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Effects of dietary protein on growth, food consumption and body composition of Nile tilapia (*Oreochromis niloticus* L.)

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Abstract

The effects of dietary protein (25, 35 & 45%) on food consumption, growth and body composition of Nile tilapia, *Oreochromis niloticus* L (mean weight 10.83±0.32g) were investigated. Individual food consumption rates were measured by X-radiography technique. The highest concentration of dietary protein (45%) enabled the fish to grow faster and more efficiently. The diet containing 45% protein appears to be more suitable for Nile tilapia compared to 35% and 25% protein levels (determined by observed rates of SGR, FC, GE, CVc and FCR). An increase in dietary protein level led to an overall improvement in wet weight gain as evidenced by better wet/gain and feed/gain ratios. Increasing dietary protein level from 25% to 45% had no significant effect on the whole-body composition (protein, lipid and ash) of Nile tilapia. The dominant fish in all three diets had better growth rates than subordinate fish. Results from the present study indicated that fast growing dominant fish consuming the most food are in fact the least efficient in terms of nutrient deposition. This may be as a result of the faster growing dominant fish consuming excess of nutrient and energy requirement for maximum lean growth (protein deposition) and there is an associated increase in protein deposition.

Keywords: Nile tilapia, Dietary protein, X-radiography, Growth

Introduction

How the fish regulate the amount of protein and deposit within their bodies is the fundamental question to our understanding of growth and development in fish and is also important in terms of the development of cost-effective diets for using in the aquaculture industry. The dietary protein requirements of several species of tilapia have been estimated to range between 20% and 56% (El-Sayed & Teshima, 1991). Most studies are confined to fry and young tilapia, although the major part of the feed is used during grow-out (Siddiqui *et al.*, 1991). Previous studies have not addressed the question of social hierarchy (dominant and subordinate fish) and its growth relationship with individual nutritional conditions. Therefore, it is important to know the optimum dietary protein requirements for tilapia during the grow-out phase in relation to feeding rank i.e. social hierarchy. In this study, this was investigated by feeding groups of juvenile Nile tilapia (*O. niloticus*) with three isoenergetic diets of varying protein concentrations and measuring different growth parameters. The aims of this study were to examine the relationship between individual protein consumption, day-to-day variability in food consumption, and feed conversion efficiencies with growth of Nile tilapia (*O. niloticus*) fed three experimental diets formulated to be nutritionally identical but with variable levels of protein.

Materials and Methods

The experiment was conducted between 10 August and 15 October 2002, for a duration of 45 days at the Department of Zoology, University of Aberdeen, Scotland, UK. Nile tilapia (*Oreochromis niloticus* L.) fingerlings (5 - 6g) were fed a commercial pelleted diet at the rate of 2.5% body weight day^{-1} until weighing 10g each. Prior to start the experiment, 20 fish were

killed for initial body composition analysis and 135 fish were randomly assigned to nine identical 65 litre (water volume 60L) glass tanks so that the stocking density was 15 fish per tank. Each individual fish was freeze branded (marked) by liquid nitrogen (Ali 2001). By using different combinations of freeze branded marks on each fish could be individually identified and therefore each fish could be monitored throughout the experiment. Three treatments each with three replications of different protein level diets were designated as: Diet 1 (45% protein), Diet 2 (35% protein) and Diet 3 (25% protein). The water temperature was maintained between 27 and 29°C and the photoperiod was regulated as 12 h light and 12 h darkness. The different protein level diets were prepared by using a raw materials premix (made up from fishmeal, oil mix, Hi-pro, corn gluten, wheat, Bio vits, Bio mins, Vit- E and mono calcium phosphate) and (Table 1). The three experimental diets were formulated to be isoenergetic but with variable concentrations of protein (Table 2.). To make the three experimental diets, all the dietary raw materials were ground and sieved to produce a fine powder and mixed. Each diet was then pelleted (2 mm) using a laboratory pellet mill and dried at 60°C to a constant weight. A part of these three mixed diets were kept to prepare X-ray labelled diets. For the measurement of individual food consumption, the normal feed was replaced by feed containing radio-opaque ballotini (size 30, 0.40 - 0.60 mm, British Optical Ltd., Walsall). The marked feed was prepared by grinding the normal feed, and ballotini (2.5% of the food wet weight) and water (15% of the food wet weight) were added. The feed was then mixed for three hours and repelleted using a California Pellet Mill (pellet size was 2 mm) and dried overnight 70°C and stored at 0°C until required. Dry weight of the experimental feed was measured following the usual procedures described by AOAC (1983). The nutritional compositions of the raw material premix were: protein 51.60 %, lipid 13.20 %, moisture11.64 %, ash 8.50 % and carbohydrates 15.06 %.

Table 1. The raw material used for the three experimental diets (Source: Biomar Feed Company, Scotland, UK)

Ingredients	Diet 1 (%)	Diet 2 (%)	Diet 3 (%)
Raw material premix	87.00	68.00	49.00
Fish oil	2.00	5.00	8.00
Starch	5.30	20.50	35.06
Non nutritive bulk	5.00	4.60	4.69
Mono Calcium Phosphate (MCP)	0.70	1.68	2.80
Vitamin premix	-	0.12	0.25
Mineral premix	-	0.10	0.20

The fish were fed with the three experimental diets at the rate of 2.5% body weight once a day (7 days a week) in the morning between 9:00 - 10:00 h and the ration was adjusted after every sampling occasion.

The dry matter, protein, fat, and ash contents of the three experimental diets and fish were determined (AOAC, 1983). Briefly, the dry matter of the diets (n = 3) and whole body of the initial fish (n = 18) and final sample of fish (n = 10 fish diet^{-1}) were determined by freeze-drying to a constant weight. The protein concentration of the fish and the diets were determined using the Lowry (Lowry et al., 1951) method. Lipid content of the fish and diets were determined by the Soxhlet method. Ash contents were determined by combustion at 550°C to a constant weight. The chemical compositions of the three experimental diets are shown in Table 2.

Table 2. Nutritional composition of the three experimental diets

Dietary constituents (%)	Diet 1	Diet 2	Diet 3
Protein	45.46	35.43	25.14
Lipid	14.10	14.30	15.20
Carbohydrate	12.52	23.24	34.14
Ash	17.95	17.43	17.08
Dry matter	96.03	95.03	93.25
Gross Energy (kJ g^{-1})	18.45	18.01	17.94

To measure individual rates of food consumed by fish, the experimental diets were used to produce labelled diets suitable for use with X-radiography and containing ballotini glass beads (size 8/9, 0.040-0.445 mm; Jencons Scientific Ltd.). The diets were homogenised with a blender and ballotini glass beads added at 2.5% of the weight of the food. The diets were then mixed thoroughly, 10% distilled water added and the mixture was re-pelleted using a laboratory pellet mill (2 mm) and then dried at 60°C to a constant weight. To calculate the relationship between the amount of food consumed by each fish and the number of glass beads present in the digestive tract of each fish, a calibration line was calculated for each of the experimental diets. Individual food consumption, body weight, and body length were measured on days 25, 32 and 39. On these days, the ballotini labelled diets were fed to the fish at the same time and in the same manner as usually adopted. Approximately one-hour post feeding, the fish were anaesthetised (4% benzocaine in ethanol, 0.15 g l^{-1}) and X-rayed. The fish were handled quickly and immediately returned to the tanks after the procedure. The exposed X-ray films were then developed and the number of glass beads presents within the digestive tract, clearly visible on the X-rays, was used to calculate amount of food eaten from the known relationship between the numbers of glass beeds for per milligram of food (McCarthy *et al.* 1993).

The amount of food (dry) consumed (FC) by each fish was calculated from the diet calibration equation for each of the diets (McCarthy *et al.*, 1992). The share of the group meal (SM, %) was calculated as the proportion of the total feed consumed by the group, which is consumed by an individual fish. As repeat measurements of consumption were made in this study, the mean share of the group meal (MSM, %) for each fish over the experiment was calculated (McCarthy *et al.*, 1992). The intra-fish variation in food consumption was calculated using the coefficient of variation (CV_c, %) (Jobling *et al.*1995). The equation (Ricker, 1979) used to calculate the specific growth rate (SGR, % day^{-1}). The growth efficiency (GE, %) is calculated from the daily rates of consumption (% bw day^{-1}) and the daily growth rates (% day^{-1}): GE (%) = $SGR/CM \times 100$; Where, SGR is the specific growth rate (% day^{-1}) and CM is the daily dry food consumption rates (% bw day^{-1}).

One way analysis of variance (ANOVA) was used to compare initial and final body weights of the groups of fish fed the experimental diets (using SPSS 9.0 statistical package for Windows 95/98). Coefficient of variation (CV, %) was used to assess the degree of inter-fish variation in SGR (CV_{SGR}), MSM (CV_{MSM}), GE (CV_{GE}) and consumption (CV_C). Regression equation was used to investigate the relationships between SGR, MSM, GE and weight specific consumption. A probability level of 5% ($P < 0.05$) was considered significant in all tests.

Results and Discussion

There were no significant differences in the mean initial wet weights of the three experimental groups of fish used in the experiment. By the end of the experiment, the groups of the fish fed diet 1 (45% protein) had significantly higher mean wet weights followed by the groups fed diet 2 (35% protein) and then diet 3 (25% protein) (Table 3).

Table 3. Mean (± S.E, n = 15) initial, final mean body weight, specific growth rate, food consumption rate, growth efficiency, feed conversion ratio and also intra-fish variation (CV) in consumption and specific growth rate for fish fed the three experimental diets. Mean values sharing a common superscript (down columns) are not statistically different at the 5% significance level (Scheffe's multiple comparison test)

Diets	Initial weight (g)	Final weight (g)	SGR_m (% day^{-1})	FC_m (% bw day^{-1})	GE_m (%)	FCR_m (mg g^{-1})	CV_{CON}	CV_{SGR}
Diet 1 (45% P)	11.09 ± 0.28^a	21.19 ± 1.14^a	1.65 ± 0.11^a	1.86 ± 0.14^a	93.26 ± 5.74^a	1077 ± 80^c	14.54 ± 2.87^a	41.64 ± 4.37^c
Diet 2 (35% P)	10.83 ± 0.32^a	16.63 ± 0.74^b	1.10 ± 0.08^b	2.00 ± 0.16^a	62.33 ± 4.05^c	1798 ± 263^{ab}	24.41 ± 4.09^b	54.10 ± 4.37^{ab}
Diet 3 (25% P)	10.81 ± 0.30^a	16.20 ± 0.67^b	1.03 ± 0.08^b	2.00 ± 0.18^a	54.69 ± 4.54^c	1916 ± 142^b	23.09 ± 3.94^b	60.65 ± 5.73^{ab}

There were no significant differences in the protein, lipid, and ash content of the initial sample of fish compared to the final sample of fish fed different protein level diets (Table 4). Over the experimental period the carcass moisture content of the fish decreased with an associated decrease in protein levels for fish fed on diets 2 and 3 respectively.

Table 4. Initial and final mean (± S.E, n = 10 fish $diet^{-1}$) whole body composition of experimental fish. Mean values sharing a common superscript (down columns) are not statistically different at the 5% significance level (Scheffe's multiple comparison test)

Whole body composition	Initial (%)	Diet 1 (45% P)	Diet 2 (35% P)	Diet 3 (25% P)
		Final (%)	Final (%)	Final (%)
Moisture	74.61 ± 1.01^a	73.32 ± 0.68^a	71.64 ± 0.75^b	70.98 ± 0.59^b
Protein	11.48 ± 0.53^a	11.11 ± 0.56^a	11.17 ± 0.48^a	11.07 ± 0.35^a
Lipid	08.43 ± 0.62^a	09.33 ± 1.04^a	09.27 ± 0.90^a	09.82 ± 0.61^a
Ash	19.59 ± 1.15^a	17.95 ± 1.14^a	17.35 ± 1.00^a	17.08 ± 2.00^a

The mean specific growth rates (SGR % day^{-1}) of the groups of fish fed diet 1 were significantly higher than the mean specific growth rates for the groups of fish fed diets 2 and 3. Fish fed diet 1 had significantly higher growth efficiencies (GE, %) and significantly lower feed conversion ratios (FCR, mg dry food g^{-1} wet weight fish) than fish fed diet 2 and 3 (Table 3).

Table 3 shows the coefficient of variation (CV, %) in food consumption (FC, % bw day^{-1}), specific growth rate (SGR, % day^{-1}) and mean share of meal (MSM, %) for all groups of fish. The groups of fish fed diet 1 showed significantly lower mean coefficient of variations in consumption, specific growth and mean share of meal compared to fish fed diet 2 and followed by diet 3.

The mean share of meal of individual fish in each diet is shown in Fig. 1. There were no obvious differences in the feeding hierarchies between the groups of fish eating diet 1, 2 and 3.

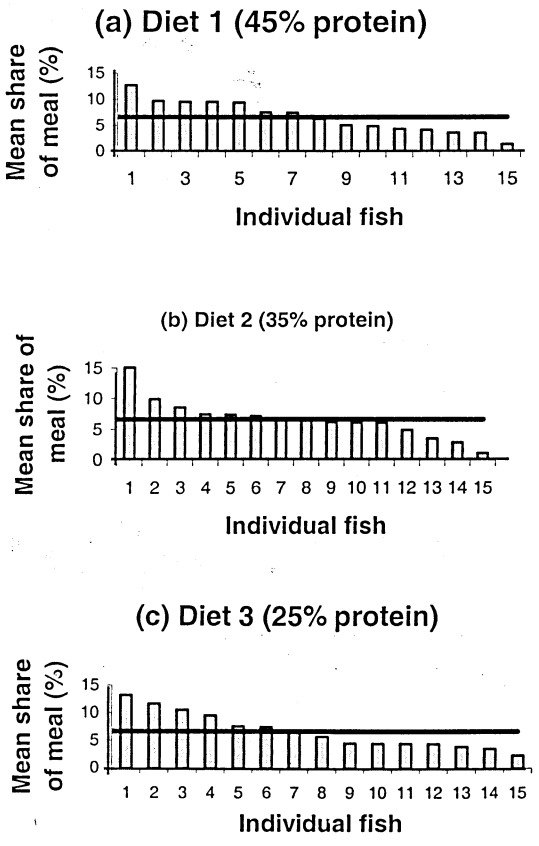

Fig. 1. Mean share of the meal (MSM, %) for individual fish ranked according to social rank in the three experimental diets of fish fed diet 1 in (a), diet 2 in (b) and diet 3 in (c) calculated from 3 X-rays. The horizontal line across the histograms is the equal share for 15 animals i.e. a 7% share of the meal

Figs. 2a, b and c shows the relationship between the specific growth rates and food consumption in fish fed diet 1, 2 and 3, respectively. In all cases, there is a significant positive relationship between rates of specific growth and food consumption.

Figs. 3a, b and c shows a significant negative relationship between the mean share of the meal (MSM %) and growth efficiencies (GE, %) in fish fed diets 1, 2 and 3 respectively.

Fig. 4 shows the significant positive relationship between the amount of protein consumed (mg protein g^{-1} of fish day^{-1}) and the amount of protein deposited (mg protein g^{-1} of fish day^{-1}) by individual fish fed diets 1, 2 and 3. There were significant differences in the regression coefficients between the fish fed diet 1 compared to diets 2 and 3 (no significant differences in the regression coefficients between fish fed diet 2 and 3). Significantly better regression coefficients observed for fish fed diet 1 clearly indicated that fish consuming diet 1 deposited significantly more protein compared with those fed diets 2 and 3.

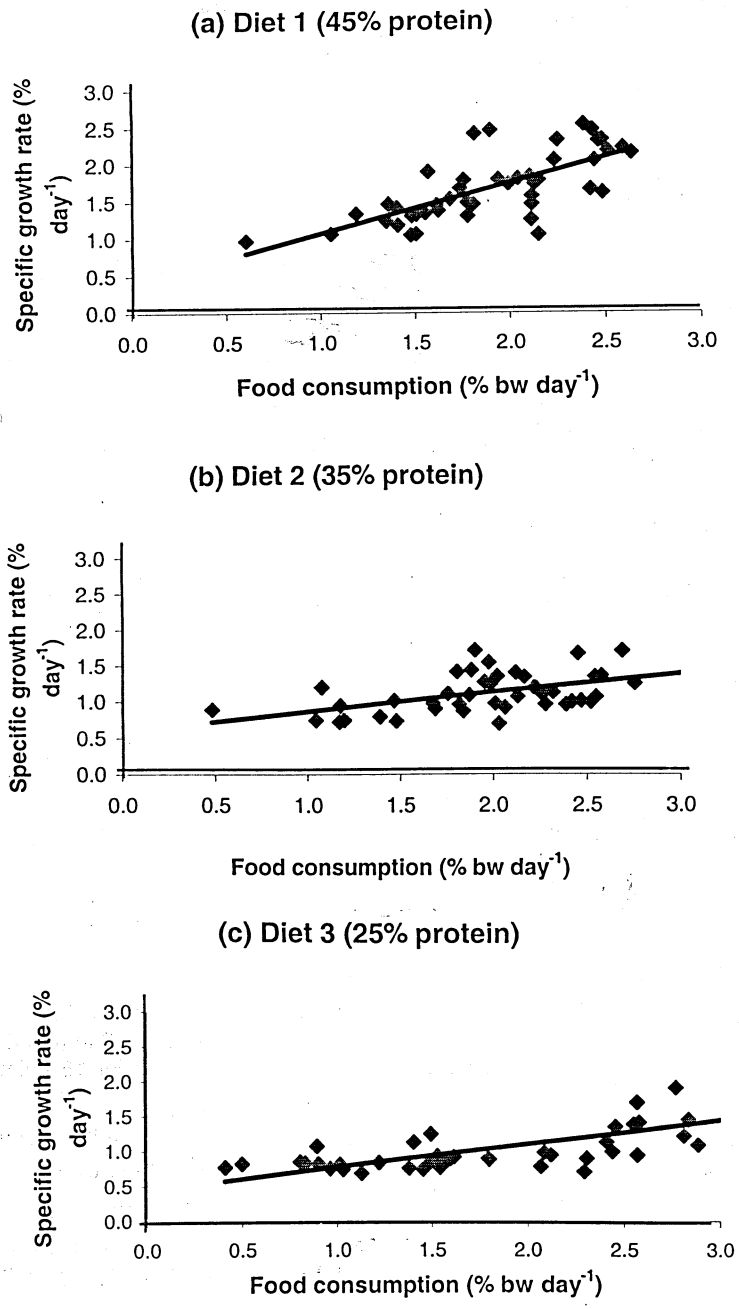

Fig. 2. Relationships between the mean specific growth rates (SGR, % day^{-1}) and the mean rates of food consumption (FC, % bw day^{-1}) in individual fish fed three experimental diets. Data are pooled from the 3 tanks (n = 45) for each diet

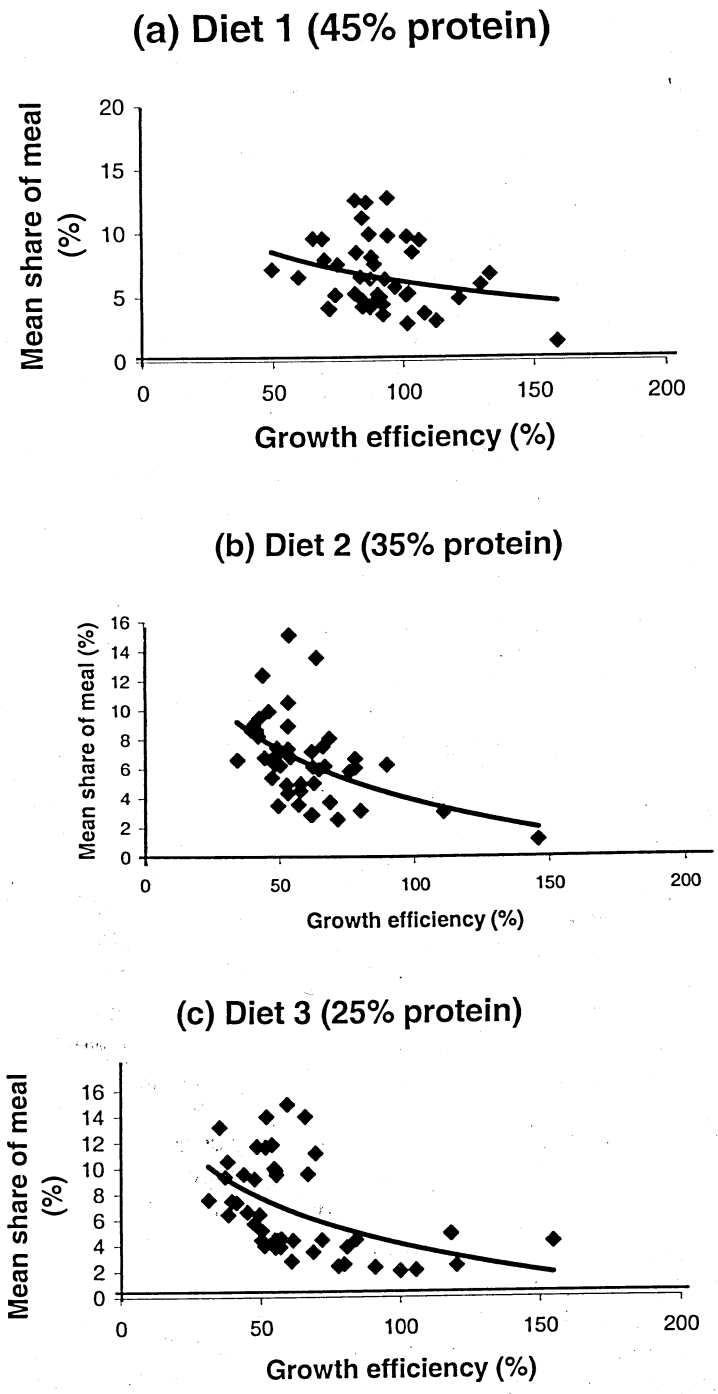

Fig. 3. Relationships between mean share of meal (MSM, %) and growth efficiency (GE, %) of the experimental fish fed diet 1 in (a), diet 2 in (b) and diet 3 (c). Data are pooled from the 3 tanks (n = 45) for each diet

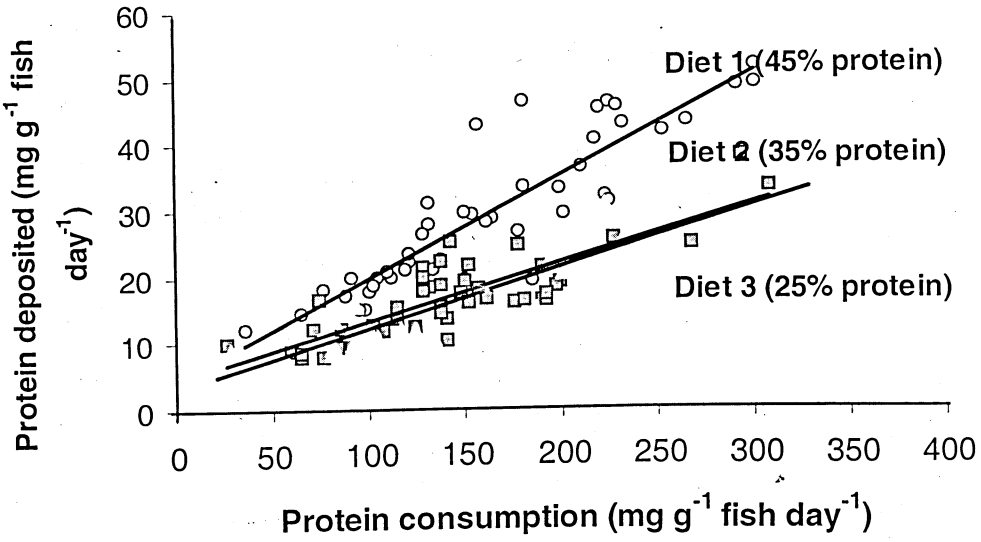

Fig. 4. The relationship between protein consumption (PC) and protein deposition (PD) in experimental fish fed diets 1, 2 and 3. Regression equation for **diet 1** (PD = 0.043952 + 0.109762 × PC, R^2 = 0.8257, P < 0.001, n = 45), **diet 2** (PD = -0.023389 + 0.109762 × PC, R^2 = 0.7938, P < 0.001, n = 45) and **diet 3** (PD = -0.020563 + 0.109762 × PC, R^2 = 0.6860, P < 0.001, n = 45). Data are pooled from the 3 tanks (n = 45) for each diet

The results presented in Table 3 clearly showed that the higher dietary protein concentration in diet 1 resulted in better growth compared with diets 2 and 3. The associated increase in growth was not due to higher daily consumption of diet 1 (not significantly different from diet 2 and 3) but from more efficient deposition of nutrients (significantly higher growth efficiencies and lower feed conversion ratios) in diet 1. Table 3 showed that the fish consuming the highest protein level diet (diet 1) had significantly lower variation in consumption as indicated by the CV values for both consumption and specific growth rate compared to values for fish fed diets 2 followed by 3. This indicates that the fish fed diet 1 were not only growing faster and more efficiently but were also growing more evenly due to less variation in their daily consumption rates when compared to fish fed diets 2 and 3, which suggests a lower establishment of a social hierarchy in diet 1. The body composition results (Table 4) showed that the only differences between fish fed the three diets was significantly higher moisture contents of fish fed diet 1, this was probably due to these fish growing at a faster rate than diet 2 and 3. Al Hafedh (1999) reported that the whole-body composition of Nile tilapia was influenced significantly by the dietary protein level when dealing with small fish (initial weight 0.51g). However, in Al Hafedh's (1999) study when dealing with adult fish (45-96g), no apparent influences of dietary protein level was found on whole-body composition which is in agreement with the present study where increasing dietary protein level had no significant effects on the whole-body composition of Nile tilapia. In addition, the results of the present study agree with Wee & Tuan (1988), where they reported growth of *O. niloticus* increased with increasing dietary protein level from 20% to 50%. Chang *et al.*, (1988) also reported a better growth in tilapia fed a high-protein (44%) diet rather than low-protein diets (21 and 27%

protein). For other tilapia species, the growth of *O. mossambicus* (Peters) fry declined at a dietary protein concentration above 40% (Jauncey, 1982), and for *T. zillii* (Gervias) above 35% (Mazid *et al.*, 1979). Considerable variations have been reported in the optimum dietary protein requirement for maximum growth for tilapia. These variations appear to be the results of different experimental conditions, and fish species, size, age of fish, stocking density, protein quality, hygiene, and environmental conditions, particularly temperature, which has been found to influence dietary protein requirement in tilapia (El-Sayed & Teshima, 1991).

In the present study, food consumption increased with increasing specific growth rates for fish fed all three diets (Figures 3) and feed conversion ratios (FCR) decreased with increasing dietary protein level (Table 1). A significant negative correlation was found in fish fed diet 1 (Figure 3a) but no such relationships were observed in fish fed diet 2 and 3 (Figures 3b & 3c). In several species of fish, the social rank of individuals has been found to depend upon the initial weight of the fish when placed within a group, with larger fish obtaining a higher social position than smaller fish. This suggests that the larger size of the dominant fish at the end of the experiment was as a result of dominance rather than its cause. This may have occurred because there was little variation in initial body weight and the dominant fish became larger due to their aggressive nature and urge to feed rather than having the advantages of initially being larger. There was a close relationship between MSM and growth rates, suggesting that MSM may be a useful indicator of dominance position in a social group (McCarthy *et al.*, 1999). It is generally accepted that when fish grow fast the efficiency of nutrient deposition is at its highest (Mazid *et al.*, 1979). Results from this present study indicate that fast growing dominant fish consuming the most food are in fact the least efficient in terms of nutrient deposition. This may be as a result of the faster growing dominant fish consuming excess of nutrient and energy requirement for maximum lean growth (protein deposition) and there is an associated increase in protein deposition.

References

Ali M.S. 2001. The effects of salinity and food consumption on growth and protein turnover in nile tilapia (O*reochromis niloticus* linnaeus). PhD Thesis. Department of Zoology, University of Aberdeen, UK.

Al Hafedh Y.S. 1999. Effects of dietary protein on growth and body composition of Nile tilapia, *Oreochromis noloticus* L. *Aquaculture Research* **30,** 385-393.

AOAC (Association of Official Agricultural Chemists), 1983. Official Methods of Analysis, 13th Edition, AOAC, Washington, DC.

Chang S., Huang C. & Liao I. 1988. The effect of various feeds on seed production by Taiwanese red tilapia. **In:** *The Second International Symposium on Tilapia in Aquaculture* (eds. R.S.V. Pullin; T.Rhukasawan; K. Tonguthal & J.L. Maclean), pp. 319-322. ICLARM, Manila, Philippines.

El-Sayed A-F.M. & Teshima S. 1991. Tilapia nutrition in aquaculture. *Reviews in Aquatic Science* **5,** 247-265.

Jauncey K. 1982. The effects of varying dietary protein level on the growth, food conversion, protein utilisation and body composition of juvenile tulips (*Saratherodon mossambicus*). *Aquaculture* **27,** 43-54.

Jobling M., Arnesen A.M., Baardvik B.M., Christiansen J.S. & Jorgensen. E.H. 1995. Monitoring voluntary feed intake under practical conditions, methods and applications. *Journal of Applied Ichthyology* **11 (3-4),** 248-262.

Lowry O.H., Rosebrough N.J., Farr A.L. & Randall R.J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193,** 265-275.

Mazid M.A., Tanaka Y., Katayama T., Asadur Rahman M., Simpson K.L. & Cichister C.O. 1979. Growth response of *Tilapia zillii* fingerlings fed isocaloric diets with variable protein levels. *Aquaculture* **18,** 115-12.

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

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

McCarthy I.D., Gair D.J. & Houlihan D.F. 1999. Feeding rank and dominance in *Tilapia rendalli* under defensible and indefensible patterns of food distribution. *Journal of Fish Biology* **55,** 854-867.

Ricker W.E. 1979. Growth rates and models. **In:** *Fish Physiology* **Vol. VIII,** (eds. W.S. Hoar, D.J. Randall & J.R. Brett), pp. 677-743. Academic press Inc., New York.

Siddiqui A.Q., Howlader M.S. & Adam A.A. 1991. Management strategies for intensive culture of Nile Tilapia (*Oreochromis niloticus*) in tanks using drainage water in Al Hassa region of Saudi Arabia. *Arab Gulf Journal of Scientific Research* **9,** 149-163.

Wee K.L. & Tuan N.A. 1988. The effects of dietary protein level on growth and reproduction in Nile tilapia (*Oreochromis niloticus*). **In:** *The Second International Symposium on Tilapia in Aquaculture* (eds. R.S.V. Pullin, T. Bhukaswan, K.Tonguthal & J.L. Maclean), pp. 401-410. ICLARM, Manila, Philippines.