Ammonia and urea excretion rates of juvenile Australian short-finned eel (Anguilla australis australis) as influenced by dietary protein level

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Abstract

This study aimed to determine excretion rates of ammonia and urea of Australian short-finned elvers as influenced by varying dietary crude protein intake. Elvers (2.3 ± 0.02 g) were fed diets containing dietary crude protein levels of 25% (P25), 35% (P35), 45% (P45) and 55% (P55) dry matter equivalent to 14.17, 19.24, 20.57 and 26.39 g CP/MJ, respectively (pairs of diets P25, P35 and P45, P55 were isoenergetic). Elvers were fed twice a day to a total of 6% BW/day and nitrogenous excretory products (ammonia- and urea-nitrogen) measured during the following 24 h and peak excretion rates occurred 4–8 h following both the morning and afternoon feed. Daily ammonia-nitrogen excretion was significantly (P < 0.05) higher on the P55 diet compared to the P35 and P45 diets. Increasing dietary protein intake resulted in increasing ammonia- (y = 0.022 x + 0.058; n = 12; r² = 0.88; P < 0.001) and urea-nitrogen (y = 0.0044 x + 0.426; n = 12; r² = 0.55; P < 0.01) excretion. The highest urea-nitrogen excretion as a percentage of consumed nitrogen was measured for fish fed the P25 diet (41.99 ± 2.62%) and compared with 30.29 ± 3.58%, 25.76 ± 1.41% and 23.57 ± 1.54% for diets P35, P45 and P55, respectively. The Australian short-finned eel appeared to be similar to other teleost and eel species in terms of the magnitude of ammonia-nitrogen excretion following feeding. However, higher rates of urea-nitrogen excretion indicates that urea is an important excretory end-product in this species. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Ammonia; Australian short-finned eel; Anguilla australis australis; Excretion; Protein intake; Urea


1. Introduction

The main end-product of protein metabolism in teleosts is ammonia and a significant proportion of nitrogenous waste is also excreted as urea (Wood, 1993). Consequently, measurements of ammonia and urea excretion have been used as indicators of the effects of various environmental and nutritional factors on protein metabolism and can give an insight into the nitrogen balance of fish (Rychly and Marina, 1977; Jobling, 1981; Beamish and Thomas, 1984; Perera et al., 1995). Therefore, quantification of ammonia- and urea-nitrogen excretion for fish species in relation to nutrition is important for intensive fish culture operations because protein metabolism partly defines the success of a particular nutritional regimen (Dosdat et al., 1995; Gélineau et al., 1998).

The rate of ammonia excretion increases rapidly in response to feed intake (Savitz, 1971; Brett and Zala, 1975; Jobling, 1981; Ballestrazzi et al., 1994) and the majority of the nitrogen excreted is derived from deamination of amino acids from dietary proteins (Wood, 1993; Brunty et al., 1997). Excretion peaks some hours after feed intake and is mainly dependent upon nitrogen intake, temperature and fish species (Lied and Braaten, 1984; Ramnarine et al., 1987; Kaushik and Cowey, 1990). Dosdat et al. (1996) used the same diet to show that ammonia excretion patterns were related to nitrogen intake in three species of marine fish and indicated no inter-species difference. Conversely, urea-nitrogen excretion rates were species specific in turbot and gilthead sea bream (Dosdat et al., 1996). Although in early studies, urea-nitrogen excretion was not found to correlate with nitrogen intake in the same way as ammonia-nitrogen excretion (Brett and Zala, 1975), several authors have now demonstrated a linear relationship in flatfish (Kikuchi et al., 1991; Carter et al., 1998; Verbeeten et al., 1999) and eel (Knights, 1985). The mechanism behind this is not clear but the adaptive significance of urea synthesis in some teleosts appears to be ammonia detoxification during times when ammonia cannot be freely excreted into the environment, such as a high environmental ammonia concentration (Walsh, 1998).

Studies on the relationship between dietary crude protein and ammonia- and urea-nitrogen excretion in eels are limited. However, the effects of dietary protein to energy ratios, feeding frequency and ration size on nitrogenous excretion by several eel species have been reported (Gallagher and Matthews, 1987; Poxton and Lloyd, 1989; Owen et al., 1998). This study aimed at demonstrating the effect of increasing dietary crude protein content on ammonia- and urea-nitrogen excretion in juvenile Australian short-finned eel kept in a recirculating culture system in which accumulation of nitrogenous excretory products can cause deterioration of water quality. Due to demand for eels, mainly in Europe and Japan, the aquaculture of this species is of interest in Australia (Skehan and De Silva, 1998). However, there is little information available on its nutritional requirements, feed utilisation or ammonia- and urea-nitrogen excretion. Therefore, quantification of nitrogenous excretion in relation to dietary protein is of importance in optimising feeding regimes in recirculating systems and provides an opportunity to compare excretion by the Australian short-finned eel with other eel species.
2. Materials and methods

2.1. Fish and experimental conditions

Wild elvers of the Australian short-finned eel (Anguilla australis australis, Richardson) supplied by the Inland Fisheries Commission, Tasmania, were first weaned onto a commercial eel diet (Chinda Enterprise, Taiwan) and kept in 380-l round fibreglass holding tanks until used. The experiment was conducted in a recirculating system, which consisted of 12 19-l carboys. There were three trickle tray biofiltration units per four carboys in the recirculating system. Twelve elvers (2.33 ± 0.02 g) were randomly allocated to each carboy. During weight measurements, the elvers were anaesthetised (80 mg/l benzocaine) and blotted dry. Fish were acclimatised to the experimental diets and conditions for 1 week before the excretion rates were measured (Beamish and Thomas, 1984; Jayaram and Beamish, 1992). Uneaten feed and faeces were siphoned out daily before beginning measurements of excretion. Water temperature, D.O. and pH levels were maintained at 25.0 ± 1°C, 5.9 ± 0.2 mg O₂/l and 6.77 ± 0.1, respectively. Water exchange (normally it was 1.1 ± 0.1 l/m) was not utilised throughout the experimental sampling period (see below). Photoperiod was 12 h:12 h light/dark.

2.2. Diets

Four experimental diets were formulated to contain 25%, 35%, 45% and 55% crude protein on a DM basis (\(^\text{1}\)). Diets were formulated to make the pairs P25 and P35 or P45 and P55 isoenergetic. Diets contained fish meal and fish oil from jack mackerel, Trachurus picturatus (Gibson’s, Tasmania, Australia), dextrin (Bunge Bioproducts, NSW, Australia) and the other ingredients by Sigma-Aldrich (Australia). L-ascorbyl-2-polyphosphate (Stay-C, Roche Pharmaceuticals, Switzerland) was used with vitamin and mineral mixtures as described by De la Higuera et al. (1989). Diets were prepared by mixing dry ingredients in a food mixer for 25 min followed by fish oil for a further 30 min. Diets were analysed for crude protein (Kjeldahl, selenium catalyst; Nx6.25), crude fat by chloroform and methanol extraction (AOAC, 1990) and energy using a bomb calorimeter (LECO AC 350 calibrated with benzoic acid). Ash contents were determined by burning the test diets at 550°C in a furnace for 16 h.

2.3. Experimental procedure and measurements

Treatments, diets with varying crude protein content, were replicated three times. Fish were fed twice a day, at 0900 and 1700 h at a feeding rate of 6% BW/day. It was ensured that fish ate all the diet presented in each feeding time. Daily rations were prepared by mixing the experimental diets with water and divided in half. After the morning feed, the second half was kept at −20°C until the afternoon feed. Sampling of carboys for ammonia- and urea-nitrogen was blocked over time so that all diets were sampled concurrently and three 8-h sampling periods (0900–1700; 1700–0100; 0100–0900 h) were used. During sampling, water flow to carboys was turned off and each carboy of each treatment was sampled over one 8-h period in each
Table 1
Formulation (g/kg diet) and chemical composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P25</td>
</tr>
<tr>
<td>Fish meal</td>
<td>385.0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>176.0</td>
</tr>
<tr>
<td>Dextrin</td>
<td>135.0</td>
</tr>
<tr>
<td>Bentonite</td>
<td>232.8</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>13.0</td>
</tr>
<tr>
<td>CMC</td>
<td>40.0</td>
</tr>
<tr>
<td>Minerals</td>
<td>12.5</td>
</tr>
<tr>
<td>Vitamins</td>
<td>5.0</td>
</tr>
<tr>
<td>Stay-C</td>
<td>0.5</td>
</tr>
<tr>
<td>B.H.A</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Proximate composition (g/kg DM)

- Moisture: 71 ± 0.7, 73 ± 2.1, 76 ± 3.5, 78 ± 2.8
- Crude protein: 264 ± 0.7, 368 ± 0.7, 464 ± 0.9, 583 ± 1
- Crude fat: 227 ± 9.9, 187 ± 2.1, 160 ± 1.4, 139 ± 1.4
- Ash: 284 ± 0.8, 245 ± 4.8, 182 ± 1.3, 164 ± 1.6
- Gross energy (MJ/kg): 18.6 ± 2.12, 19.1 ± 1.53, 22.5 ± 0.25, 22.1 ± 0.22

*aMineral mixture (g/kg food). According to De la Higuera et al. (1989): CaH₂PO₄ = 3.424, CaCO₃ = 3.265, KH₂PO₄ = 2.384, KCl = 0.24, NaCl = 1.442, MnSO₄·H₂O = 0.089, FeSO₄·7H₂O = 0.36, MgSO₄ = 1.201, KI = 0.0046, CaSO₄·5H₂O = 0.012, ZnSO₄·7H₂O = 0.06, CoSO₄·7H₂O = 0.007, Na₂MoO₄ = 0.002, Na₂SeO₃ = 0.0018, Al₂O₃·18H₂O = 0.004.

*bVitamin mixture (g/kg food). According to De la Higuera et al. (1989): calcium pantothenate = 0.13, thiamine = 0.044, riboflavin = 0.109, pyridoxine = 0.033, inositol = 0.874, biotin = 0.001, folic acid = 0.011, choline chloride = 2.623, nicotinic acid = 0.219, cyanocobalamin = 0.002, ascorbic acid = 0.874, retinol = 0.044, menadione = 0.022, α-tocopherol = 0.007, cholecalciferol = 0.009. Individual ingredients were supplied by Sigma-Aldrich and ICN Biochemicals, Australia.

*cStay-C (L-Ascorbyl-2-polyphosphate).

The sampling day. The experiment was conducted over 5 days so that each tank was sampled over each of the three 8-h periods, with a day in between sampling. It was previously shown that ammonia levels remained well below toxic concentrations (Wedemeyer, 1996). Triplicate 10-ml water samples were collected for ammonia and urea measurements at 4 and 8 h in each sampling period by pipetting water samples from the middle of a carboy to provide data every 4 h over a 24-h period. Excretion was calculated from the change in ammonia or urea concentration and the water volume in the carboy.

The concentration of ammonia in samples was determined by the phenol-hypochlorite method (Solorzano, 1969). Urea was analysed by the urease method (Elliott, 1976). Total ammonia-nitrogen concentration was calculated using a standard curve prepared from ammonium chloride solution. The difference between ammonia concentration before and after urease treatment was used to calculate urea concentration.

2.4 Statistical analyses

Data are presented as means ± S.E.M. throughout the text. Peak rates of ammonia-nitrogen excretion following feeding were compared by one-factor ANOVA. When a
significant treatment effect was observed, a Tukey–Kramer HSD test was used to compare means. The relationship between daily nitrogenous excretion rates and dietary protein levels, as dietary percentage, was described by linear regression of the form \( y = a + bx \), where \( y \) is the excretion rate of ammonia- or urea-nitrogen and \( x \) is the dietary protein content (%DM). Regression analysis was also used to describe the relationships between nitrogen intake and nitrogenous excretion rates for individual tanks in each treatment. Significance was accepted at probabilities of 0.05 or less.

3. Results

3.1. Diurnal ammonia- and urea-nitrogen excretion rates

Daily ammonia-nitrogen excretion rates increased 4 h after the morning feed (Fig. 1). Excretion rates were significantly \( (P < 0.05) \) higher for treatments P45 and P55 than for P25 and P35. The first peak occurred 4–8 h after the morning feed for all treatments (Fig. 1). Following the afternoon feed, the excretion rates decreased in all of the treatments except P45 (Fig. 1). The ammonia-nitrogen excretion rates just before the next mornings’ feeding were higher than the rates at 0500 h in each treatment. The peak excretion rates following feeding in treatments P45 and P55 were significantly \( (P < 0.05) \) higher than P25 and P35 (Fig. 1). However, there was no significant difference between treatments P25 and P35 or between P45 and P55.

Fig. 1. Fluctuations of daily ammonia-nitrogen (NH\(_3\)-N) excretion by the Australian short-finned eel fed varying dietary protein levels. Values are means ± S.E.M. \((n = 3)\) for each treatment. "Represents initial mean ammonia-nitrogen values for each treatment.
Table 2
Mean daily rates of nitrogenous excretion by the elvers of *A. australis australis* in relation to dietary protein levels and as a percentage of consumed nitrogen ($C_N$)*

<table>
<thead>
<tr>
<th>Diets (%crude protein levels)</th>
<th>P25</th>
<th>P35</th>
<th>P45</th>
<th>P55</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_N$ (consumed nitrogen, mg N/kg/day)</td>
<td>1330 ± 20*</td>
<td>1854 ± 40b</td>
<td>2340 ± 10c</td>
<td>2940 ± 30d</td>
</tr>
<tr>
<td>$E_i$ (energy intake, kJ/kg/day)</td>
<td>16520 ± 80*</td>
<td>16960 ± 90c</td>
<td>19990 ± 120d</td>
<td>19640 ± 260d</td>
</tr>
<tr>
<td>Ammonia-nitrogen (mg NH$_3$-N/kg/day)</td>
<td>572 ± 30*</td>
<td>853 ± 110bc</td>
<td>1079 ± 80ad</td>
<td>1225 ± 140ad</td>
</tr>
<tr>
<td>Urea-nitrogen (mg Urea-N/kg/day)</td>
<td>559 ± 40</td>
<td>561 ± 60</td>
<td>603 ± 110</td>
<td>693 ± 110</td>
</tr>
<tr>
<td>Total nitrogen (mg N/kg/day)</td>
<td>1131 ± 70*</td>
<td>1415 ± 130bc</td>
<td>1681 ± 110bc</td>
<td>1919 ± 170c</td>
</tr>
<tr>
<td>Ammonia-nitrogen (%$C_N$)</td>
<td>43.00 ± 2.47</td>
<td>46.03 ± 5.97</td>
<td>46.10 ± 3.96</td>
<td>41.65 ± 4.40</td>
</tr>
<tr>
<td>Urea-nitrogen (%$C_N$)</td>
<td>41.99 ± 2.62b</td>
<td>30.29 ± 3.58*</td>
<td>25.76 ± 1.41c</td>
<td>23.57 ± 1.54a</td>
</tr>
<tr>
<td>Total nitrogen (%$C_N$)</td>
<td>84.98 ± 5.09b</td>
<td>76.32 ± 7.20ab</td>
<td>71.86 ± 5.36bc</td>
<td>65.22 ± 5.04*</td>
</tr>
<tr>
<td>Urea-N/ammonia-N + urea-N (%$C_N$)</td>
<td>37.13 ± 0.23c</td>
<td>21.37 ± 2.24bc</td>
<td>15.32 ± 0.25ab</td>
<td>12.32 ± 0.86c</td>
</tr>
</tbody>
</table>

* Values are means ± S.E.M. ($n = 3$) and means in the same row with different superscripts are significantly different ($P < 0.05$).

Urea-nitrogen excretion accounted for between 30–50% of total ammonia-nitrogen excretion rates at each treatment (Table 2). The variability in excretion rates was not as pronounced as ammonia-nitrogen rates over a 24-h sampling period (Fig. 2) and rates were found to be the highest in sampling periods, 8 h following each feeding except the...
treatment P35. The excretion rates in treatments P25 and P35 were the same at 2100 and 0100 h, respectively.

Fig. 3. The relationship between nitrogen intake and (a) ammonia-nitrogen ($A_N$) ($y = 0.066 + 0.41x; n = 12; r^2 = 0.88; P < 0.001$); (b) urea-nitrogen ($U_N$) ($y = 0.424 + 0.085x; n = 12; r^2 = 0.57; P < 0.01$) for the Australian short-finned eel in individual tanks. ●: P25, ○: P35, ▼: P45, △: P55.
3.2. The relationships between dietary crude protein and nitrogenous excretion rates

Mean daily ammonia-nitrogen excretion rates tended to increase with increasing dietary crude protein (Table 2). The excretion rate in treatment P25 was significantly \((P < 0.05)\) lower than the other treatments. The highest mean daily excretion rate was \(1225 \pm 0.14 \text{ (mg NH}_3\text{-N/kg/day)}\) in treatment P55 and it was similar to the rate obtained in the treatment P45. Ammonia-nitrogen excretion as a percentage of consumed nitrogen \((C_N)\) increased steadily with increasing crude protein levels. However, there was no significant difference between the treatments (Table 2). The relationship between dietary crude protein \((x, \%DM)\) and ammonia-nitrogen excretion \((y, \text{mg NH}_3\text{-N/kg/day})\) was described by \(y = 0.058 + 0.022x \ (n = 12; \ r^2 = 0.88; \ P < 0.001)\).

Urea-nitrogen excretion rates did not differ significantly between the treatments (Table 2). Urea-nitrogen excretion rates, as percentage of nitrogen intake, was significantly \((P < 0.05)\) higher in treatment P25 than the other treatments. However, there was no significant difference between the treatments P35, P45 and P55 (Table 2). The highest daily urea-nitrogen excretion as percentage of the daily total nitrogen excretion was obtained on P25 \((42 \pm 2.61\%)\) and compared to \(30.3 \pm 3.56\%, 25.8 \pm 1.45\%\) and \(23.6 \pm 1.54\%\) for P35, P45 and P55, respectively. The relationship between dietary percentage of crude protein \((x, \%DM)\) and mean daily urea-nitrogen excretion \((y, \text{mg urea-N/kg/day})\) rates was described by \(y = 0.426 + 0.0044x \ (n = 12; \ r^2 = 0.55; \ P < 0.01)\). The relationship between the mean daily nitrogen intake \((C_N)\) and nitrogenous excretion rates for ammonia- and urea-nitrogen was linear (Fig. 3). Total mean nitrogen (ammonia-nitrogen + urea-nitrogen) excretion as percentage of nitrogen intake decreased with increasing crude protein (Table 2). The highest value \((84.98 \pm 5.09\%)\) was on the treatment P25 and was significantly \((P < 0.05)\) higher than the other treatments. Values were \(75.39 \pm 8.10\%, 71.86 \pm 5.36\%\) and \(65.22 \pm 5.04\%\) for treatments P35, P45 and P55, respectively. The proportion of urea-nitrogen to total nitrogen excretion rates in treatments also decreased with increasing dietary crude protein (Table 2).

4. Discussion

Ammonia excretion rates are directly related to dietary nitrogen and protein intake in teleosts (Rychly, 1980; Beamish and Thomas, 1984; Kaushik and Cowey, 1990). Feeds for some fish species typically have a high protein content that supplies a large proportion of dietary energy and results in high nitrogenous excretion. Increasing the dietary level of non-protein digestible energy increases nitrogen retention by decreasing nitrogen losses (Cho and Kaushik, 1985; Kaushik and Oliva-Teles, 1985; Médele et al., 1995). The increase in ammonia excretion with increasing dietary protein was in agreement with previous findings for eels (Gallagher and Matthews, 1987; Degani and Levanon, 1988). For the isoenergetic diet pairs, the higher excretion rates for treatments P35 and P55 compared with treatments P25 and P45, respectively, are probably explained by the protein-sparing effect of non-protein energy yielding substrates at lower dietary protein (Lied and Braaten, 1984; Jobling, 1994; Rodehutscord and Pfeffer, 1999). Gallagher and Matthews (1987) found that when American eels were fed
increasing crude protein/energy diets, ammonia excretion increased significantly with increasing crude protein in experimental diets. In their study, the lowest excretion (0.07 ± 0.05 mg NH₃-N/g/h) was obtained at the lowest crude protein/energy ratio diet (16.75 mg/kJ) and the mean increase of 0.05 mg/g/h in ammonia excretion was recorded in between the crude protein/energy ratio levels tested in their experiment (Table 3). Degani and Levanon (1988) also reported an increase in ammonia excretion when the protein/energy ratio was maintained. However, overall excretion was lower than found in the present study, and measuring excretion under continuous water flow, lower stocking density or the use of larger tanks in their study may have impacted on excretion rates. Biochemical mechanisms of nitrogenous excretion in the Australian short-finned eel, as demonstrated for several fish species (Walsh and Milligan, 1995; McGoogan and Gatlin, 1999), could have related to reduced glutaminase activity on higher dietary non-protein energy diets as ammonia is an end-product of the metabolism of glutamine to glutamate.

The amplitude and time of appearance of peak excretion rates are dependent upon fish size, water temperature and nitrogen intake (Kaushik and Cowey, 1990). There was an immediate increase in ammonia excretion rates following both the morning and afternoon feeds, and two visibly distinct peaks occurred 4–8 h after feeding in all the treatments in this study. Clearly, two peaks of excretion were related to the two meals fed to the elvers. Large variations in the timing and the magnitude of peak excretion have been reported for different fish (Brett and Zala, 1975; Rychly and Marina, 1977; Ramnarine et al., 1987; Verbeeten et al., 1999), including the European eel (Poxton and Lloyd, 1989; Owen et al., 1998) and the American eel (Gallagher and Matthews, 1987). Brett and Zala (1975) found that following the single morning feed, the excretion rate in sockeye salmon rose sharply to a peak, falling rapidly thereafter in an almost exponential decrease to the early morning base level. Rychly and Marina (1977) demonstrated an increase in blood ammonia levels within 1 h of feeding rainbow trout and proposed that endogenous circadian rhythms in both ammonia excretion and nitrogen metabolism were dependent upon feeding times and nitrogen intake. Feeding twice a day, as used in this study, had a cumulative effect on ammonia excretion in each treatment and rates after a 24-h period remained higher compared to pre-feeding levels. This was probably due to the increased anticipatory metabolic activity or diel feeding rhythms shown by the elvers. In a study with juvenile Atlantic cod, peak excretion occurred 6.5–27.0 h after feeding depending on ration size and feeding frequency, and declined to pre-feeding levels after 4 days (Ramnarine et al., 1987). This could be both related to the water quality parameters like temperature and pH or diel feeding rhythm of Atlantic cod adapted to a stable feeding regimen. According to Poxton and Lloyd (1989), two meals per day led to the lowest overall production of ammonia by the European eel and that the lowest peak concentration occurred when these feeds were widely spaced out (Table 3). Owen et al. (1998) reported that feeding ad lib once a day at high ration increased the ammonia-nitrogen excretion more than tenfold of pre-feeding levels by the European eel and the peak excretion rate occurred 5 h following the start of feeding (Table 3). Feeding American eels once a day, also increased the ammonia excretion to a peak 4 h following feeding and returned to pre-feeding level 10 h thereafter (Gallagher and Matthews, 1987).
### Table 3
Summary of maximum rates of nitrogen excretion by Anguillid eel species under different nutritional regimens

<table>
<thead>
<tr>
<th>Species</th>
<th>Weight (g)</th>
<th>Temperature (°C)</th>
<th>CP (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ration (%BW/day)</th>
<th>Ammonia-nitrogen (mg N/kg/h)</th>
<th>Urea-nitrogen (mg N/kg/h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. rostrata</td>
<td>0.75</td>
<td>22–23</td>
<td>30</td>
<td>satiation, 1</td>
<td>17.5</td>
<td>NA</td>
<td>Gallagher and Matthews (1987)</td>
</tr>
<tr>
<td>A. anguilla</td>
<td>&lt; 10</td>
<td>25</td>
<td>50</td>
<td>satiation, 1</td>
<td>53.0</td>
<td>18.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Knights (1985)</td>
</tr>
<tr>
<td>A. anguilla</td>
<td>16.4</td>
<td>23</td>
<td>45</td>
<td>2.5, 2</td>
<td>25.0</td>
<td>NA</td>
<td>Poxton and Lloyd (1989)</td>
</tr>
<tr>
<td>A. anguilla</td>
<td>45</td>
<td>25</td>
<td>45</td>
<td>0.5, 1</td>
<td>0.9</td>
<td>1.0</td>
<td>Owen et al. (1998)</td>
</tr>
<tr>
<td>A. anguilla</td>
<td>25</td>
<td>17</td>
<td>NA</td>
<td>Unfed</td>
<td>NA</td>
<td>38.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Masoni and Payan (1974)</td>
</tr>
<tr>
<td>A. australis</td>
<td>2.3</td>
<td>25</td>
<td>25</td>
<td>6.0, 2</td>
<td>29.0</td>
<td>27.5</td>
<td>Present study</td>
</tr>
</tbody>
</table>

<sup>a</sup>Dietary crude protein (%N×6.25).
<sup>b</sup>30% saltwater.
<sup>c</sup>Freshwater.
<sup>d</sup>Saltwater.
Fluctuations in daily urea-nitrogen excretion followed the same trend in each dietary treatment, however the overall excretion rates tended to increase with increasing dietary protein (Table 2). The level of urea excretion was 30–50% of total nitrogen excretion rates. Hourly excretion rates were slightly higher 4 h following each feeding. Other workers have found lower and more constant levels (Olson and Fromm, 1971; Brett and Zala, 1975; Cockroft and Du Preez, 1989). For example, Brett and Zala (1975) found that urea-nitrogen excretion by sockeye salmon averaged $2.2 \pm 0.2 \text{ mg N/kg/h}$ independent of feeding or dark–light switching of the photoperiod control. There is little known about the urea-nitrogen excretion rates in eels. It appears that many previous studies have concentrated on only the ammonia excretion rates in feeding and nutritional studies with eels (Degani and Levanon, 1988; Degani et al., 1985; Gallagher and Matthews, 1987) However, available studies confirmed our results indicating that urea-nitrogen can be an important additional component of nitrogenous excretion in some species, including eels under certain conditions (Masoni and Payan, 1974; Knights, 1985). Masoni and Payan (1974) found significantly lower branchial clearance values in seawater adapted European eel than freshwater adapted fish indicating a reduced branchial permeability to urea in saltwater (Table 3). Knights (1985) also indicated that salinity may have to be taken into account in nitrogenous excretion studies with eels since the author found that urea-nitrogen was higher in freshwater than in seawater (by 120–180% in unfed fish and by 300–330% on one satiation meal of commercial feed; over 50% crude protein) (Table 3). This might result from the greater potential for flushing out urea in the higher urine flow rates expected in a hypo-osmotic medium (Eddy, 1981). Highly variable urea-nitrogen excretion and increased urea-nitrogen with increased feed intake were also reported with several other teleosts (Kikuchi, 1995; Harris and Probyn, 1996; Carter et al., 1998; Verbeeten et al., 1999).

The total nitrogenous excretion, expressed as a proportion of consumed nitrogen ($C_N$), allows an indirect estimate of the proportion of nitrogen retained as growth plus faecal N. Therefore, the current experiment showed a decrease in nitrogen retention, due to increased urea but not ammonia excretion (as a proportion of nitrogen intake), as dietary protein: energy decreased. This result is difficult to explain since higher nitrogen retention efficiency would be predicted at lower dietary protein intake. Part of the explanation may relate to dietary fat content and the ability of eels to use it (Sanz et al., 1993). At higher dietary fat levels, the eels may have had a lower ability to use the fat and therefore used a larger proportion of the protein as an energy source. Because of the changing metabolic patterns in the life cycle of this species (Lecomte-Finiger, 1983), young eels could have a higher protein requirement and reduced tendency towards fattening than the older ones (Sanz et al., 1993). The optimum protein requirement for similar sized Japanese and European eels and larger American eels ($8.11 \pm 0.07$) was estimated as between 45% and 50% on DM basis (Nose and Arai, 1972; Degani et al., 1985; Tibbetts et al., 1999). Determination of optimum protein/energy ratio for the Australian short-finned eel would, therefore, be a useful tool in understanding the pattern of urea excretion observed in the present study. Higher urea loss, as the proportion of total nitrogenous excretion in the treatment P25 and P45 compared to P35 and P55, may also indicate that urea-N excretion in the Australian short-finned eel is more responsive to nutritional variables as was demonstrated for some flatfish species.
and lake trout (Jayaram and Beamish, 1992; Dosdat et al., 1995, 1996; Verbeeten et al., 1999).

In conclusion, this study showed that mean daily ammonia-nitrogen excretion rates of juvenile Australian short-finned eel increased when fed increasing dietary crude protein in paired isoenergetic diets. Mean daily urea-nitrogen excretion also tended to increase and accounted for 30–50% of total daily nitrogenous excretion indicating that urea-nitrogen is an important nitrogenous excretory end-product in the Australian short-finned eel. Further studies on the effects of nutritional variables to nitrogenous excretion by the Australian short-finned eel should be used to detail the protein metabolism in this species.

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References


