The effect of replacing fishmeal with kikuyu leaf meal on the activity of digestive enzymes and growth in *Tilapia rendalli, Oreochromis mossambicus and Clarias gariepinus*

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ABSTRACT

The effect of replacing fishmeal with kikuyu on digestive enzyme activities was investigated in *Tilapia rendalli, Oreochromis mossambicus* and *Clarias gariepinus*. Five diets (30% CP: 20 MJ GE kg-1 DM) were formulated. In the control diet, fishmeal was replaced at 25, 50, 75 and 100% by kikuyu leaf meal. Each diet was assigned to three groups of 20 fish (per species). Fish were fed to apparent satiation three times daily for 60 days. There was a significant decrease (P<0.05) in protease, amylase and lipase activities at higher kikuyu inclusion. Protease activities were higher in *T. rendalli* followed by *O. mossambicus* and *C. gariepinus* had the lowest activities. The inability of the fish to produce enough enzymes at higher kikuyu inclusions resulted in significantly lower growth performance and poor protein digestibility. This decrease was more pronounced in *C. gariepinus* than the tilapias.

Key words: Amylase, Catfish, Lipase, Protease, Tilapia.

INTRODUCTION

Most studies which investigated the replacement of fishmeal with plant-based proteins have focused on growth performance. However, the digestibility of these ingredients is influenced by the ability of the fish to secrete the requisite digestive enzymes. Understanding the functioning of the digestive tract in different species can provide critical information for the optimisation of the dietary nutrients.

Knowledge of the digestive capability of a fish species is essential for adjusting dietary formulation to the functionality of the digestive tract. The ability of fish to utilise a diet is largely a function of the fish's enzyme profile (Essa *et al*., 2010) and temperature (Gupta, 2002). Thus, the characterisation of specific enzyme activities may provide information about the digestive capacity and the efficiency of different species to utilise dietary components (Buddington *et al*., 1997). Enzyme secretion is dependent on a number of factors, including the quality of the diet, fish size or life stage, time after feeding, and phylogeny (Montoya *et al*., 2010; Chaudhuri *et al*., 2012). Recently, some genotype-diet interactions for growth have been reported in fish fed plant-based diets (Le Boucher *et al*., 2011). The existence of such interactions indicate that it is possible to select fish with a better ability to utilise plant-based diets.

The demand for aquaculture products is on the rise because of the overfishing of the world's waters (Gao *et al*., 2016; Karatas, 2016) This leads to and increased demand for the replacement of fishmeal with cheaper, locally available plant resources is now a global research priority. *Pennisetum clandestinum* (kikuyu grass) has a relatively high protein content (26%), a balanced amino acid profile and low concentrations of anti-nutrients (Hlophe and Moyo, 2014a). Previous work showed that this grass has a potential to partially replace fishmeal in the diets of Tilapia *rendalli* (Hlophe and Moyo, 2014a), *Oreochromis mossambicus* (Hlophe and Moyo, 2014b) and *Clarias gariepinus* (Hlophe and Moyo, 2014c). In this study, we examine the effect of kikuyu leaf meal on digestive enzyme activities in *T. rendalli, O. mossambicus* and *Clarias gariepinus*.

MATERIALS AND METHODS

Feed preparation: The study was conducted in 2016, at the Aquaculture Research Unit, University of Limpopo, South Africa. Fresh kikuyu grass was harvested from the Unit's lawn and dried under a shade, then milled using a hammer mill. Five isonitrogenous (crude protein 30%) and isoenergetic (gross energy 20 MJ kg^{-1}) diets were formulated. The control diet did not contain kikuyu leaf meal (Table 1). In the experimental diets, fishmeal was substituted at 25, 50, 75 and 100% with kikuyu leaf meal in diets designated KLM 25, KLM 50, KLM 75 and KLM 100, respectively. In each diet, 0.5% chromic oxide (Cr_2O_3) was added as an inert marker. The diets were formulated using Winfeed 3, EFG Software (Natal). All dry ingredients were mixed, then oil was added. Water was added at 10-20% v/w. The mixture was passed through a Hobart pelletiser with a 1.0 mm die. Pellets were shade dried and stored at -20°C until used.

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Table 1: Ingredients (%) and proximate composition of experimental diets.

	Control	KLM 25	KLM 50	KLM 75	KLM 100
Fishmeal replaced (%)	**0**	**25**	**50**	**75**	**100**
Ingredient composition					
KLM	0.00	7.50	15.00	22.50	30.00
Fishmeal	10.62	7.73	5.25	2.77	0.00
Soybean meal	7.32	7.32	7.32	7.32	7.32
Canola meal	18.00	18.00	18.00	18.00	18.00
Sunflower meal	16.80	16.80	16.80	16.80	16.80
Maize gluten	11.60	12.00	12.00	12.00	12.00
Wheat middlings	2.17	2.17	2.17	2.17	2.17
Maize meal	28.56	23.45	18.37	13.30	8.25
Canola oil	1.00	1.00	1.00	1.00	1.00
Mineral premix	1.00	1.00	1.00	1.00	1.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00
Binder	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Proximate composition					
Dry matter (%)	94.69	92.40	92.32	91.32	91.40
Crude protein (%)	30.03	29.87	29.96	29.71	30.07
Energy (MJ kg^{-1})	20.21	20.34	20.48	20.53	20.47
Fat (%)	3.94	4.65	4.52	4.31	3.93
Crude fibre (%)	7.04	8.07	8.88	10.31	12.91

Experimental design, diet allocation and feeding: Forty-five 1.5 m^3 fibreglass tanks (15 tanks per fish species x 3 fish spices) a recirculating system were filled with aged water up to 1 m^3. Air was diffused through air stones in each tank. *Tilapia rendalli* (12.0 ± 1.0 g), *Oreochromis mossambicus* (12.0 ± 1.5 g) and *Clarias gariepinus* (31.3 ± 1.8 g) were stocked separately at 20 fish per tank. Each diet was allocated to 3 tanks per fish species. All fish were hand fed to apparent satiation three times daily (09:00, 13:00 and 17:00 hours). Feed intake was recorded daily. Faecal samples were siphoned from each tank 2 hours after each feeding. Faecal samples from the same dietary treatments were pooled and stored at -20°C. The feeding trial lasted for 60 days, after which all fish were weighed and five fish from each tank (15 per dietary treatment) were sacrificed for enzyme analysis. Intestines were removed for intestinal enzyme activity determination. Dissolved oxygen ranged between 6.02 and 6.80 mg L^{-1}, water temperature 25.6 -28.4°C, pH 7.0 - 8.2 and the photoperiod was natural.

Proximate composition of diets and faecal analysis: Proximate composition analyses were done according to AOAC International (2012) methods. Chromic oxide in diets and faeces was calculated as: Weight of chromic oxide in sample = (Absorbance – 0.0032 / 0.2089). Chromic oxide (%) = [weight of chromic oxide/ sample weight (mg)] x 100%.

Specific growth rate (SGR) was calculated as:

$$SGR \ (g/day) = \frac{[InW_t - InW_0]}{t} \times 100\%$$

where: W_t = final body weight (g), W_0 = initial body weight (g), t = time feeding period (days), ln = natural Logarithm

(log)$^{-10}$. Feed utilisation was estimated as feed conversion ratio (FCR) = food consumed (g)/weight gained (g); Protein efficiency ratio (PER) = weight gained (g)/ protein consumed (g); Apparent digestibility coefficient (ADC) for protein was calculated as:

Protein ADC (%) = 100

$$\left[1-\left(\frac{\%Cr_2O_3 \ diet}{\%Cr_2O_3 \ in \ faeces}\right) \times \left(\frac{\% \ protein \ in \ faeses}{\% \ protein \ in \ diet}\right) \right]$$

Crude enzyme preparations: The whole intestine from *C. gariepinus, T. rendalli*, and *O. mossambicus* fed each diet were separately homogenised. Protease, amylase and lipase enzyme activities were determined according to procedures outlined in Hlophe *et al*. (2014).

Statistical analysis: One way analysis of variance was used to determine effect of diet of enzyme activities. Linear regression was performed on SGR, FCR, PER and apparent digestibility of protein for each fish species. The regressions were significant when P<0.05. Analysis of covariance (ANCOVA) was used to determine if the linear regressions for each parameter were different between the fish species. All statistical analysis was done on IBM SPSS statistics 21 software.

RESULTS AND DISCUSSION

Effect of diets on digestive enzyme activities: The addition of kikuyu leaf meal in the diet led to a decrease in the digestive enzyme activities. In fish fed the control diet, protease activity was highest in *O. mossambicus*, followed by *T. rendalli* then *C. gariepinus* (Fig 1). Protease activity in all three fish species was not significantly affected (P>0.05) when fishmeal was replaced with 25% kikuyu leaf
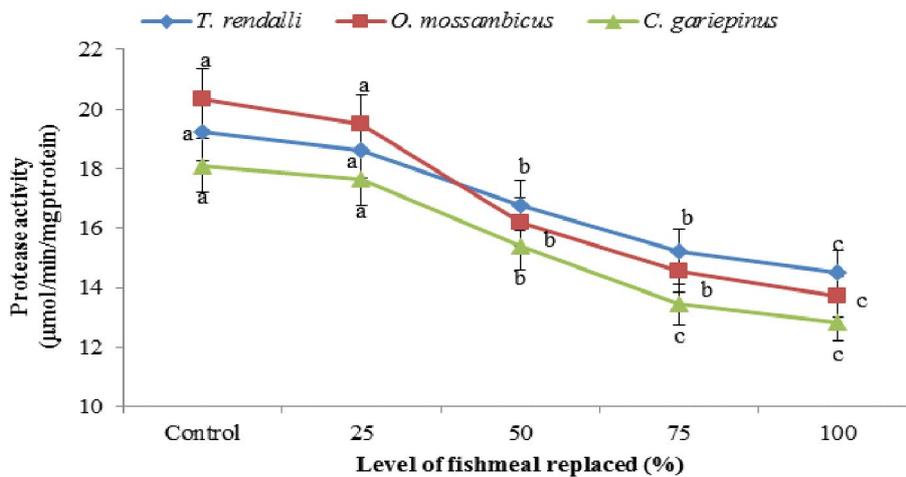
Fig 1: Effect of replacing fishmeal with kikuyu leaf meal on protease activities in *Tilapia rendalli*, *Oreochromis mossambicus* and *Clarias gariepinus* intestines (n=15). Data points represent mean of 3 replicates ± standard error, points with different letters are significantly different (P<0.05)

meal. However, a significant (P<0.05) decrease in protease activity was observed when kikuyu leaf meal replaced more than 25% fishmeal in all fish species. Protease activity is an important indicator of the fish's ability to utilise dietary protein. The present results show that even though all fish were fed diets with the same protein level (30% CP), protease activity decreased with increasing KLM levels. This decrease may indicate a reduced bioavailability of protein at high leaf meal inclusion. According to Le Moullac *et al.* (1996), protease activity is influenced by protein quality and quantity. Protease activities were higher in the tilapias than in *C. gariepinus* regardless of the diet. This may be a genetic adaptation by the herbivorous tilapias to secrete higher protease levels (Chaudhuri *et al.*, 2012) for the digestion of plant proteins which are more difficult to breakdown compared to animal protein which dominates the catfish's natural diet. At higher inclusion levels of kikuyu in the diets, *T. rendalli* showed higher protease activity than *O. mossambicus* and *C. gariepinus*. This implies that *T. rendalli* is more capable of utilising plant-based protein more efficiently than the other two species.

Amylase activity did not differ significantly (P>0.05) between the tilapias when fed the control diet (Fig 2). When kikuyu leaf meal replaced >25% fishmeal in the diet, amylase activity decreased significantly (P<0.05) in all three fish species. This decrease may be a result of the observed reduction in feed intake with increasing kikuyu levels in the diet, which may have resulted in reduced levels of secreted digestive enzyme. German *et al.* (2004) reported that amylase enzyme activity is affected by the quantity of the diet. Amylase activities were also higher in the tilapias compared to those recorded in *C. gariepinus* in all the experimental diets. Several authors report that amylase activity is dependent on the natural diet of each species, and

that herbivorous fish have higher amylase activity than carnivores (Hofer *et al.*, 1982; Hidalgo *et al.*, 1999). Amylase activities in *O. mossambicus* fed higher levels of plant meal in the diet were lower than those recorded for *T. rendalli* fed the same diets. This again confirms that *T. rendalli* is better equipped to utilise plant-based diets. These results concur with German *et al.* (2004) who reported that digestive enzyme activities in fish vary according to the species and composition of the diet.

Lipase activity decreased in all fish species with increasing leaf meal levels (Fig 3). Lipase activities were higher in *C. gariepinus* than in both tilapias in all dietary levels. In *C. gariepinus* and *O. mossambicus* lipase activities only showed a significant decrease (P<0.05) when fishmeal was replaced with more than 50% kikuyu. However, there was no significant decrease in lipase activity in *T. rendalli* across all the dietary treatments. The high lipase activity was observed in *C. gariepinus* probably because its natural diet has higher lipid levels than that of tilapias. Lin and Luo (2011) reported that fish have the capacity to adjust their digestive enzymes to different soybean inclusion levels. This is supported by Karasov (1992)'s adaptive modulation hypothesis which states that "variation in diet confers upon an animal the ability to modulate their digestive enzyme activity accordingly". These results support Sethuramalingam and Hariffa (2002) who reported that lipase activity in fish is influenced by diet composition and was lower in herbivorous fish than in carnivorous fish.

Effect of diets on growth performance: The decrease in the digestive enzyme activities is confirmed by a corresponding decrease in growth performance. The control diet had the highest feed intake (Fig 4). Inclusion of KLM in the diet had a significantly (P<0.05) negative linear
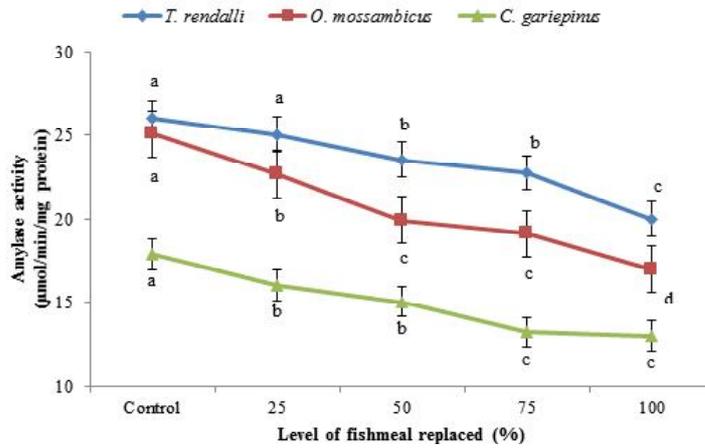
Fig 2: Effect of replacing fishmeal with kikuyu leaf meal on amylase activities in *Tilapia rendalli*, *Oreochromis mossambicus* and *Clarias gariepinus* intestines (n=15). Data points represent mean of 3 replicates ± standard error, points with different letters are significantly different (P<0.05)
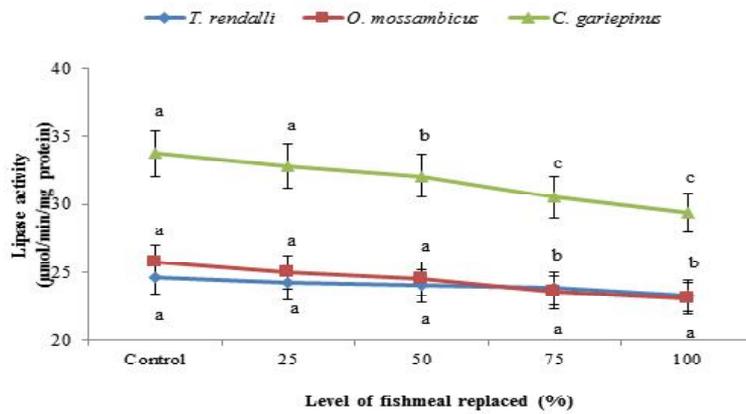
Fig 3: Effect of replacing fishmeal with kikuyu leaf meal on lipase activities in *Tilapia rendalli*, *Oreochromis mossambicus* and *Clarias gariepinus* intestines (n=15). Data points represent mean of 3 replicates ± standard error, points with different letters are significantly different (P<0.05).
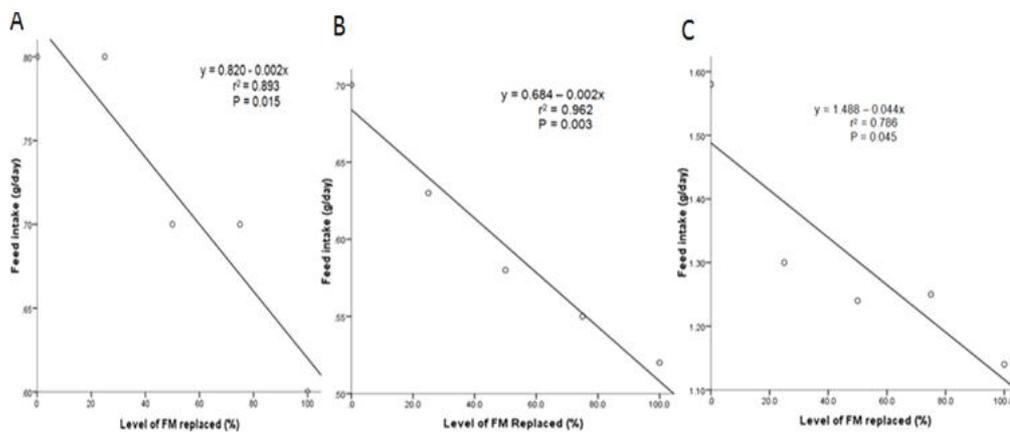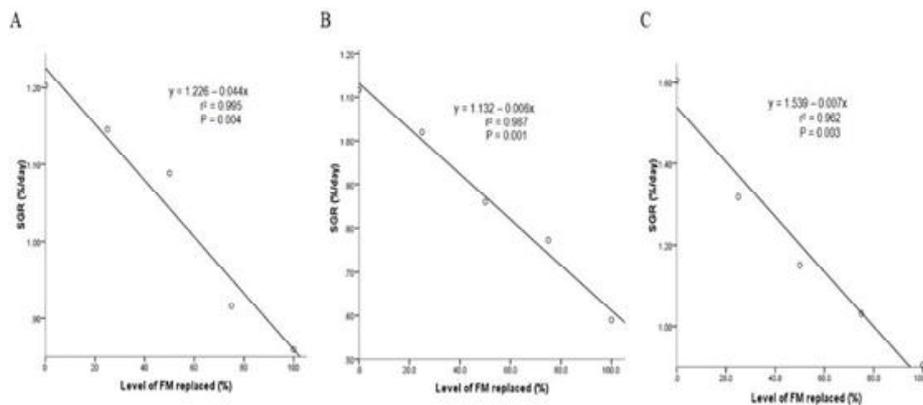

Fig 4: Effect of replacing fish meal with kikuyu leaf meal on feed intake in A) *Tilapia rendalli*, B) *Oreochromis mossambicus* and C) *Clarias gariepinus*.

relationship with feed intake. The decrease in feed intake was more pronounced in *C. gariepinus* followed by that of *O. mossambicus* and *T. rendalli* respectively. Specific growth rate decreased with increasing levels of KLM in the diet (Fig 5). A significant (P<0.05) negative linear regression for SGR with increasing KLM inclusion was observed in all fish species. The analysis of covariance showed that regression of SGR on the level of fishmeal replaced was significantly (P<0.05) higher in *C. gariepinus* followed by that of *O. mossambicus* and lowest in *T. rendalli*.

The best feed conversion ratio was recorded in fish fed the control diet in all fish species (Fig 6). The ability to utilise the diet decreased significantly (P<0.05) when fish meal was replaced with KLM in the diet. A significant (P<0.05) and positive linear relationship between feed conversion ratio and the level of fishmeal replaced by KLM in the diet was recorded in all three fish species. Poorer FCR was observed in *C. gariepinus* followed by *O. mossambicus* and *T. rendalli*.

The highest PER was recorded in fish fed the control diet (Fig 7). Protein utilisation also decreased with increasing fishmeal replacement in all fish species. The decrease in PER was significantly higher (P<0.05, ANCOVA) in *C. gariepinus* followed by that of *O. mossambicus* and lowest in *T. rendalli*. Apparent digestibility coefficient for protein also decreased as the level of KLM in the diet increased (Fig 8). The negative linear relationship between ADC for protein and the level of fish meal replaced was significant (P<0.05) for all three fish species.

Higher protease, amylase and lipase activities recorded in all fish fed the control diet led to a higher growth performance in fish fed this diet. The decrease in digestive enzyme secretion may also be attributed to the presence of anti-nutritional factors, as their concentration most likely increased with increasing leaf meal levels. The presence of anti-nutritional factors in plant diets hampers activities of digestive enzymes and reduce the capacity to digest nutrients (Chong *et al.*, 2002). Anti-nutrients found in kikuyu leaf meal
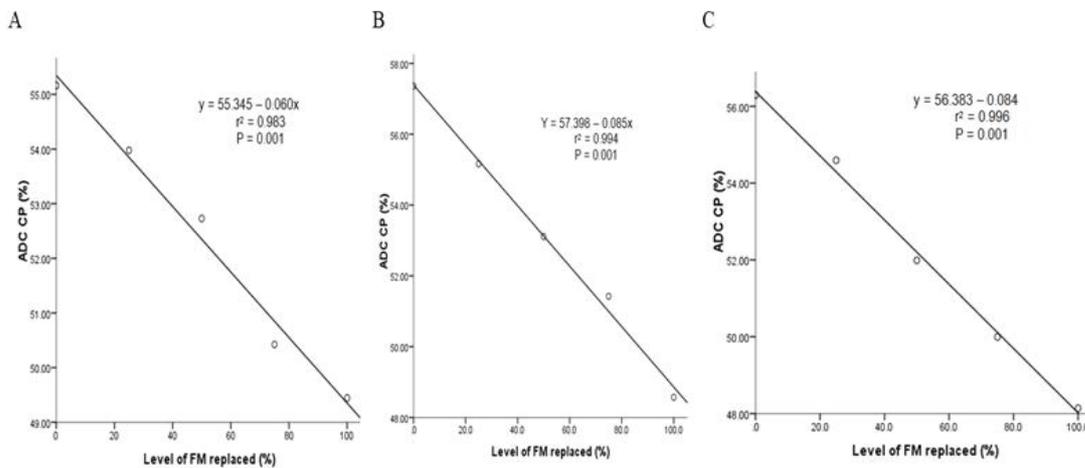

Fig 5: Effect of replacing fish meal with kikuyu leaf meal on the specific growth rate in A) *Tilapia rendalli*, B) *Oreochromis mossambicus* and C) *Clarias gariepinus*.
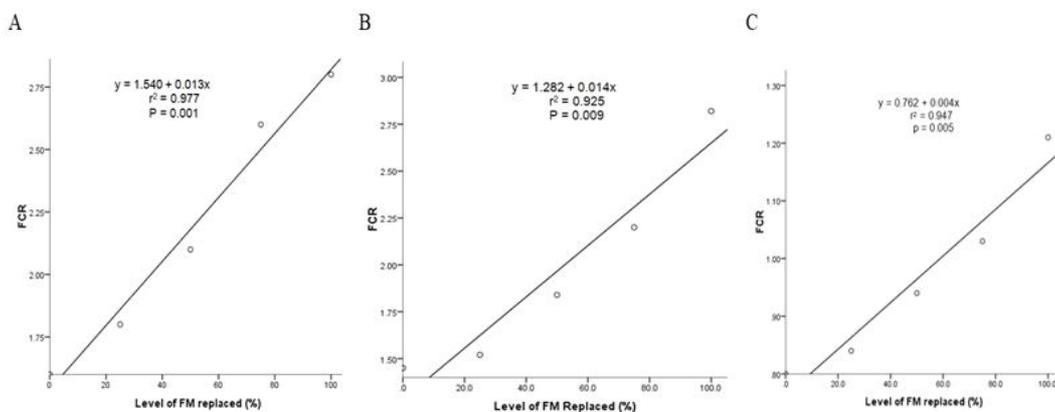
Fig 6: Effect of replacing fish meal with kikuyu leaf meal on feed conversion ratio in A) *Tilapia rendalli*, B) *Oreochromis mossambicus* and C) *Clarias gariepinus*
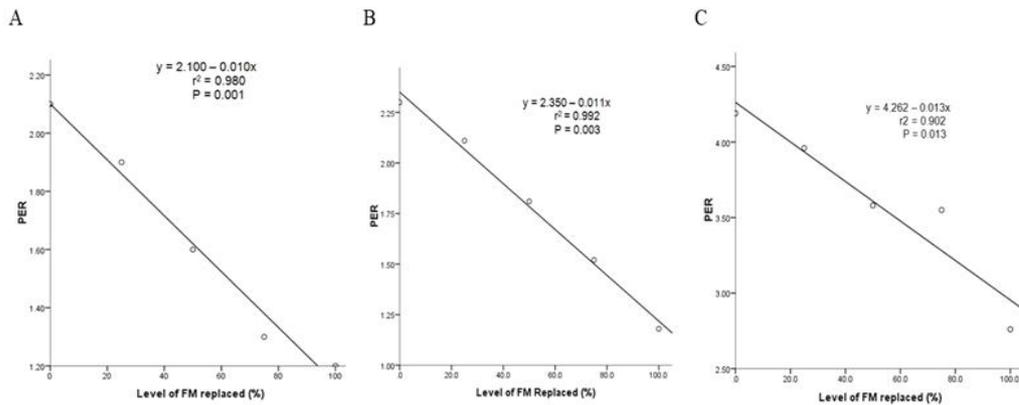
Fig 7: Effect of replacing fish meal with kikuyu leaf meal on the protein efficiency ratio in A) *Tilapia rendalli*, B) *Oreochromis mossambicus* and C) *Clarias gariepinus*.

Fig 8: Effect of replacing fish meal with kikuyu leaf meal on the apparent digestibility coefficient for protein in A) *Tilapia rendalli*, B) *Oreochromis mossambicus* and C) *Clarias gariepinus*.

include polyphenols and tannins (Hlophe & Moyo, 2014a). Tannins hinder the digestive process by binding to digestive proteases and thus reducing their bioavailability. This may explain the reduction in protease activity in all fish species. Bairagi *et al*. (2004) and Ritcher e*t al*. (2003) also attributed the reduced growth performance of fish fed plant-based diets to anti-nutritional factors.

CONCLUSION

The current study clearly shows that the inability of the fish to produce enough enzymes may be the main reason for poor feed utilisation and ultimately reduced growth. It is therefore concluded that digestive enzymes could potentially set a limit upon the digestive capacity of individual fish and determine their maximal growth rate and food utilisation efficiency. These results also show that the different fish species exhibit distinctly different patterns of brush-border enzyme activity when fed plant-based protein resources than when fed fishmeal-based diets and that the activity of digestive enzymes is an important indicator of a fish's ability to utilise its feed.

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