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Use of Kikuyu Grass as a Fishmeal Substitute in Practical Diets for *Tilapia rendalli*

S.N. Hlophe, N.A.G. Moyo and J.R. Sara

Aquaculture Research Unit, Faculty of Science and Agriculture, School of Agricultural and Environmental Sciences, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727, South Africa

Corresponding Author: S.N. Hlophe, Aquaculture Research Unit, Faculty of Science and Agriculture, School of Agricultural and Environmental Sciences, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727, South Africa

ABSTRACT

Plant based protein sources may be the only solution to reduce high feed costs in aquaculture. The use of kikuyu grass meal as a dietary protein replacement for fishmeal in practical diets for *Tilapia rendalli* was evaluated. To determine the optimum substitution level, kikuyu grass meal was used to replace 20, 40, 60 and 80% of fishmeal in isonitrogenous (CP = 16.70%) and isocaloric (GE = 15.20 MJ kg^{-1}) diets. The test diets were fed to triplicate groups of fish held in 1 m^3 fibre glass tanks at 10 (36±2 g) fish per tank for 60 days. The best specific growth rate (1.60 g day^{-1}) and feed conversion ratio (1.86) were recorded for fish fed diets with 20% kikuyu grass meal. The lowest specific growth rate (1.29 g day^{-1}) and feed conversion ratio (2.56) were recorded for fish fed diets with 80% kikuyu grass meal. When the level of kikuyu grass meal was more than 20% in the diet, growth performance was reduced. However, there were no statistical differences in the growth performance indices measured across the tested diets. The observed reduction in growth for diets containing higher kikuyu grass meal is explained by the decreasing amino acids levels (particularly methionine and lysine) and increasing fibre content. This suggests that kikuyu grass meal is a suitable protein replacement for the expensive fishmeal in *T. rendalli* practical diets when it constitutes up to 20% of the dietary protein.

Key words: Fishmeal replacement, practical diets, feed utilization, growth performance, amino acids levels

INTRODUCTION

Fishmeal has been used as the main protein source in aquaculture feeds because of its high protein value, fatty acids, vitamin and mineral composition (Nguyen *et al.*, 2009). However, fishmeal is the most expensive ingredient in aquafeeds. Furthermore, the availability of fishmeal in future can no longer be guaranteed because the ocean stocks have been depleted. Moreover, Olvera-Novoa *et al.* (2002) pointed out that using animal protein in the diets of herbivorous fish is unnecessary.

There has been a vigorous search for alternative ingredients in aquaculture feeds in recent years. A number of studies have been carried out on the replacement of fishmeal with alternative sources (Enami, 2011). These include poultry by-product meal (Soltan, 2009) terrestrial plant seeds such as soybean (Nguyen *et al*., 2009; Goda, 2007; Nyirenda *et al*., 2000); a combination of soybean and cottonseed (El-Saidy and Saad, 2011) a combination of soybean, sunflower, and cottonseed, (Chebbaki *et al*., 2010; Kangombe and Brown, 2008) soybean meal and wheat meal based diets (Jalal *et al*., 2000); soybean meal fortified with phytase (Hassan *et al*., 2009). Use of rapeseed meal (Davies *et al*., 1990) and aquatic plants such as duckweed (Chowdhury *et al*., 2008; Skillicorn *et al*., 1993; Fasakin *et al*., 1999) and *Azolla* spp. (Fiogbe *et al*., 2004; El-Sayed, 1999) have also been investigated.

Only a few studies have focused on the use of plant leaves as dietary protein sources in fish feeds. These include the use of leucaena leaf meal (Osman *et al*., 1996) cassava leaf meal (Ng and Wee, 1989) and leaf protein concentrate such as Rye grass and alfalfa leaf protein concentrate (Olvera-Novoa *et al*., 1990). However, the results from a majority of these studies are contradictory, this contradiction may be due to differences in the protein content, amino acid profile, digestibility, palatability or the presence of antinutritional factors in the diets tested (Ogunji, 2004).

Soybean is widely used as a fishmeal replacement, owing to its high protein levels and good amino acid profile (El-Sayed, 2006). Moreover, its steady supply guarantees its future availability. However, other plant protein sources for inclusion in Tilapia feeds have to be explored to further reduce feed costs. Plants with high protein levels are preferred because dietary protein affects the growth performance in Tilapia (Musuka *et al*., 2009). Successful fishmeal replacement with cheaper and readily available plant sources will make Tilapia culture applicable and sustainable in small scale semi-intensive systems. Incorporating less expensive and readily available, relatively high protein plant sources such as kikuyu grass in *T. rendalli* diets, would be beneficial in reducing feed costs.

The replacement of fishmeal with dried kikuyu grass meal in *Tilapia* feeds has not been evaluated before. Kikuyu grass has several attributes, which make it a suitable fishmeal replacer in the diet of the macrophagous *T. rendalli*. This grass has relatively high protein levels and a good amino acid profile. It is widely available and used as a lawn grass and therefore of little or no financial value. It is easy to cultivate and maintain and is available all year round in most parts of southern Africa. The main objective of this study was to evaluate the effect of including different levels of kikuyu grass on the growth performance of *T. rendalli*.

MATERIALS AND METHODS

The study was carried out at the Aquaculture Research Unit of the University of Limpopo, the experiment ran for 60 days, from the 1st of February, 2011 to the 2nd of April, 2011.

Formulation of the experimental diets: Kikuyu grass was harvested from the University of Limpopo's Aquaculture Research Unit's grounds. The fresh grass was dried and milled with a hammer mill before being passed through a 250 µm sieve. Five practical diets were formulated using commercially available ingredients (Table 1). The diets were formulated to be isonitrogenous and isocaloric with crude protein being 16.70% of dry matter and gross energy 15.20 MJ kg^{-1}.

The test diets were formulated by substituting fishmeal with kikuyu meal at levels of 80% (diet 1), 60% (diet 2), 40% (diet 3), 20% (diet 4) and 0% (diet 5). Commercial pellets (diet 6) were used as a control. The crude protein and energy content of the different test diets were equalized by adjusting the level of maize meal, brewer's dry grain, sunflower meal, soybean meal, fishmeal and fish oil.

The dry ingredients were passed through a 250 µm sieve, weighed, and thoroughly mixed by hand for approximately 10 min. Fish oil and water were added to the mixture to attain a

Table 1: Formulation, proximate composition (%) and amino acid of the experimental diets

Item	Inclusion level (%) of kikuyu meal					
	80	60	40	20	0	C.P
Diet	1	2	3	4	5	6
Ingredients (%)						
DL-methionine	0.00	0.49	0.99	1.48	1.97	-
Monocalcium phosphate	1.00	1.00	1.00	1.00	1.00	-
Vitamin premix	2.00	2.00	2.00	2.00	2.00	-
Fishmeal	0.00	1.63	3.27	4.90	6.53	-
Brewer's dry grain	0.00	0.25	0.50	0.75	1.00	-
Maize	0.00	10.50	21.00	31.50	42.00	-
Soybean 44	0.00	4.53	9.05	13.58	18.10	-
Sunflower 33	0.00	1.00	2.00	3.00	4.00	-
Fish oil	2.00	2.13	2.25	2.38	2.50	-
Kikuyu meal	80.00	60.00	40.00	20.00	0.00	-
Potato starch	15.00	14.25	13.50	12.75	12.00	-
Benzonite	0.00	2.22	4.45	6.67	8.90	-
Proximate composition						
Crude protein (%)	15.72	16.24	16.87	17.11	17.57	34.48
Lipid (%)	1.37	2.56	3.22	3.57	4.40	2.97
Crude fibre (%)	17.38	13.89	10.72	6.77	2.50	2.30
Gross energy	15.31	15.06	15.46	15.31	14.87	17.00
Ash	11.26	12.75	12.35	12.41	11.94	10.78
Essential amino acids (g 100 g^{-1})						
Arginine	0.69	0.75	0.87	0.98	1.04	2.17
Methionine	0.30	0.54	1.11	1.64	1.69	0.57
Tyrosine	0.67	0.26	0.66	0.72	0.64	0.88
Histidine	0.34	0.28	0.33	0.81	0.41	0.73
Threonine	0.48	0.45	0.52	0.53	0.55	1.19
Isoleucine	0.48	0.48	0.56	0.56	0.59	1.32
Phenylalanine	0.57	0.60	0.62	0.63	0.65	1.40
Lysine	0.85	0.85	0.90	0.98	1.01	1.69
Valine	0.59	0.58	0.62	0.65	0.67	1.77
Leucine	0.95	0.92	1.01	1.06	1.20	2.75

CP: Commercial pellets

consistency appropriate for passing through a hand operated extruder. The paste was homogenised by kneading for an additional 10 min. A hand extruder was used to produce pellets of about 2 mm diameter. The extruded strands were oven dried at 30°C for 48 h, thereafter broken into approximately 5 mm lengths and stored in plastic bags at room temperature.

Proximate composition of experimental diets: The proximate analysis of the formulated diets was done according to procedures stipulated by the AOAC International (2003). The diets were analysed for energy, crude protein, crude fat and crude fibre. Dry matter was determined by freeze-drying each sample for 72 h. Nitrogen content of the dry matter of the feed and animal tissue was determined using a LECO FP2000 Nitrogen Analyser using the Dumas combustion with protein content calculated as % nitrogen ×6.25. Lipid content was assessed by SoXhlet extraction of the freeze-dried samples with petroleum ether at 40-60°C. Gross energy was established with a DDS isothermal CP 500 bomb calorimeter. Amino acid composition of the diets was determined using a Beckman Amino Acid Analyser System 6300.

Experimental design: Eighteen 1 m^3 fibre glass tanks housed in a green house were used. These were connected to a recirculating system. Air was continuously supplied using a cyclone blower and diffused through air stones in each tank.

Sub-adult *T. rendalli* (36±2 g) were selected and randomly distributed in the eighteen tanks, at 10 fish per tank. Each diet was replicated three times. Fish were hand fed their allocated diet at 5% body weight per day, three times daily at 0900, 1300 and 1700 h. All fish from each treatment were weighed once every 30 days. The feeding trial lasted for 60 days.

Water quality monitoring: Daily measurements were taken for temperature (°C), dissolved oxygen (mg L^{-1}) and pH, using a hand held YSI (556 MPS) multi-meter.

Growth performance and feed utilization parameters: Average Weight Gain (AWG), Average Daily Gain (ADG), Relative Growth Rate (RGR) Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) *T. rendalli* were calculated for all treatments.

Statistical analysis: One-way Analysis of Variance (ANOVA) on the Statistical Package and Service Solutions (SPSS version 17.0) was used to determine significant differences ($p<0.05$) in the Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Weight Gain (WG), Average Daily Gain (ADG) and Relative Growth Rate (RGR).

RESULTS

Specific growth rate showed a decreasing trend with increasing levels of kikuyu grass in the diet (Table 2). The highest SGR was recorded in fish fed diet 6 (commercial pellets) followed by fish fed diet 5 (0% kikuyu), diet 4 (20% kikuyu), diet 3 (40% kikuyu), diet 2 (60% kikuyu) and fish fed diet 1 (80% kikuyu) had the lowest specific growth rate. However, the differences in the SGR for *T. rendalli* fed the different experimental diets were not statistically significant ($p>0.05$, ANOVA). Food conversion ratio decreased with decreasing levels of kikuyu in the diet (80-0%). The lowest FCR was recorded for fish fed diet 6, followed by those fed diet 5, diet 4, diet 3 and diet 2 and fish fed diet 1 had the highest FCR. These differences were not statistically significant ($p>0.05$, ANOVA).

Table 2: Growth performance indices measured in *Tilapia rendalli* fed kikuyu meal based diets

	Diet					
Parameters	1 (80%)	2 (60%)	3 (40%)	4 (20%)	5 (0%)	6 (commercial)
Initial weight (g)	368.73	385.90	386.27	343.69	338.17	387.30
Final weight (g)	800.40	860.57	878.77	881.33	880.86	1080.45
SGR (%/day)	1.29	1.33	1.37	1.53	1.60	1.71
FCR	2.56	2.48	2.26	2.10	1.86	1.67
RGR	117.03	122.79	127.50	154.66	161.48	179.15
AWG (g)	43.17	47.47	49.25	53.76	54.27	9.32
ADG (g)	7.19	7.91	8.21	8.96	9.04	11.55

AWG: Final weight (g) - initial weight (g)/number of fish. ADG: Final weight (g) - initial weight (g)/ experimental period (days). RGR: [Final weight (g)-initial weight (g)/ initial weight (g)]. SGR: [(Final body weight (g)- initial body weight (g))/ feeding period (days)]x100%. FCR: Food consumed (g)/weight gained (g)

Relative Growth Rate (RGR), Average Weight Gain (AWG) and Average Daily Gain (ADG) increased with decreasing level of kikuyu in the diet (Table 2). These growth parameters were highest in *T. rendalli* fed diet 6, followed by those fed diet 5, 4, 3, 2 with the lowest RGR, WG and ADG observed in fish fed diet 1. RGR, WG and ADG were not significantly different ($p>0.05$, ANOVA) between treatments.

Average water temperature was 26.1±2.2°C. The pH was 7.2-7.7 and dissolved oxygen ranged between 6.24-7.1 mg L^{-1}. Ammonia ranged between 0.62-0.87 ppm. All these parameters are within the acceptable limits for *T. rendalli*.

DISCUSSION

There was no significant difference in the growth performance of *T. rendalli* when kikuyu grass meal was used to replace fishmeal. This implies that kikuyu may be a suitable replacement for fishmeal. However, there was an inverse relationship between the growth of *T. rendalli* and dietary kikuyu inclusion. The best specific growth rate and feed conversion ratio was attained at the lowest kikuyu grass inclusion level (20%), with the highest inclusion level (80%) yielding the poorest growth rates.

The depression of growth performance when the amount of kikuyu grass increased may be attributed to the decrease in amino acid content. This is possible because with every increase in kikuyu grass meal, a decrease in the amount of essential amino acid (especially methionine and lysine) was observed. The results of this study support by Siddiqui *et al.* (1988) theory, that fish require the right combination of essential amino acids and do not necessarily have a specific need for crude protein. This implies that the amino acid profile of the dietary protein is more important than the total protein value. The nutritional value of proteins is based on the amino acid composition of the protein source, especially the indispensable amino acids and the biological availability of the amino acid from the protein (Stickeny *et al.*, 1983). Similarly, Olvera-Novoa *et al.* (2002) and Garcia-Gallego (1998) also reported a reduction in essential amino acids when sunflower meal was included beyond 20 and 50%, respectively.

The decline observed in the growth of *T. rendalli* when the dietary level of kikuyu meal was increased may also have been caused by the increase in the fibre. High fibre levels have been associated with poor utilization of feed and reduced growth rates because it hinders nutrient digestibility (Anderson *et al.*, 1984). The fibre content of the experimental diets increased with increasing amount of kikuyu grass meal in the diets, ranging from 2-5% in diet 5 (0% kikuyu) to 17.38% in diet 1 (80% kikuyu). The higher dietary fibre content may have been the cause of the reduced growth performance attained when these diets were fed. Anderson *et al.* (1984) reported a drastic reduction in growth, PER, FCE and whole body fat in *Nile tilapia* when more than 10% α-cellulose was included in the diet. Similarly, Fasakin *et al.* (1999) reported that higher inclusion levels of duckweed (*Spirodela polyrrhiza*) in the diet lowered growth performance. The increased fibre content may have resulted in an increase in stomach-emptying time, thus reducing the time for nutrient assimilation (Shiau, 1997).

Reduced growth performance in *T. rendalli* with increasing kikuyu levels is consistent with other studies where plant based protein was used to replace fishmeal in Tilapia diets. For example, Garduno-Lugo and Olvera-Novoa (2008) evaluated the effects of replacing fishmeal with peanut (*Arachis hypogaea*) leaf meal in practical diets for all male *O. niloticus* and found that higher inclusion levels of peanut leaf meal (>20%) reduced growth performance. Sheeno and Sahu (2006) reported reduced growth performance and nutrient utilization when >50% of Azolla protein concentrate and Spirogyra mixture was included in diets of *Labeo rohita* fry. Similarly, Olvera-Novoa *et al*. (1990) reported better growth rates in diets with 35% alfalfa leaf protein than when a fishmeal based diet was fed. However, increasing the level above 35% decreased the growth rate.

Antinutritional factors were not identified in the current study, however, their concentration in the diet is likely to have increased with increasing levels of kikuyu grass meal. The higher concentration of antinutritional factors in the diets at high kikuyu levels may have also contributed to the decreased growth performance. Antinutritional factors are any substance which may reduce intake or utilization, these may include Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF), saponins, phenolics and phytic acid (Akande *et al*., 2010). The presence of antinutritional factors in plant based protein sources restricts the level at which these plants can be included in *Tilapia* feeds. Kikuyu grass is associated with some indigestible material such as lignin, NDF, cellulose and hemicellulose (Marais, 2001). However, it is not possible to accurately compare the results obtained in this study with previous work as there has never been any published work on the utilization of kikuyu grass in Tilapia feeds.

The growth rates of *T. rendalli* observed in this study were slightly lower than those reported in other studies. This may be due to differences in fish size or the type of production system. For example, Kangombe and Brown (2008) reported SGRs ranging from 2.1 to 3.6% day in *T. rendalli* fed different plant based diets, these results were better than those obtained from the current study (1.29-1.71). Firstly these authors used juvenile (mean, 4.7 g) fish, whilst 36±2 g fish were used in this study. Secondly Kangombe and Brown (2008) experiments were conducted in fertilized ponds where natural food is abundant and thirdly, the protein content of the formulated diets was much higher than that used in this study. In these studies, the level to which the alternative leaf meals could be used in Tilapia diets was low, probably because of the lower protein content of the plants used. The results of the current study support the findings of these various workers, in that leaf meal protein can be used as fishmeal replacement in Tilapia diets at low inclusion levels without adverse effects on fish growth.

CONCLUSION

The findings of this research suggest that kikuyu grass meal may prove to be a suitable replacement for fish meal protein in *T. rendalli* diets. In view of the favourable amino acid profile of the grass and its wide and ready availability throughout the tropics and subtropics, kikuyu can be considered as a potential feed component with high nutritive value for fish. However, more investigations must be done on a larger production scale for longer periods of time and for different size groups of *T. rendalli*. No measures were taken to quantify or destroy any anti-nutrients that may be present in kikuyu grass. It is hypothesized that processing of the grass before formulation of the diets may increase growth performance of *T. rendalli*.

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