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Effect of dietary protein on the nitrogen excretion and growth of the African catfish, *Clarias gariepinus*

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Abstract

The rates of growth and nitrogen efflux (total nitrogen and ammonia) of individual *C. gariepinus* ($\bar{x} = 32.2\ g$; $S.D. = 4.8\ g$) kept under 4 feeding regimens, following a 48 h imposed fast (phase 1), were measured periodically. In phase 2 (35 *d*), groups A, B and C were fed a 49.75%, 45.55% or 41.10% protein diet respectively at a ration of 0.5% body weight ($1\ d^{-1}$). Group D were not fed. In phase 3 (25 *d*) all groups were fed the 41.10% diet. In phase 1, the ammonia efflux rates were lower than any of the values found in either phase 2 and 3. In phase 2, group A (49.75%) had a higher mean ammonia efflux rates than the other groups and ammonia comprised 60-100% of the total nitrogen efflux in all groups. Group D showed a direct relationship between ammonia efflux rate and length of fast. In phase 3, refeeding with the 41.1% protein diet caused the ammonia efflux rates of groups B and D to converge within 7 days to values no different from those of group C, but group A maintained a significantly higher mean value until day 25. During phase 2, the growth rates in B (45.55%) were greatest, but, none of the among groups differences were significant. Group D fish (unfed) lost approximately 30% of their initial weight during phase 2. Nitrogen efflux rates, notably ammonia, showed a pattern of excretion that was directly related to the protein content of the diet, but that the source of dietary protein, dietary energy and the total available energy also influenced nitrogen metabolism. The small differences in growth found were related to between diet differences in composition. The weight loss in the unfed group of fish was probably attributable to the utilisation of lipid and/or protein reserves as metabolic fuels.

Keywords: *Clarias gariepinus,* excretion, growth, protein.

Effets des protéines alimentaires sur l'excrétion azotée et la croissance du poisson-chat africain, Clarias gariepinus.

Résumé

Les taux de croissance et d'azote (azote total et ammoniaque) ont été étudiés chez *C. gariepinus* ($\bar{x} = 32.2\ g$; $S.D. = 4.8\ g$) élevés avec 4 régimes alimentaires, suivant un jeûne de 48 h (phase 1). En phase 2 (35 jours), les groupes A, B et C ont été nourris respectivement à 49,75 %, 45,55 % ou 41,10 % de protéines avec une ration de 0,5 % du poids vif (1 par jour). Le groupe D n'a pas été nourri. En phase 3 (25 jours) tous les groupes ont été nourris à base du régime protéinique 41,10 %. En phase 1, les taux de rejet d'ammoniaque étaient plus bas que tous ceux observés en phase 2 et 3. En phase 2, le groupe A (49,75 %) avaient une plus haute moyenne de rejet d'ammoniaque que les autres groupes et un taux d'ammoniaque compris entre 60 et 100 % des flux d'azote total dans tous les groupes. Chez le groupe D, il y a une relation directe entre le taux d'ammoniaque et la durée du jeûne. En phase 3, le nourrissage à 41,1 % de protéines provoque des rejets d'ammoniaque sensiblement égaux chez les groupes B et D 7 jours plus tard et similaires au groupe C mais plus important pour le groupe A jusqu'au 25^{e} jour. Durant la phase 2, les taux de croissance du groupe B (45,55 %) étaient les plus élevés mais il n'y avait pas de différence significative entre les groupes. Les poissons du groupe D (non nourris) ont perdu approximativement 30 % de leur poids initial durant la phase 2. Les taux d'azote, d'ammoniaque

notamment, sont directement liés à la composition en protéines du régime alimentaire ; mais la source de protéines alimentaires en énergie métabolique et l'énergie totale disponible influence aussi le métabolisme azoté. Les petites différences de croissance observées sont liées à la différence de composition des régimes alimentaires, la perte de poids du groupe de poissons non nourri peut être attribuée probablement à l'utilisation des réserves en lipides et/ou en protéines comme source d'énergie métabolique.

Mots-clés : *Clarias gariepinus*, excrétion, croissance, protéine.

INTRODUCTION

Dietary protein is of fundamental importance to aquaculture systems; representing considerable economic investment and a central factor determining fish growth. If the maximum utilisation of dietary protein for growth is to be achieved, the selection for quality and quantity of protein is important. The processes of synthesis and deposition of body protein are interlinked to many processes of nitrogen and energy metabolism.

Absorbed dietary amino acids, in excess of those required for growth and maintenance cannot be stored and are degraded (Campbell, 1991). This, or other catabolic use of amino acids, results in the production of nitrogenous waste products, of which ammonia is the predominant form in teleost fish (Smith, 1929; Wood, 1958; Randall and Wright, 1987; Kaushik and Cowey, 1990). A large proportion of the ammonia production occurs in the liver (Pequin and Serfaty, 1963), although the necessary enzymes have been found also in the kidneys, gills and skeletal muscle tissue (Goldstein and Forster, 1970; McBean *et al.*, 1966; Walton and Cowey, 1977). Ammonia is secreted into the blood and is voided to the surrounding environment, primarily through the gills (Sayer and Davenport, 1987), by simple diffusion or ionic exchange (Evans, 1985; Randall and Wright, 1987; Heisler, 1990).

The most limiting water quality parameter to the production of a fish farm, after adequate water oxygenation, is the toxic effects of dissolved ammonia (Colt and Armstrong, 1981). Safe levels of ammonia for non-salmonid fish have been recommended as 10 mg $NH_4^+ l^{-1}$ (Haywood, 1983), although the specific toxic effects associated with ammonia pollution are stongly influenced by both environmental and biological factors. Effects include histopathological changes to the gills (Smart, 1978; Solderberg *et al.*, 1984; Kirk and Lewis, 1993), and to the liver (Smith and Pyper, 1975), reduced resistance to disease (Burrows, 1964) and suppressed growth rate (Colt and Tchobanoglous, 1978; Guillen *et al.*, 1993).

The African catfish, *Clarias gariepinus* (Burchell, 1822) is recognised as an ideal canditate for aquaculture due to its continued reproductive performance under intensive rearing conditions, ability to efficiently utilise commercial feedstuffs and the capacity to use atmospheric oxygen (Haylor, 1989). It is also becoming an increasingly important commercial species in parts of Africa and Europe (FAO, 1992). The intensification of culture systems necessitates the prediction of fluctuations in daily nitrogenous effluxes for estimating potential waste loads (Cowey and Cho, 1991), in the interests of water quality management. This study was designed to evaluate the effect of change in dietary protein intake on the nitrogen excretion (ammonia+total nitrogen) and growth of *C. gariepinus*

METHODS

Clarias gariepinus ($\bar{x} = 32.2\ g$; $S.D. = 4.8\ g$), were kept in multiple, interconnected, black plastic tanks (161 volume) at a stocking density of $8\ g \cdot l^{-1}$. The tanks were supplied with a single biological gravel filter and recirculating (flow rate = $10\ l \cdot min^{-1}$; recirculation = $6\%\ min^{-1}$), aerated, fresh water (25°C). Water in the holding tanks and the experimental chambers (in a water bath) was maintained at 25°C by a 300 W thermostat. Before the experiment all fish were acclimatised to control diet C ($41 \cdot 10\%$ protein, pelleted diet). Experimental fish ($n = 24$) were randomly divided into four groups (A-D; $n \geq 6$). Each fish was given a blue ink identification mark on the underside of its body using a Panjet marker (Hart and Pitcher, 1969).

The experiment comprised three phases (P1-P3), within each of which, periodically, the total ammonia ($TA = NH_4^+ + NH_3$) and total nitrogen (TN) effluxes and the wet weight (nearest 0.1 g; Mettler top balance: PJ400) of individual fish were measured periodically. The fish were not anesthetised before weighing. Fish were transferred to the experimental chambers following 1 h post-feeding and then allowed to acclimate for 2 h before the experiment started. This acclimation period was in order to minimize any stress effect which may be caused by experimental conditions, which could ultimately lead to stress-related elevated ammonia efflux rates (Jobling, 1994). The same fish ($n > 6$) were used within each group for all experiments. Water pH was monitored throughouth the experiment and was not found to alter substantially. The diets used in the experiments were commercial dry, pelletted diets stored in a cool dry place. Fish

Table 1. – Proximate composition of diets A-C (dry matter).

Nutrient	Proximate composition (%)		
	Diet A	Diet B	Diet C
Protein	49.75	45.55	41.10
N.F.E ([1])	16.05	20.40	28.51
Lipid	12.05	16.85	8.00
Fibre	2.00	2.35	4.45
Moisture	8.10	6.05	7.95
Ash	12.05	8.80	9.99
Estimated kcal DE/100 g ([2])	396	445	371
% by Crude Protein	56.5	46.1	49.9

([1]) N.F.E. Nitrogen Free Extract = 100 – (crude protein + crude lipid + moisture + ash + crude fibre).
([2]) DE Digestible Energy.

meal comprised the major dietary protein source in all diets.

Phase 1 (P1): No food was given for a period of 48 h and then each fish was transferred to an individual container (2 l, 25°C) with supplemental aeration, and water samples (1 ml × 2) for subsequent measurement of TA and TN effluxes were taken after 2, 4 and 6 h. The water samples were frozen immediately (–20°C) for later analyses. Each fish was blotted with absorbent paper and its wet weight taken. Fish were then returned to their original holding tanks.

Phase 2 (P2): For 35 d following **P1** fish in groups A-C were fed diets at a rate of 0.5% body weight per day. Protein content of the diets A-C was 49.75%, 45.55% and 41.10% respectively and dietary composition is shown in *table* 1. Fish in group D were not fed in P2. At day 7, 14, 21, 28 and 35, TA, TN and weight data of individual fish were redorded as in P1.

Phase 3 (P3): At the end of **P2**, all fish were fed the original diet C (41.10% protein) at a rate of 0.5% body weight per day. At day 1, 7, 9 and 25 TA and TN efflux data were collected.

Total dissolved ammonia levels in the water samples (1 ml) were measured using a flow injection gas diffusion technique (Hunter and Uglow, 1993). This method consists of a 0.01 M NaOH carrier stream, separated by a polytetraflouroethylene (PTFE) gas permeable membrane, from a 0.5 g l^{-1} solution of bromothymol blue. The ammonia released, following sample injection into the 0.01 M NaOH solution, diffuses across the PTFE membrane and alters the pH and colour of the bromothymol blue. The colour change is then detected colorimetrically. Concentrations of samples were calculated with reference to standards of $NH_4(SO_4)_2$. Total nitrogen levels were determined using a modified micro Kjeldahl technique (Yang, 1993) which converts all the nitrogen-containing compounds in the sample to ammonia. Each sample (1 ml) and 10% (v/v) of the reducing agent (200 ml of 96% H_2SO_4, 134 g K_2SO_4, 2 g $HgSO_4$ and made up to 1 l with distilled water) were heated at 120°C for 12 h and the digest analysed for TN using the flow injection gas diffusion method outlined above. Weight specific values of TA and TN efflux were calculated by the following equation:

$$[\text{Final value } (\mu\text{mol l}^{-1})\text{-Initial value } (\mu\text{mol l}^{-1})] / (\text{Fish weight (g)}) / (\text{Time (h)} \times \text{Volume (l)}).$$

The effects of diet and time on TN, TA efflux rates and growth were assessed statistically using ANOVA; Tukeys test for multiple comparison (Zar, 1984), although fish were individually identified, statistical comparisons were made between groups of fish. The computer package used was SPSS inc. (Windows, MS DOS 6) and statistical significance was taken at $p < 0.05$.

RESULTS

Figure 1 illustrates the ammonia efflux rate data of *C. gariepinus* under the different feeding regimens, for the duration of the experiment (P1-P3). After a period of 48 h starvation (P1) the TA efflux rate of *C. gariepinus* had a grand mean of 0.41 ± 0.02 μmol TA g^{-1} h^{-1}, and no significant among group differences were found. This efflux rate represents the endogenous excretion rates after 48 h on the previous dietary regimen (41.10% protein) of *C. gariepinus* and was lower than any of the values found for the postprandial or fasting efflux rates measured during P2 and P3. All groups showed an increase in TA efflux rate overt P1, during P2 and P3.

During P2 and P3 the control group C (41.10% protein) showed no significant differences in efflux rate and the grand mean of 0.56 ± 0.01 μmol TA g^{-1} h^{-1}, although lower than that of any of the experimental groups, was significantly higher than the mean of TA efflux rates at P1 (*fig.* 2*a*).

Fish fed the 45.55% protein diet (B) also showed a trend of gradual increased efflux rates (day 7-28) during the experiment (*fig.* 2*b*). By day 7, the mean TA efflux rate was significantly higher than that during P1 and by day 28 the mean TA efflux rate was again significantly higher than that of day 7. A rapid, but insignificant, drop in TA efflux rate from 1.17 ± 0.17 to 0.81 ± 0.14 μmol TA g^{-1} h^{-1} followed the start of P3 and efflux rates remained reasonably constant throughout P3. In absolute terms, the TA efflux rate of group B fish (45.55% protein), was intermediate between those of the A and C group fish throughout P2.

In P2 the diet A group (49.75% protein) showed a sharply increased mean TA efflux rate (*fig.* 2*c*). By day 7, such rates doubled the P1 values and levels remained at *ca.* 1.2 μmol TA g^{-1} h^{-1} between day 14-P2 and day 9-P3. By day 25 of P3 the mean

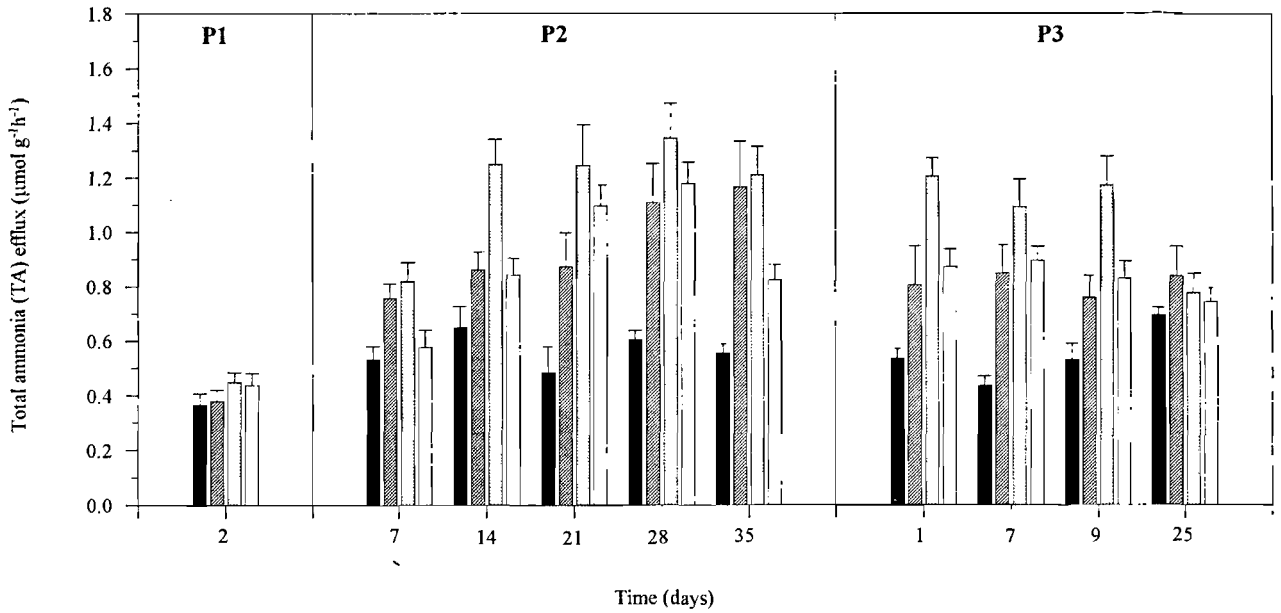

Figure 1. – The effect of diet on the ammonia efflux rates (μmol g^{-1} h^{-1}) *C. gariepinus* under the different feeding regimes. P1: phase 1-48 h fast, P2: phase 2 – a. 41.10% protein diet b. 45.5% protein diet c. 49.75% protein d. non-fed 35 d P3: phase 3 – diet C (41.10% protein).

TA efflux rate for diet A (49.75% protein) group had dropped to 0.78 μmol TA g^{-1} h^{-1}, and was insignificantly different from any of the other groups. In comparison to the other experimental groups, the TA efflux rates of group A (49.75% protein) fish had the highest value which was statistically greater than group C fish (41.10% protein) at least, between P2-day 7 and P3-day 9.

The unfed fish also showed a large increase in TA efflux rate during P2 until day 28. An abrupt decrease then occurred and this reduced rate was maintained throughout P3, despite the recommencement of feeding with diet C (*fig.* 2*d*).

In these studies, the TN efflux rate measured ranged from 0.53-2.01 μmol TN g^{-1} h^{-1} and varied temporally following a similar pattern to that of the relevant TA efflux rate (*fig.* 2). In relative terms, the mean contribution of TA of the TN output ranged between *ca.* 60-100% for all the diet groups throughout the experiment, altthough only statistically significant at P3-day 1, the proportion of TN excreted as TA (%TA/TN) was greatest when fish were fed the 49.75% protein diet.

In P2, the fish fed diet B (45.55% protein) increased their initial weight by *ca.* 32% and showed the highest growth rates (*table* 2). However, none of the values for relative weight gain or SGR among the fed fish groups were significant. By the end of P2 the fasting group of fish incurred a mean weight loss of 31.2% of their initial weight and exhibited a negative SGR of 1.14.

DISCUSSION

Ammonia efflux rates of teleost fish invariably are related to dietary protein intake (Kaushik, 1980; Rychly, 1980; Beamish and Thomas, 1984), and in *C. gariepinus* the levels of TA efflux are also directly related to the proportion of protein in the diet. Porter *et al.* (1987) proposed a dependence of fish TA production upon their feeding regimes – including the source, amino acid balance of the proteins and the proportion of protein to carbohydrate and lipid present.

Modification of protein metabolism in response to dietary change is mediated *via* the activity of enzymes involved in intermediary metabolism. In these studies, following a change in diet, the fish appeared to pass through a dietary acclimation period in terms of nitrogen excretion. This was most noticeable when there was a 10% protein increment. In *C. gariepinus*, high levels of the digestive enzymes pancreatic amylase, gastric lysozyme and gastric and pancreatic protease were found (Uys and Hecht, 1987) and the species is classified as an opportunistic omnivore, which further reflects its possession of many different enzymes to aid digestion of different dietary components (Uys and Hecht, 1987; Uys 1989). However, the change in dietary protein constant – causing an acclimatory response – may have caused changes to the existing enzymes by allosteric or covalent modifications (Lupianez *et al.*, 1989).

Love (1980) suggested that, during fasting, there are differential rates of mobilisation within and between body constituents. The duration of the fasting period will influence the choice of metabolic energy, as

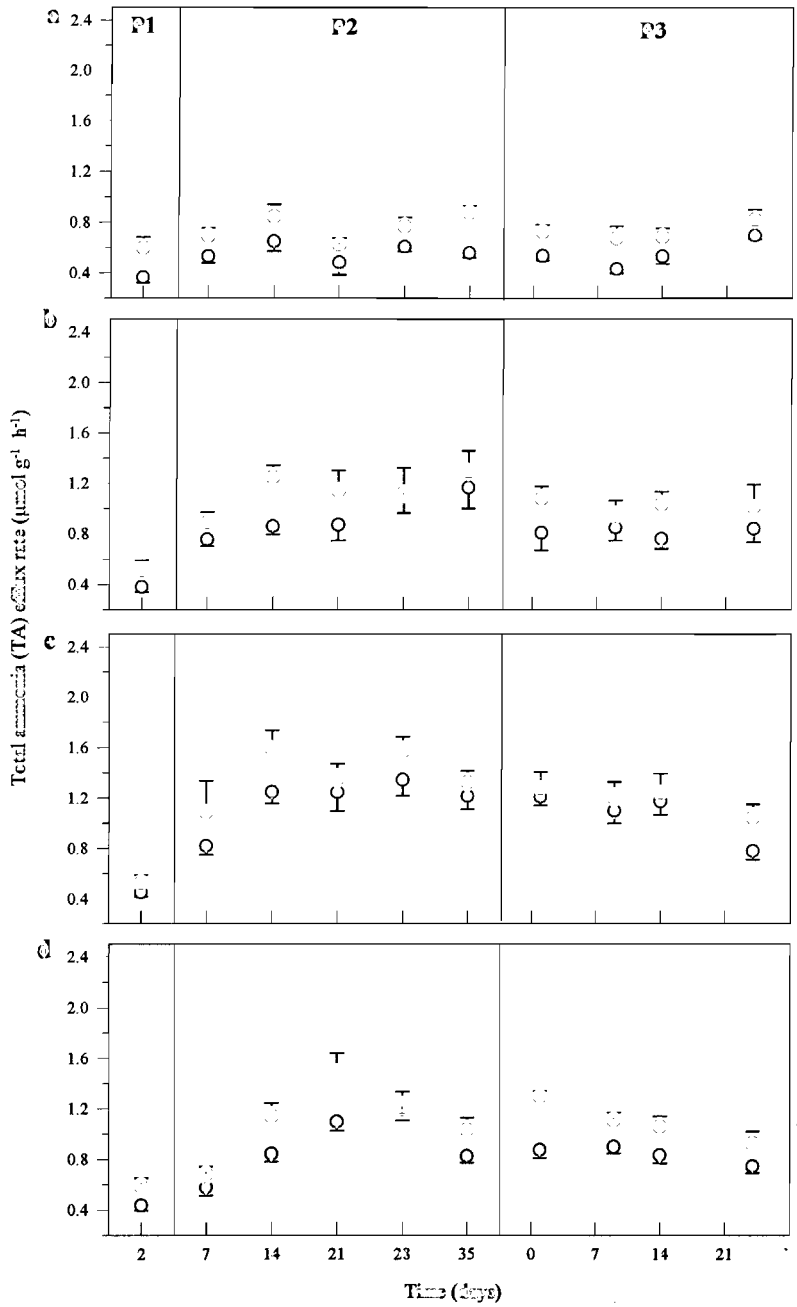

Figure 2. – The effect of diet on total ammonia (TA: ○) and total nitrogen (TN: ●) efflux rates (μmol g^{-1} h^{-1}) of *C. gariepinus*. P1: phase 1-48 h non-fed, P2: phase 2 – **a.** diet C (41.10% protein), **b.** diet B (45.55% protein), **c.** diet A (49.75% protein), **d.** non-fed 35d. P3: phase 3 - diet C (41.10% protein).

will other metabolic factors shuc as protein turnover (McMillan and Houlihan, 1992). Both protein and lipid are known to support fasting metabolism in *C. gariepinus* (Hogendoorn, 1993), and the utilisation of these metabolic resources is presumably reflected by the *ca.* 31% weight loss shown by the fasted group over the 35 d period of P2. The increase in TA efflux rate found at 2-7 d, after the onset of fasting was unlikely to be caused by the mobilisation, and catabolism of muscle protein, but was probably due to the utilisation of availabe amino acids from an unknown endogenous source. This early increase in TA efflux in fasting *C. gariepinus* contrasts with the findings for sockeye salmon, *Oncorhynchus nerka,* (Brett and Zala, 1975) which showed no elevation of TA efflux over a 22 day fast, and for *Cyprinus*

Table 2. – Comparison of the growth of *C. gariepinus* under the four feeding regimens of the experiment throughout phase 2 (35 days). No significant differences observed between the dietary treatments, among the various variables.

Fedding Regime	Initial Weight (g) $\bar{x}$ S.E.	Final Weight (g) $\bar{x}$ S.E.	FW ([1])-IW ([2]) (g) $\bar{x}$ S.E.	% FW-IW/IW $\bar{x}$ S.E.	SGR ([3]) $\bar{x}$ S.E.
49.75% Protein (A)	29.9 (1.5)	36.6 (1.4)	6.8 (0.7)	23.3 (3.1)	0.6 (0.1)
45.55% Protein (B)	29.4 (2.2)	38.9 (2.7)	9.55 (1.2)	32.7 (4.7)	0.8 (0.1)
41.10% Protein (C)	36.0 (1.4)	45.7 (1.8)	9.6 (0.8)	26.8 (2.2)	0.7 (0.1)
Fasted (D)	33.7 (1.7)	23.3 (1.8)	−10.3 (0.5)	−31.2 (2.3)	−1.1 (0.1)

([1]) FW = final weight.
([2]) IW = initial weight.
([3]) SGR = specific growth rate = (ln final wgt − ln initial wgt) × 100 / No. of day

carpio L. where a decrease in nitrogen excretion occurred over 6 fasting days (Infante, 1974). The fasting *C. gariepinus* exhibited sessile behaviour, which is similar to the response of other species during fasting *e.g. Perca fluviatilis* (Mehner and Weiser, 1994), and presumably represents an energy-conserving response.

Ammonia is the principal, but not necessarily the only, nitrogenous metabolic end-product excreted by teleost fish. In this study, the non-TA fraction of the TN efflux within diet groups remained reasonably constant and was not greatly influenced by dietary variations within the experiment, even though the absolute amount of TA and TN excreted were diet-dependent. This agrees with Brett and Zala's (1975) findings for sockeye salmon in which the urea-N fraction accounted for only 21% of the total nitrogen excreted (urea + ammonia) and was independent of feeding patterns. Also, Tatrai (1981) found a continuous rate of urea production that was independent of food quality, quantity and temperatures in *Abramis brama.*

Fish require a well balanced mixture of essential and non-essential amino acids for maximum growth. Uys (1989) found that juvenile and sub-adult *C. gariepinus* had dietary protein requirements of 44-48%, and Machiels and Henken (1985), working with purified feed ingredients, concluded that, irrespective of dietary energy level, this species requires a dietary protein content > 40% for maximum growth. Although no significant differences in growth rate were observed with the fed groups, the highest growth rate was associated with the diet (B) which contained 16% more energy than the other diets. This difference, in addition to between diet-differences in the relative proportions of non-protein energy (carbohydrates and lipids), is intensified at the reduced and fixed feed intake used in these experiments. Diet A had a comparatively high ash content which may have caused a mineral imbalance to occur and thus reduce the growth rate of the group fed this diet. The protein energy of diet A was greater than that in the other diets which could explain the higher ammonia efflux rates shown in this group. The actual growth rates found in these studies are modest in comparison with other published data, probably due to the sub-optimal feeding ration and feeding frequency used (Hogendoorn *et al.*, 1983; Machiels and Henken, 1985). This species is also particularly disposed towards social interactions, hierarchies and gender growth differences – all of which would have been influenced by the low stocking densities used in these experiments.

CONCLUSION

These data show that specific dietary protein levels are a major determinant of nitrogen excretion, particularly ammonia excretion, in *C. gariepinus.* Small differences in relative protein intake evoke distinct patterns of ammonia efflux over a range of dietary protein levels. However, the source of dietary protein, dietary energy and the total available energy also influence nitrogen metabolism. In view of the modest feeding regime used (0.5% BW; 1 meal d^{-1}) and the substantial diet-dependent variability of TA efflux observed, there are clear implications in terms of the intensive feeding of the fish and for the interests of water quality maintenance.

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