have been adjusted to include the liver and stomach. Numbers of fish in each group are the same as in Table 2. * $P<0.05$; ** $P<0.01$ compared to time zero

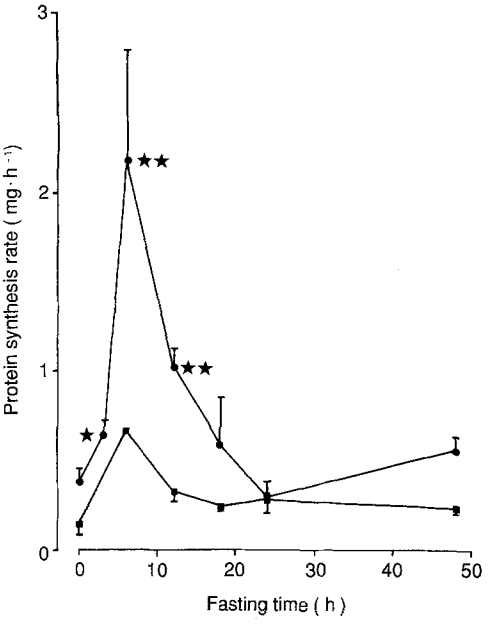

Fig. 2. Absolute protein synthesis rates (A_s) in the liver *(circles)* and the stomach *(squares)* of cod at various times after refeeding. Values are means ± SEM which have been corrected to the respective mean weights of the tissues; 4.8 g for the liver and 3.1 g for the stomach. For the stomach, no data were available at 3 h post-feeding and the 6-h point is the mean of two values. Number of individuals per time-point are the same as in Table 2. * $P<0.05$; ** $P<0.01$ compared to time zero

Statistical analyses. Comparisons between groups were made using one-way analysis of variance. Significant differences were confirmed using a two-sample *t*-test. In all cases significance was accepted at probabilities of 0.05 or less.

Results

Refeeding: whole-body $\dot{M}O_2$ and protein synthesis

Examination of $\dot{M}O_2$ after a meal (Fig. 1) shows that respiration was significantly elevated 3 h post-feeding ($P<0.05$) and continued to increase to a peak at 12 h post-feeding, at which point it was approximately twice the unfed value. The respiration rate decreased after this, remaining significantly higher ($P<0.05$) than that of unfed fish up to 24 h post-feeding, but returning to pre-feeding levels by 48 h after the meal.

Fractional rates of protein synthesis also increased rapidly after refeeding (Table 2), becoming significant at only 3 h post-feeding ($P<0.01$). Synthesis rates continued to rise until 18 h after the meal, this peak being some four times the prefed value. Subsequent to this the synthesis declined, although it remained significantly higher than that in unfed fish up to 48 h post-feeding ($P<0.01$). There were no appreciable differences in whole-body protein content between groups ($P>0.05$), and consequently the calculated rates of absolute protein synthesis followed a very similar pattern to that of the fractional synthesis rates (Fig. 1), although a significant increase was not detected until 6 h after feeding.

Table 2. Fractional rates of protein synthesis (k_s, % per day) in the whole bodies and tissues of cod refed a single meal after a 6-day fast. Values are means ± SEM (*n*). The whole-body synthesis rates were adjusted to a standard-sized fish of 180 g weight. The liver and stomach synthesis rates have been adjusted to their respective mean weights of 4.8 g and 3.1 g

Time after feeding (h)	Tissue		
	Whole body	Liver	Stomach
0	0.50 ± 0.05 (6)	1.15 ± 0.20 (6)	1.16 ± 0.27 (6)
3	1.06 ± 0.15 (7)**	8.53 ± 1.43 (7)**	–
6	1.31 ± 0.06 (6)**	18.32 ± 6.99 (6)**	7.82 (2)
12	1.16 ± 0.12 (12)**	6.96 ± 1.12 (12)***	3.82 ± 0.69 (5)*
18	2.16 ± 0.28 (6)**	2.86 ± 0.58 (6)*	1.38 ± 0.17 (6)
24	1.78 ± 0.19 (6)**	1.65 ± 0.31 (6)	1.24 ± 0.25 (6)
48	1.57 ± 0.49 (6)**	2.37 ± 0.42 (6)*	3.09 ± 0.36 (6)**

* $P<0.05$; ** $P<0.01$; *** $P<0.001$ compared to time zero

Tissue protein synthesis

There was a significant rise in the fractional protein synthesis rate of the liver by 3 h post-feeding ($P<0.05$; Table 2), with a peak at 6 h and then a return to pre-feeding values within 24 h. Synthesis rates in the stomach also showed a peak at 6 h post-feeding with a rapid return to initial values. Calculated absolute rates of synthesis followed the same pattern in both cases (Fig. 2). This is clearly different from the situation in the body as a whole, suggesting that changes in protein synthesis rate after feeding differ in their timing between the various

Table 3. Absolute protein synthesis rates and RNA:protein ratios in the whole body and tissues of cod fasted for up to 14 days. Values are means ± SEM. Other details are as described in Table 2

Tissue	Days fasted	n	k_s % · day^{-1}	A_s mg · h^{-1}	RNA:protein ratio μg · mg^{-1}
Liver	6	3	9.93 ± 2.47	1.15 ± 0.38	45.94 ± 4.22
	10	4	12.93 ± 3.77	1.19 ± 0.35	54.45 ± 5.15
	14	6	5.95 ± 2.47	0.60 ± 0.30	92.37 ± 19.97
Stomach	6	3	2.64 ± 0.63	0.098 ± 0.040	57.17 ± 10.79
	10	4	2.54 ± 0.75	0.053 ± 0.005	53.87 ± 0.57
	14	6	2.84 ± 0.70	0.076 ± 0.018	48.44 ± 3.34
Whole body	6	3	0.44 ± 0.06	3.42 ± 0.48	9.95 ± 0.82
	10	3	0.52 ± 0.07	3.64 ± 1.14	8.77 ± 1.15
	14	6	0.43 ± 0.12	3.38 ± 0.48	7.45 ± 1.06

body tissues. The liver is particularly interesting, since at 6 h post-feeding it contributed 31% of the protein synthesized by the whole animal.

Fasting: $\dot{M}O_2$ and protein synthesis

After 6 days without food, whole-animal $\dot{M}O_2$ was significantly lower than that observed at 24 h post-feeding ($P<0.05$), but similar to valves at 48 h post-feeding ($P>0.05$). Respiration rate showed no further change between 6 and 14 days after the last meal ($P>0.05$). Similarly, whole body fractional rates of protein synthesis were stable between 6 and 14 days post-feeding ($P>0.05$; Table 3), but were significantly lower than those of fish 48 h after a meal ($P<0.01$). As before, the absolute rates of protein synthesis closely reflected the fractional rates. The amount of protein synthesized by a cod after 6 days of fasting was 3.42 mg · 180 g^{-1} · h^{-1}, with no significant change from this value being detected up to 14 days post-feeding ($P>0.05$). These observations together suggest that the fish were in a post-absorptive state at 6 days post-feeding and that they had attained this status at some time after 2 days post-feeding. Analysis of the stomachs and livers of starving fish revealed very much the same pattern as described above for whole bodies, with low, stable protein synthesis rates which showed no significant changes over the period examined (Table 3). There was no significant correlation between individual $\dot{M}O_2$ and protein synthesis for the fasted fish ($P>0.1$).

RNA : protein ratios

In the refeeding experiment, although there were significant differences in RNA : protein ratio between some of the groups ($P<0.01$; Table 4), none were elevated compared with the unfed values for either the whole bodies or the stomachs, indicating that the observed increases in protein synthesis in these samples must have occurred as a result of increased ribosomal activity (McMillan and Houlihan 1988; Fig. 3). However, significantly higher RNA : protein ratios were detected in the livers of refed fish after 3, 24 and 48 h post-feeding. The reason for the difference at 3 h is not clear, since the lack of any difference in RNA : protein ratio between unfed fish and those at 6, 12 and 18 h suggests that feeding was not the cause of the observed discrepancy. With respect to the increases seen at 24 and 48 h after the meal, it is possible that these reflect a feeding-induced rise in the concentration of liver RNA. No changes were observed in the RNA : protein ratios of either the whole bodies or the tissues (liver and stomach) from fasted fish up to 14 days post-feeding (Table 3). In both experiments RNA : protein ratios were found to be highest in the liver and the stomach, which both had fairly similar values, and were lowest in the whole bodies, excluding the tissue samples (Tables 3 and 4). Figure 3 shows the relationship between the fractional rate of protein synthesis (k_s) and the RNA : protein ratio in starved and fed fish. There is significant relationship for the fed fish ($y = 0.39 + 0.02x$, $r=0.79$, $P<0.001$) but not for the fasted fish ($y = -0.51 + 0.26x$, $r=0.37$, $P>0.05$). The slopes of the lines correspond to the amount of protein synthesized per unit RNA (k_s/RNA; Millward et al. 1973), so that the lack of a significant relationship for the unfed data reflects the similarity in synthesis rates between the tissues in the unfed group. The marked increase in the slope upon refeeding indicates that there is a substantial increase in k_s/RNA.

Table 4. RNA : Protein ratios (μg · mg^{-1}) of whole bodies and tissues from cod refed after a 6-day fast. Values are means ± SEM. Number of individuals per group is the same as in Table 2

Time after feeding (h)	Tissue		
	Whole body	Liver	Stomach
0	13.70 ± 1.20	26.29 ± 0.61	38.89 ± 3.28
3	6.37 ± 0.51**	37.45 ± 2.53**	–
6	9.28 ± 2.63	32.41 ± 2.77	37.98
12	12.06 ± 1.10	33.01 ± 2.89	34.31 ± 1.49
18	11.09 ± 0.81	71.27 ± 15.39	29.54 ± 3.16
24	9.21 ± 0.89**	82.91 ± 12.92**	27.23 ± 1.33**
48	7.09 ± 0.94**	28.88 ± 0.74*	40.73 ± 0.68

* $P<0.05$; ** $P<0.01$ compared to unfed group

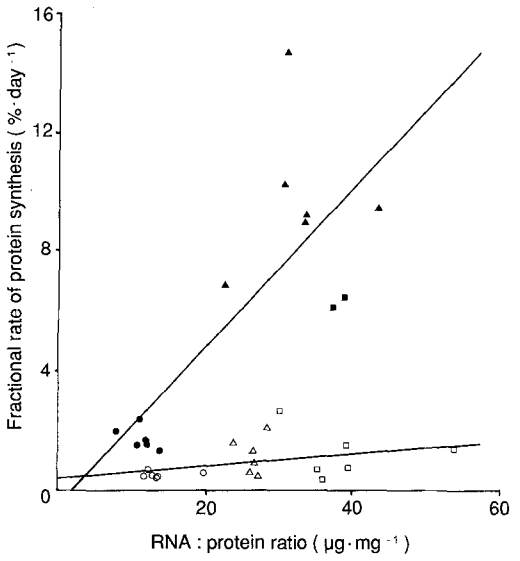

Fig. 3. Relationship between fractional rate of protein synthesis (k_s) and RNA : protein ratios in cod after a 6-day fast *(open symbols)* and at maximal rates of protein synthesis (*closed symbols;* 18 h post-feeding for whole body, 6 h post-feeding for liver and stomach). *Circles,* whole body; *triangles,* liver; *squares,* stomach. The equations of the *lines* are described in the text

Calculated contribution of protein synthesis to oxygen consumption

The magnitude of the ASDA obtained from the present study was 8.031 mmol O_2. Conversion of the observed absolute protein synthesis rates to oxygen equivalents using a theoretical value of 36 mmol ATP · g protein^{-1} gave a figure of 1.88 mmol O_2, indicating that protein synthesis accounts for at least 23% of the observed rise in $\dot{M}O_2$ evident after feeding. Furthermore, the value of 71 mmol ATP · g protein^{-1}, calculated from the data of Aoyagi et al. (1988), gives a figure for the ASDA of 3.53 mmol O_2, implying that protein synthesis contributes some 44% of the post-feeding increment in $\dot{M}O_2$.

Discussion

The influence of feeding on $\dot{M}O_2$ in fish is well documented, most studies indicating an increase in respiration following a meal, which may be of considerable magnitude [for reviews see Jobling (1981, 1983)]. The timing of the peak $\dot{M}O_2$ may be anywhere between 2 and 24 h after the meal (Saunders 1963; Muir and Niimi 1972; Beamish 1974; Vahl and Davenport 1979; Jobling and Davies 1980; Beamish et al. 1986; Le Grow and Beamish 1986), with much of the variation probably being attributable to differences in ration size (Beamish 1974; Jobling and Davies 1980) and temperature. In the present study, it could be argued that some of the post-prandial increase in $\dot{M}O_2$ may be due to changes in activity. Previous work on largemouth bass avoided this criticism by swimming fish against a constant load (e.g. Tandler and Beamish 1979), although the effects of activity on appetite and digestion in fish are not known. Unfortunately, cod will not consistently swim voluntarily against a water current (Soofiani and Priede 1985; Axelsson and Nilsson 1986), and to select only those fish which could do so would lead to a potentially biased sample. However, it was found that once settled in the respirometers the cod remained stationary for long periods both before and after feeding, with only occasional bouts of activity. Data collected when the fish were active were excluded from further analyses, so that any effect of activity on the recorded changes in $\dot{M}O_2$ was minimized.

The effects of diet composition on the ASDA have previously been investigated by several workers (e.g. Jobling and Davies 1980; LeGrow and Beamish 1986), and in general it was found that the magnitude of the ASDA increased with meal size, energy content and protein content. In the present study the ASDA was considerably greater than that reported by Jobling and Davies (1980) for plaice fed on a fish-paste diet, and this difference was probably attributable to the larger ration size used here, since the protein and moisture content of the two diets was similar. However, the higher lipid (8% compared to 4%) and energy (7.16 kJ · g^{-1} compared to 5.19 kJ · g^{-1}) contents of the sandeels may also have contributed to the elevated ASDA in some degree.

The decline from the peak $\dot{M}O_2$ to prefeeding values was relatively rapid in the present study, with respiration rates having returned to unfed values at 48 h after the meal. This is in contrast to earlier work on cod, which indicated that $\dot{M}O_2$ took up to 7 days to return to prefeeding levels (Saunders 1963). Results from other species are very variable, partly due to differences in experimental procedure, but in some cases $\dot{M}O_2$ remained elevated for up to 80 h post-feeding (Beamish 1974; Jobling and Davies 1980). Beamish (1964) established for both brook trout and white suckers that $\dot{M}O_2$ fell to a roughly constant value after 3–10 days without food. The present study suggests that $\dot{M}O_2$ cod had declined to a stable value at 2 days after the meal, and it seems reasonable to suggest that the fish were in a post-absorptive state after a 6-day fast.

The marked effect of refeeding on protein synthesis rates was anticipated, as McMillan and Houlihan (1988) had previously demonstrated a rapid response of protein synthesis to refeeding in various tissues of rainbow trout. The current work agrees well with that of McMillan and Houlihan (1988), who observed the liver and stomach to respond fastest to feeding, in contrast to the three muscle types examined which did not attain maximum synthesis rates before 12 h post-feeding. The whole-body protein synthesis rates measured here are taken to reflect mainly muscle protein synthesis, since white muscle makes up the major proportion of the body tissue of cod (Houlihan et al. 1988).

The stomach and liver both exhibited a spike in protein synthesis rate at 6 h post-feeding, returning to prefed values by 18 h after the meal. This is similar to the pattern observed in rainbow trout liver on refeeding (McMillan and Houlihan 1988), although in trout the peak occurred earlier at 3 h post-feeding. It is noteworthy that at 6 h post-feeding in cod the liver accounts, in

absolute terms, for some 31% of whole-body protein synthesis. This compares to a fish fasted for 6 days where the liver contributed only 12% of the total protein synthesis. If the stomach is also considered, the two tissues account for over 40% of the synthesis in a 180-g cod 6 h after refeeding. The combined increment of their protein synthesis rates at 6 h post-feeding is 2.313 mg protein, and translation of this value into O_2 equivalents using the data of Aoyagi et al. (1988) give a value for $\dot{M}O_2$ of 27.37 μmol $O_2 \cdot h^{-1}$, which is equivalent to 11% of the increment in respiration at that point. Clearly, the viscera are likely to play an important part in determining both the protein synthesis rate and respiration rate of the whole animal during the first few hours after refeeding.

It is well known that protein synthesis rates in fish decline when the animals are deprived of food, with white muscle being the tissue most sensitive to fasting (Smith 1981; Pocrnjic et al. 1983; Loughna and Goldspink 1984; Houlihan et al. 1988; Lowery and Somero 1990). Studies of protein synthesis in rainbow trout (Loughna and Goldspink 1984) and radiotracer incorporation in barred sand bass (Lowery and Somero 1990) have shown that for white muscle both these variables decline to a plateau after 10–14 days without food. The results obtained here for the whole bodies and tissues of cod fasted for up to 2 weeks indicate that protein synthesis rates reached a stable level some time between 2 and 6 days post-feeding, although it was not possible to tell exactly when this occurred. Cod fed a single meal of 4% body weight took approximately 4 days for ammonia excretion to return to pre-prandial values (Ramnarine et al. 1987). Thus, it seems probable that in the present study the fish were not in a truly post-absorptive state (i.e. all processes affected by feeding returned to pre-prandial values) until at least 4 days post-feeding, since they were fed a larger ration than this (6% body weight).

There has been speculation for some time as to the basis of the ASDA in fish, with various explanations having been suggested, including food processing in the gut, elimination of excess amino nitrogen and biosynthesis of macromolecules. It seems unlikely that processing of food in the gut makes more than a minor contribution to the ASDA, since work on plaice showed no detectable increase in $\dot{M}O_2$ after an indigestible meal of kaolin (Jobling and Davies 1980). Furthermore, in large-mouth bass fed at 3% body weight, pre-absorptive work accounted for about 10% of the ASDA, with a decline in this proportion for larger rations (Tandler and Beamish 1979). The possibility that production of nitrogenous waste products might account for the ASDA in fish is also doubtful. In higher vertebrates this could be important because the major nitrogenous excretion is urea, which requires a substantial input of energy for its synthesis (Meijer et al. 1990). However, in most teleost fish the main waste product is ammonia (Brett and Zala 1975; Jobling 1981) which is passively eliminated into the ambient water across the gills (Randall and Wright 1987) and requires very little energy for its synthesis compared to urea. Although up to 20% of nitrogen excreted by teleosts may be urea (Brett and Zala 1975), this is primarily derived from uricolysis (Van Waarde 1983), a less costly process than the urea cycle of mammals, whilst the amount of urea excreted is unaffected by feeding (Brett and Zala 1975; Wiggs et al. 1989) suggesting that it plays only a minor role in the ASDA. In contrast, the synthesis of macromolecules, especially proteins, can be very costly in energetic terms, and so could be a substantial contributer to post-prandial metabolism. Grisolia and Kennedy (1966) suggested that protein synthesis might be an important contributor to the ASDA in man, while Ashworth (1969) proposed that in children recovering from malnutrition, the increase in post-prandial metabolic rate reflected the cost of growth. The possibility that ASDA is related to growth in fish was suggested by Jobling (1981, 1983, 1985), but his line of argument was not based on direct evidence. Brown and Cameron (1991) have recently explored the effect of amino acid infusion on $\dot{M}O_2$ and protein synthesis in channel catfish, and concluded that the increased respiration induced by such infusions was almost entirely accounted for by protein synthesis.

The present study indicates that protein synthesis, which represents the major process involved in animal growth, is a very important determinant of $\dot{M}O_2$ after feeding in cod. On the basis of protein synthesis costs derived from the literature (Aoyagi et al. 1988) it is evident that it might contribute as much as 44% of the observed post-prandial rise in $\dot{M}O_2$, implying that a substantial part of the ASDA represents the unavoidable costs of growth related to the synthesis of new body tissue. In the light of this evidence, there is probably little value in trying to reduce the ASDA of cultured fish in an attempt to increase the efficiency of production, since this would probably lead to decreased growth rates.

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