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The Effect of Dietary Protein and Water Temperatures on Performance of *T. rendalli* Juveniles Reared in Indoor Tanks

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Abstract: An experiment was conducted at Bunda College of Agriculture, Lilongwe, Malawi for 14 weeks (from August to November 2005) to determine the combined effect of dietary protein (30 and 40%) and water temperatures (22.8, 26 and 30°C) on the performance of *Tilapia rendalli* juveniles reared in eighteen 200-L tanks with stocking rates of 15 fish (average wt 9.25g/fish) per tank. Results showed that the treatment of 40% crude protein at 30°C temperature produced significantly ($p<0.05$) greater growth, with a weight gain of 220.55%. Whole body moisture, protein and ash content increased and differences among treatments were highly significant ($p<0.05$) among treatments. Whole body lipid decreased in the treatment with 40% crude protein fed at 26°C, while moisture, protein, ash and energy increased in all treatments. Blood glucose and haematocrit values did not differ significantly across all treatments. Dietary protein and water temperature significantly influenced changes in body weight, Specific Growth Rate (SGR) and feed conversion ratio of *T. rendalli*. The experiment showed that high dietary protein and water temperature produced the best feed conversion ratio (FCR) of 2.74, but this was still higher than that recommended by many researchers. Temperature in particular, had a significant effect on the growth of *T. rendalli* at different dietary protein levels. Growth of fish increased with an increase in dietary protein and water temperature. Survival was over 90% across treatments.

Key words: *Tilapia rendalli,* feed utilization, water temperature, dietary protein level, body composition, blood glucose, haematocrit

INTRODUCTION

Tilapia rendalli (common name: red-breasted tilapia), the second commonly cultured species in Malawi (Masuda *et al*., 2004) is important for: commercial aquaculture, game fish and aquarium (Fish Base, 2006). It is predominantly herbivorous, feeding on higher aquatic plants. Juveniles feed on plankton while adults feed on macrophytes, algae, insects, crustaceans, aquatic invertebrates and small fish (Konings, 1990; Lowe-McConnell, 2000; Skelton, 2001). With respect to prepared fish diet, dietary protein is always considered to be the most important nutrient component of complete formulated fish feeds (Jauncey, 2000). The protein requirements are also higher for fish reared at high density (recirculating aquaculture) than in low-density (pond aquaculture) systems (Craig and Helfrich, 2002). Temperature is a factor of great importance for aquatic ecosystems, as it affects the organisms as well as the chemical and physical characteristics of water (Delince, 1992). It has a powerful effect on growth, both through its influence on rates of biochemical processes of metabolism and other factors such as feed intake (Ross, 2000). All biological and chemical processes in aquaculture are influenced by temperature (Buttner *et al*., 1993). This study was conducted to determine the effect of dietary protein levels and water temperatures on growth, feed utilization, whole body composition, blood glucose and haematocrit in *T. rendalli*.

MATERIALS AND METHODS

A 14 week study was conducted in the wet laboratory at Bunda College Fish farm, Lilongwe, Malawi to determine effects of dietary protein (30 and 40%) and water temperatures (22.8, 26 and 30°C) on the performance of juvenile *Tilapia rendalli* (Mean SD wt. 9.25±1.90 g) using eighteen 200-L clear circular plastic tanks.

The study was a 3 by 2 design factorial (3 temperatures ×2 crude protein levels) run in a completely randomized experimental design with three replicates. Two hundred and seventy fish were randomly distributed among 18 tanks at a density of 15 fish per tank. Treatment combinations were: 30% crude protein with 22.8°C, 30% crude protein with 26°C, 30% crude protein with 30°C, 40% crude protein with 26°C, 40% crude protein with 30°C and 40% crude protein with 22.9°C. Automatic aquarium heaters (Tetratec® HT 200) were placed directly into each of twelve tanks to maintain water temperature at 26.21 and 30.23°C while daily average ambient temperature was 22.84°C. The fish were acclimatized for a week in the experimental tanks before the start of the experiment. Fish were not fed 24 h prior to sampling.

Two practical diets (30 and 40% crude protein levels) with the same dietary energy level (19.51±0.04 kJ/g) were formulated using Pearson Square Method from fishmeal, soybean meal, rice bran, maize bran, wheat bran, Kazinga vegetable oil, vitamin and mineral

premixes. Snowflake wheat flour was used as binder. Feed was presented to fish in form of pellets of 2.5 mm diameter twice (09:00 and 14:00 h), at 4% body weight per day throughout the experimental period.

Selected water quality parameters were measured in each tank. To maintain suitable water quality, fish excreta and leftover feed were siphoned daily after fish had ceased feeding. Daily partial replacement of water was done in all tanks, while partial cleaning of tanks was done twice per week and complete cleaning was done fortnightly during fish measurement. The growth of fish was monitored fortnightly. Body weights and lengths for all fish from each tank were recorded after 24 h without feeding. A graduated measuring board was used for length (cm) measurement of fish and an electronic scale was used for fish weight to 3 decimal units. At the termination of the study, fish were harvested, weighed and measured individually to obtain a final mean body weight and length. Various procedures were used to assess growth, feed utilization and weight gains of *T. rendalli*.

Specific Growth Rate (%SGR) expressed as percentage body weight per day was calculated using the equation illustrated by Westers (2001). SGR (%/day) = 100 [(ln Wt-ln W_1)/t], where: ln (W_t) is the natural logarithm of the weight at time t and ln (W_1) is the natural logarithm of the initial weight. Feed Conversion Ratio (FCR) was calculated by dividing the dry weight of feed offered in a given period of time by the wet weight gained (g) (Wee and Shu, 1989; Stickney, 1994):

$$FCR = \frac{\text{Total dry feed offered (g)}}{\text{Total live weight gained by fish (g)}}$$

At the beginning of the experiment 32 fish were randomly sampled after 24 h of food deprivation to determine their initial carcass composition.

At the end of the experiment, a random sample of 60 fish (10 from each treatment) were collected and killed to determine their final whole body composition. This was intended to determine changes in body composition of the fish. The initial blood glucose value of thirteen fish randomly selected from the holding tank was determined using end filling method and analyzed by MediSense Optium Sensor. At the end of 14 weeks growing period, blood samples were collected from ten fish in each treatment for blood glucose determination. Fourteen fish were randomly selected from the holding tank for initial haematocrit determination. The base of the tail was severed with a scalpel blade and blood was drawn into a heparinized capillary tube of 1.1-1.2 mm internal diameter. The end of the tube was closed with Critoseal (a commercial sealant). Micro-haematocrit centrifuge (Hawksley) was used to centrifuge blood samples at 12,000 revolutions/minute for 4 min. Haematocrit reading was observed visually using the percentage volume (i.e., packed cell volume) of a haematocrit tube. Each capillary tube was slid along the chart until the meniscus of the plasma crossed the 100% and height of the packed red cell column was read off directly as percentage cell volume. At the end of the growing period, 60 fish (10 from each treatment) were randomly selected for haematocrit determination.

SPSS (Version 12) for windows was used to analysis the data. Analysis of variance (ANOVA) was used to determine significant effects of dietary protein levels and water temperatures on fish growth. Duncan Multiple-Range Test (DMRT) was used to determine significant differences ($p<0.05$) between treatment means.

RESULTS

There were highly significant differences ($p<0.05$) in growth of fish among treatments. Fish in a treatment with 40% crude protein and 30°C showed the best ($p<0.05$) growth while growth in the treatment with 40% crude protein and 22.89°C was the lowest (Fig. 1). The final average weight gain for the treatment with 40% crude protein and 30°C was 220.55%, followed by the treatment with 40% crude protein and 26°C with a percent weight gain of 208.10%. The treatment with 30% crude protein and 26°C produced average percent weight gain of 180.85%, followed by the treatment with 30% crude protein and 30°C with 174.10%. The treatment with 30% crude protein and 22.8°C, had an the average percent weight gain of 88.85%. Finally, the treatment with 40% crude protein and 22.8°C showed the lowest average weight gain of 75.71% (Table 1).

The final mean body weight of fish varied from 16.06±1.25-31.19±1.25 g with highly significant differences between treatments ($p<0.05$) (Table 1). Treatments with 40% crude protein and 26°C as well as 40% crude protein and 30°C were significantly different from each other and also from the rest, but treatments 30% crude protein and 26°C and 30% crude protein and 30°C were not. Similarly, treatments with 30% crude protein and 22.79°C and 40% crude protein and 22.89°C were not significantly different ($p>0.05$).

A similar response was observed with specific growth rate. It was highest in the treatment with 40% crude protein and 30°C (1.19%/day), followed by treatment with 40% crude protein and 26°C (1.15%/day), then treatment with 30% crude protein and 26°C (1.05%/day), treatment with 30% crude protein and 30°C (1.03%/day) then treatment with 30% crude protein and 22.79°C (0.65%/day) and lastly, treatment with 40% crude protein and 22.89°C (0.58%/day (Table 1). In general, performance of fish was satisfactory and they responded well to dietary protein and water temperature.

Effect of dietary protein and water temperature on feed utilization (FCR): Feed Conversion Ratio (FCR) results followed the same pattern as other previous parameters (Table 2). The best feed conversion rate

Table 1: Initial and final mean body weights, weight gain, weight gain/day, percent weight gain, specific growth rate (SGR), Feed conversion ratio (FCR) and CV of *T. rendalli* juveniles cultured in indoor tanks for 14 weeks*

	Treatments					
Parameter	30%CP, 22.79°C	30%CP, 26°C	30%CP, 30°C	40%CP, 26°C	40%CP, 30°C	40%CP, 22.89°C
Initial mean weight (g/fish)	*9.24±0.29[a]	9.19±0.29[a]	9.19±0.29[a]	9.01±0.29[a]	9.73±0.29[a]	9.14±0.29[a]
Final mean weight (g/fish)	17.45±1.25[a]	25.81±1.26[b]	25.19±1.25[b]	27.76±1.26[c]	31.19±1.25[d]	16.06±1.25[a]
Weight gain (g/fish)	8.21	16.62	16.00	18.75	21.46	6.92
Weight gain/day	0.08	0.17	0.16	0.19	0.22	0.07
Weight gain (%)	88.85	180.85	174.10	208.10	220.55	75.71
SGR (%/day)	0.65	1.05	1.03	1.15	1.19	0.58
CV of individual weights (%)	21.26	38.70	33.70	34.91	37.22	16.25

*Values are means±S.E. of triplicate tanks (n=3) holding 15 fish each. Means within same column not sharing a common superscript are significantly different ($p<0.05$)

Table 2: Interactive effect of dietary protein levels and water temperatures on fish growth and Feed Conversion Ratio (FCR)

Crude protein (%CP)	Water temperature (°C)	Final wt. (g)	Mean wt (g)	Overall SGR (%/day)	Mean SGR (%/day)	FCR
30	22.79	17.45		0.65		4.54
30	26	25.81		1.05		2.97
30	30	25.19	22.82	1.03	0.91	3.13
40	22.89	16.76		0.58		4.82
40	26	27.76		1.15		2.77
40	30	31.19	25.24	1.19	0.97	2.74

Table 3: Interactive effect of water temperature and dietary protein levels on fish growth and Feed Conversion Ratio (FCR)

Water temperature (°C)	Crude Protein (%CP)	Final weight (g)	Mean weight (g)	Overall SGR (%/day)	Mean SGR (%/day)	FCR	Mean FCR
22.79	30	17.45		0.65		4.54	
22.89	40	16.76	17.11	0.58	0.62	4.82	4.68
26	30	25.81		1.05		2.97	
26	40	27.76	26.79	1.15	1.10	3.13	3.05
30	30	25.19		1.03		2.77	
30	40	31.19	28.19	1.19	1.11	2.74	2.76

(FCR) was 2.74 in treatment with 40% crude protein and 30°C. Treatment with 40% crude protein and 26°C was second, followed by treatment with 30% crude protein and 26°C, treatment with 30% crude protein and 30°C, treatment with 30% crude protein and 22.79°C and lastly, treatment with 40% crude protein and 22.89°C. Feed conversion ratio decreased with an increase in dietary protein level at low temperature (Table 2). The coefficient of variation (CV%) indicated that the treatment with 40% crude protein and 22.89°C had the lowest value of 16.25% and treatment with 30% crude protein and 26°C had the highest CV of 38.70% (Table 2).

Effect of water temperature across dietary protein levels on growth and Feed Conversion Ratio (FCR): Across the two dietary protein levels, growth increased with an increase in water temperature (Table 2). However, there was a decrease in Feed Conversion Ratio (FCR), suggesting an increase in feed conversion efficiency.

Effect of dietary protein across three water temperatures on growth and Feed Conversion Ratio (FCR): Across the three water temperatures there was an increase in mean body weight and specific growth rate

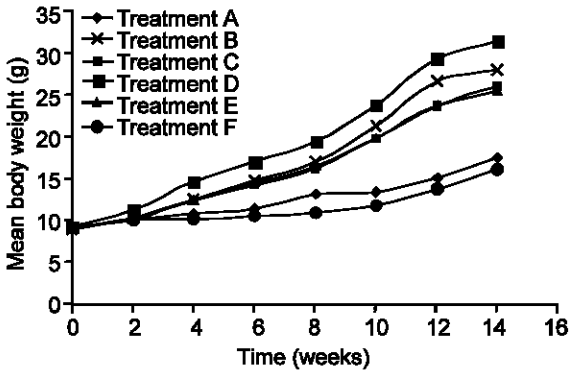

Fig. 1: Changes in mean body weight of *T. rendalli* reared in indoor tanks for 14 weeks. A = 30%CP with 22.79°C, B = 30%CP with 26°C, C = 30%CP with 30°C, D = 40%CP with 26°C, E = 40%CP with 30°C, F = 40%CP with 22.89°C

with an increase in temperature. Feed conversion ratio (FCR) also decreased as in Table 2 with an increase in temperature (Table 3).

Whole body composition: There were highly significant differences ($p<0.05$) among moisture, protein, fat, ash

Table 4: Initial and final whole body composition (%) of *T. rendalli* juveniles cultured in indoor tanks for 14 weeks (Means±SE)[1]

Crude Protein (%CP)	Treatments: Water Temp (°C)	Number of fish	Moisture	Protein	Lipid	Ash	Energy (kJ/g)
Initial		32	88.38±0.06	56.80±0.12	18.27±0.02	13.94±0.17	21.67±0.18
30	22.79	10	95.76±0.14^{c}	63.32±0.37^{b}	22.03±0.33^{d}	15.42±0.18^{b}	23.10±0.21^{c}
30	26.00	10	95.35±0.14^{b}	64.01±0.26^{b}	18.39±0.33^{b}	16.56±0.18^{c}	21.30±0.21^{b}
30	30.00	10	94.49±0.14^{a}	61.07±0.26^{a}	20.78±0.33^{d}	17.54±0.18^{d}	20.69±0.21^{a}
40	22.89	10	95.97±0.14^{c}	63.68±0.37^{b}	21.28±0.33^{d}	15.09±0.18^{b}	21.22±0.21^{b}
40	26.00	10	95.80±0.14^{c}	64.67±0.26^{c}	16.43±0.33^{a}	15.18±0.18^{b}	20.48±0.21^{a}
40	30.00	10	96.90±0.14^{d}	64.97±0.26^{c}	19.17±0.33^{c}	14.70±0.18^{a}	20.37±0.21^{a}

[1]Means within same column not sharing a common superscript are significantly different ($p<0.05$)

Table 5: Initial and final blood glucose and haematocrit of juvenile *T. rendalli* juveniles cultured in indoor tanks for 14 weeks (Mean±SE)[1]

Crude Protein (%CP)	Treatments: Water Temp (°C)	Number of fish	Glucose (mmols/L)	Number of fish	Hct (%)
Initial		12	6.11±1.04	13	41.00±1.27
30	22.79	10	8.19±0.75^{c}	10	45.80±2.29^{c}
30	26.00	10	5.96±0.75^{ab}	10	36.10±2.29^{a}
30	30.00	10	6.72±0.75^{ab}	10	37.30±2.29^{a}
40	22.89	10	7.23±0.75^{ab}	10	41.10±2.29^{ab}
40	26.00	10	5.77±0.75^{a}	10	38.50±2.29^{a}
40	30.00	10	7.13±0.75^{ab}	10	36.90±2.29^{a}

[1]Means within same column not sharing a common superscript are significantly different ($p<0.05$)

content and energy (Table 3). Moisture content showed an increase in all treatments (Table 3). Treatments with 30% crude protein and 22.79°C, 40% crude protein and 22.89°C and 40% crude protein and 26°C were not significantly different from each other while the rest were. All diets increased carcass protein, but values were significantly ($p<0.05$) different among treatments (Table 3). The lower-protein (30% crude protein) diet in particular resulted in lower carcass protein except for treatment with 30% crude protein and 26°C. Treatments with 30% crude protein and 22.79°C, 30% crude protein and 26°C as well as 40% crude protein and 22.89°C were significantly different from 40% crude protein and 26°C, 40% and 30°C and 30% crude protein and 30°C. Carcass lipid increased in all treatments. Fish from treatment with 30% crude protein and 22.79°C had the highest lipid content of 22.03±0.33% which indicated the highest lipid deposition while fish from treatment with 40% crude protein and 26°C showed the lowest value (16.43±0.33%) compared with the initial and the rest of the other treatments (Table 3). There were significant differences between treatments 30% crude protein and 22.79°C, 30% crude protein and 30°C, 40% crude protein and 22.89°C and the rest. An increase in ash was observed in all treatments with significant differences. However, a reduction in body energy level was observed in all treatments except for treatment with 30% crude protein and 22.79°C (Table 4).

Blood glucose and haematocrit: The highest blood glucose level of 8.19±0.75 mmol/L was observed in a treatment with 30% crude protein and 22.79°C (Table 5). Treatments with 30% crude protein and 26°C and 40% crude protein and 26°C showed a decline in mean blood glucose level of 5.96±0.75 and 5.77±0.75 mmol/L, respectively compared to initial values of 6.11±1.04 mmol/L (Table 5). However, the interaction effect was significant. This indicates that there were significant differences in effect of crude protein and water temperature on blood glucose. A treatment with 40% crude protein and 22.89°C had the second highest blood glucose level (7.23±0.75 mmol/L), followed by a treatment with 40% crude protein and 30°C with 7.13±0.75 mmol/L and then treatment with 30% crude protein and 30°C was fourth with 6.72±0.75 mmol/L (Table 5).

The highest haematocrit (Hct) value (45.8±2.29%) was observed in the treatment with 30% crude protein and 26°C to 45.80±2.29% in a treatment with 30% crude protein and 22.79°C (Table 5), followed by the treatment with 40% crude protein and 22.89°C (41.10±2.29%) followed 38.50±2.29% (40% crude protein and 26°C) with 37.30±2.29% (30% protein and 30°C), 36.90±2.29% (40% protein and 30°C) (Table 5). Only the treatment with 30% crude protein at 22.79°C was significantly different ($p<0.05$) from all other treatments (Table 5).

DISCUSSION

Results of the experiment showed that in order to achieve satisfactory fish growth, 40% crude protein and 30°C should be used. At this combination, growth performance was good and other indicators were well within acceptable ranges for fish of the same size. The result indicates that the physiological demand for growth

was being met. Generally, preferred water temperatures for tilapia growth are approximately 29-31°C. When fish are fed to satiation, growth at the preferred temperature is typically three times greater than at 22°C. Maximum feed consumption at 22°C is only 50-60% as great as at 26°C. Therefore, when temperatures are lower than 26°C the ideal diet should contain 30% crude protein.

The results suggest that high protein levels at low temperature may be not adequately utilized for body tissue build up due to low metabolism. For this reason, high protein levels at low temperatures would not be economically viable. Jobling (1994) reported that food consumption declines precipitously as the temperature approaches the upper thermal tolerance limits of the species. The results of the present study are in agreement with the results obtained by Kang'ombe (2004). However, in Kang'ombe's trial on the same fish species, weight gains were: 10.75 g at 32°C, 6.89 g at 28°C, 4.01 g at 24°C and 2.0 g at ambient temperature and were much lower than those obtained in this study. The inclusion of animal protein (fish meal) as opposed to the plant protein may be the reason for this difference. Research on substitution of plant protein sources for fish meal has had mixed results (Diana, 1997). Currently, fish protein sources are still most reliable in producing rapid tilapia growth on complete feeds (Diana, 1997).

A high dietary protein level had significantly influenced the mean body weight, Specific Growth Rate (SGR) and feed conversion ratio of *T. rendalli*. Gunasekera *et al*. (2000) reported that the best growth and Feed Conversion Ratio (FCR) were observed in a diet of high dietary protein to a certain point and decreased with further increase in dietary protein content.

Water temperature has a major influence on the amount of food consumed by a fish (Jobling, 1998). Barrows and Hardy (2001) concluded that the best temperature for rapid efficient growth is that at which appetite is high and maintenance requirements (or the energy cost of living) are low. Popma and Lovshin (1995) observed that preferred water temperatures for tilapia growth are approximately 29-31°C. When fish are fed to satiation, growth at the preferred temperature is typically three times greater than at 22°C. Maximum feed consumption at 22°C is only 50-60% as great as at 26°C (Popma and Lovshin, 1995).

The results of whole body composition after a proximate chemical analysis showed much variation between treatments and differences between the initial and final moisture, protein, fat, ash content and energy were observed. A reduction in body lipid content in a treatment with 40% crude protein and 26°C was observed. Goddard (1996) explained that levels of stored lipids are inversely related to the water content and varies than does the protein level, which is relatively stable for any particular species. Dietary protein levels and amino acids content may significantly influence muscle fiber: affecting growth, texture and muscle quality.

The body energy decreased in all treatments except for treatment 30% crude protein and 22.79°C. Jobling (1998) observed that in all types of studies of fish nutrition and growth, it is desirable to have information about the changes in body composition induced by any experimental treatment. This information is necessary in studies where an accurate assessment of energy partitioning is to be made and such information is also vital if the aim of the study is to examine the effects of different diets or feeding regimes upon fish that are intended to be a saleable product.

Blood glucose values were lower than those reported elsewhere. They however, did not reflect a secondary stress response. Mwera (1998) reported blood glucose level values of 7.08 (for 10 fish/tank), 8.80 (15 fish/tank) and 8.24 (for 20 fish/tank) in *Clarias gariepinus* with a sample size of 16 fish. He reported that blood glucose level did not differ significantly between 10 fish/tank and 20 fish/tank. At 15 fish/tank blood glucose differed significantly. He admitted that there was an increase in blood glucose and attributed it to a response of the fish to the effect of glucagon on the glycogenolysis process in the liver. The conclusion was that blood glucose level was higher probably showing a hyperglcaemic condition that could be a result of depressed carbohydrate metabolism.

Haematocrit (Hct) values in all treatments were in line with values obtained by other researchers. However, Barton (2000) reported a haematocrit range of 25-40% for resting stage and 40-50+ for post stress condition. Mwera (1998) obtained 35.69% (for 10 fish/tank), 28.40% (for 15 fish/tank) and 23.33% (for 20 fish/tank) in *Clarias gariepinus*. Mwera (1998) further reported that haematocrit values at lower stocking density were significantly different from medium and higher stocking densities but values in the stocking densities of 15 and 20 fish/tank did not differ significantly in that study.

Msiska (2003) also observed differences in growth of *O. karongae* with a stocking density of one fish (7-10 g mean weight) per 1 m^2 raised at Kasinthula and Domasi. Kasinthula is located in the lower Shire, which is a wetland extension of the Zambezi River. It has a mean air temperature of 25°C and a maximum of over 40°C. The second site, Domasi is 750 m above sea level with mean air temperature of 22.1°C and maximum of over 33°C. He reported that fish growth rate was slower at Domasi than at Kasinthula. Msiska's conclusion was that the growth differences between the two sites have not been recorded on any commonly cultured Malawian species, the *Oreochromis shiranus* and *Tilapia rendalli*. These findings agree with Balarin and Hatton (1979) who stated that tilapias have a distinct preference for the higher water temperatures.

Conclusion: In conclusion, higher dietary protein level had a significant influence on average weight, Specific Growth Rate (SGR) and feed conversion ratio of *T. rendalli*. The best feed conversion ratio was consistent with both higher dietary protein and water temperature levels. Haematocrit (Hct) values in all treatments were in line with values obtained by other researchers. *Tilapia rendalli* appears to be a promising species for commercial aquaculture due to its good growth rates, high survival, ease of handling and ready acceptance of formulated feeds.

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