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Performance of *Oreochromis niloticus* juveniles fed autoclaved mango seed kernel diets

Falaye Augustine Eyiwunmi[1,3], Sule Shakiru Okanlawon[1,2*], Sanogo Salimata[1], Kechicha Olufunmike Martha[1,3]

[1] *Department of Aquaculture and Fisheries Management University of Ibadan, Ibadan, Nigeria.*
[2] *Department of Forestry, Wildlife and Fisheries, Olabisi Onabanjo University, Ayetoro Campus, Ogun State, Nigeria.*
[3] *Centre for Environmental and Agricultural Resource Development, University of Ibadan, Ibadan, Nigeria.*

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ABSTRACT

Human and livestock population increase has led to escalating prices of energy feed stuffs used in aquaculture. There have been some researches on various inexpensive agro-industrial by-products as substitutes for maize in fish diets with varying degrees of success. This study investigated the growth performance of Oreochromis niloticus juveniles fed with autoclaved Mango seed formulated fish diet as a dietary energy source at different inclusion level. 150 Oreochromis niloticus juveniles with mean weight of 10.24±0.05g were randomly allocated to five dietary treatments (T1, T2, T3, T4, T5). The fish were acclimated for two weeks and were allotted to five treatments in plastic tanks (26x46x20cm). Each treatment contained 10 fish per tank. The fish were fed experimental diets at T1:0%, T2: 25%, T3: 50%, T4: 75% and T5: 100% Mango Seed Kernel Meal (MSKM) inclusion levels respectively. The highest Mean weight Gain (MWG) and lowest Feed Conversion Ratio (FCR) was recorded in Treatment 2: 25% (48.93±4.88); (0.91±0.07) while the lowest MWG and highest FCR was recorded in Treatment 5: 100% (27.30±3.99); (1.47±0.16). Specific Growth Rate (SGR) was lower in Treatment 5: 100% when compared to other treatments. Feed intake followed a decreasing order. The blood samples collected showed that there was significant differences ($p<0.05$) In conclusion, growth performance and haematological activity could be best improved by substituting mango seed kernel meal at 75% with no implication on growth and fish health.

Introduction

Maize consumption is a staple energy food for humans in many developing countries. Maize utilisation has continued to increase due to population growth leading to scarcity and escalating costs at off season as a result of inadequate storage facilities. Maize is an important feedstuff for animal feed industry (Alatise *et al.,* 2007) and major source of metabolisable energy in most compounded diets for fish and livestock (Fagbenro *et al.,* 1992; Balogun and Fagbenro, 1995). The necessity to evaluate alternative nutrient sources to improve aquaculture production and reduce competition with human for conventional ingredients is inevitable. Scarcity, human competition and high prices of feedstuffs have led to reduction in profit of farmers (Ipinjolu *et al.,* 1999). There have been documented researches on various inexpensive agro-industrial by-products as substitutes in fish diets (Falaye, 1992) with varying degrees of success (Shittu *et al.,* 2013; Joseph and Abolaji, 1997).

Utilising cheaper carbohydrates sources (Olurin *et al.* 2006) like Mango Seed Kernel Meal (MSKM) in rural areas among low income group fish farmers will increase profitability. MSKM constitute 15-20% of mango fruit (Budhwar, 2002) with annual generation of MSKM from processed and fresh consumption of mango reported at 3.4 million ton (El-Boushy *et al.,* 2000) and availability at off-season possible if processed to meal. Crop residues are under-utilised as feeds and until recognition is given to its economical utilisation in animal nutrition production objectives of farmers will decline (Williams *et al.,* 1997).

MSKM waste contain nutrient of importance (Fowomola, 2010) which can be utilised in

* Corresponding author.
Email address: okanlawon.sule@yahoo.com; sule.okanlawon@oouagoiwoye.edu.ng

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aquaculture. Processing methods have been adopted to reduce the effect of anti-nutritional factors of MSKM on livestock (Diarra and Usman 2008; Diarra *et al.,* 2010; Dakare *et al.,* 2010). The use of abundant waste from Mango consumption and processing will reduce environmental nuisance caused by indiscriminate disposal of the mango seed. This research assessed the growth performance of *Oreochromis niloticus* juveniles fed autoclaved MSKM as partial replacement for maize energy source at different inclusion levels.

Materials and Methods

Time and site

The experiment was conducted for 12 weeks (April - June, 2018) at Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria. Experimental procedure: The mango seed were collected from a local market and was processed into MSKM by autoclaving method before laboratory analysis for proximate composition. A total of 150 *O. niloticus* juveniles with a mean weight of 10.24±0.05g were purchased from a reputable fish farm in Ibadan. The fish were acclimatized for two weeks before the experiment during which they were fed the control diet at 5% body weight. The experiment was a conducted using complete randomised design and fish were allotted to five treatments of 36% crude protein diets (T1: 0%, T2: 25%, T3: 50%, T4: 75% and T5: 100%) in triplicate groups in plastic tanks measuring 26x46x20cm (25L/tank). Each treatment contained 10 fish per tank.

Proximate analysis

The proximate composition of MSKM; experimental diets and fish carcass were analysed according to (AOAC, 2005).

Table 1. Diet preparation and proximate composition of the experimental diet.

Ingredient	Treatmen				
	1	2	3	4	5
Maize	30.68	23.01	15.24	7.67	0.00
Mango seed	0.00	7.67	15.24	23.01	30.68
Fishmeal	21.44	21.44	21.44	21.44	21.44
Soya Bean Meal	21.44	21.44	21.44	21.44	21.44
Groundnut cake	21.44	21.44	21.44	21.44	21.44
Oil	3.00	3.00	3.00	3.00	3.00
Di-Calcium Phosphate	0.50	0.50	0.50	0.50	0.50
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Premix/Vitamin	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50

Measured growth parameters

The weight of experimental fish was taken biweekly using electronic weighing scale BY-B and used to adjust the feed intake. The measured parameters of growth performance parameters were modified from Muchlisin *et al.* (2016) as follows:

Mean Weight gain (MWG) = Final weight – Initial weight

Specific Growth Rate (SGR) = $\text{Log}_{e\ \text{final weight}} - \text{Log}_{e\ \text{initial weight}}$/Time * 100

Feed Conversion Ratio (FCR) = Feed fed (g)/MWG

Protein intake (PI) = Feed intake * % of protein in the diet.

Protein efficiency ratio (PER) =MWG/Protein consumed.

Haematological analysis

The haematological composition of the fish was carried out at the end of the experiment for the analysis of packed cell volume (PCV), heamoglobin (Hb), Red Blood Cells (RBC), white blood cells (WBC) and lymphocytes (L) according to (Joshi *et al.* 2002) while Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were calculated according to (Dacie and Lewis, 2001).

Determination of water quality

The water quality parameters were monitored (temperature using clinical thermometer; dissolved oxygen, nitrate, ammonia and pH) using API® Freshwater Master test kit and recorded.

Data analysis

Data resulting from the experiment was subjected to descriptive statistics (±SD) and one-way analysis of variance (ANOVA). Tukey HSD Test was used to test differences among means when significant values is observed at P<0.05 using SPSS version 20.

Results

Result of the proximate composition (Table 2) of mango seed and diets showed autoclaved MSKM crude protein value of 5.08% with crude fibre (4.60%) and low ash content. While dietary analysis showed there was no significant difference (P>0.05) in crude protein among treatments. Highest crude fibre was in T5 and lowest in T1. The moisture content ranged from lowest in T1 to highest in T5. The percentage fat varied between lowest in T5 and highest in T4. T5 had the highest ash content and energy content. Parameters (moisture content, fat, fibre ash and energy) were significantly different (P<0.05) among treatments.

The growth response of fish fed varying levels of MSKM diets (Table 3) showed significantly difference (P<0.05) in all parameters of T2 and T3 over other dietary treatments with the highest mean weight gain (48.93±4.88) in T2. The FCR was least in

T2 (0.91±0.07) and T1, T4, and T5 were significantly different from T2 and T3. Specific growth rate, protein intake, protein efficiency ratio and feed intake for T1, T4 and T5 followed similar tend and were all significantly different from T2 and T3 respectively. Specific growth rate ranged between 0.28±0.04 in treatment 5 to 0.46±0.04 in treatment 2.

The proximate composition of the experimental fish (Table 4) showed that crude protein increase was observed from initial fish carcass to final fish carcass. The percentage of the crude protein of T1 and T5 were significantly different ($P<0.05$) from other treatments. Significant difference was also observed in the crude fibre content with the highest crude fibre (6.17±0.24) in T5 while the least was in T1 (5.12±0.24). Moisture content varied between treatments with slight significant difference. Ash content decrease was observed from the initial fish towards dietary treatments with least value (0.72±1.25) in T5.

Table 2. Proximate composition of the mango seed and experimental diets.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	MSKM
Moisture Content (%)	5.67±0.01^{c}	7.10±0.02^{c}	6.28±0.02^{d}	7.28±0.03^{b}	7.82±0.03^{a}	9.80
Crude Protein (%)	36.00±0.05^{a}	35.69±0.05^{a}	35.39±0.02^{a}	35.28±0.05^{a}	34.77±0.02^{a}	5.08
Crude Fat (%)	11.10±0.03^{a}	10.01±0.02^{c}	10.70±0.03^{b}	11.59±0.03^{a}	8.20±0.04^{d}	7.10
Crude Fibre (%)	6.61±0.02^{d}	8.01±0.02^{c}	9.60±0.03^{b}	9.62±0.02^{b}	10.00±0.02^{a}	4.60
Ash Content (%)	7.30±0.01^{c}	7.29±0.01^{c}	7.19±0.01^{d}	7.43±0.01^{b}	7.88±0.01^{a}	2.00
N.F.E (%)	32.42±0.03^{a}	31.90±0.05^{b}	30.84±0.03^{c}	28.80±0.07^{d}	31.33±0.09^{b}	

*Means in the same row with the same superscript are not significantly different from each other.

Table 3. Growth performance and nutrient utilization of *O. niloticus* fed MSKM diets.

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Initial weight (g)	101.73±1.12^{a}	102.53±2.56^{a}	102.80±2.17^{a}	102.00±1.73^{a}	102.90±2.15^{a}
Final weight (g)	134.23±2.80^{b}	151.47±5.26^{a}	145.47±3.11^{b}	134.23±3.29^{b}	130.20±5.01^{b}
MWG (g)	32.50±3.18^{bc}	48.93±4.88^{a}	42.67±4.19^{ab}	32.23±3.44^{bc}	27.30±3.99^{c}
FCR	1.26±0.12^{bc}	0.91±0.07^{a}	1.01±0.11^{ab}	1.26±0.13^{bc}	1.47±0.16^{c}
SGR (%/g/day)	0.33±0.03^{bc}	0.46±0.04^{a}	0.41±0.04^{ab}	0.32±0.03^{bc}	0.28±0.04^{c}
Protein Intake (g)	12.19±0.11^{bc}	13.24±0.30^{a}	12.83±0.08^{ab}	12.07±0.13^{bc}	11.86±0.53^{c}
PER	1.08±0.10^{bc}	1.63±0.16^{a}	1.42±0.14^{ab}	1.07±0.11^{bc}	0.91±0.13^{c}
Feed Intake (g)	40.65±0.36^{bc}	44.12±1.02^{a}	42.77±0.26^{ab}	40.23±0.42^{bc}	39.53±1.76^{c}

**Mean in the same row with the same superscript are not significantly different from each other.*

Table 4. Proximate composition of initial and final fish carcass.

Parameters	Initial	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Moisture Content (%)	25.00±0.03^{ab}	23.96±0.82^{b}	25.45±0.35^{a}	25.92±0.16^{a}	25.80±1.03^{a}	25.02±0.39^{ab}
Crude Protein (%)	12.77±0.02^{c}	14.90±1.64^{b}	16.00±0.76^{a}	16.09±0.17^{a}	15.95±0.73^{a}	15.87±1.29^{a}
Crude Fat (%)	2.77±0.01^{d}	2.39±0.16^{b}	2.60±0.21^{a}	2.48±0.28^{ab}	2.48±0.13^{ab}	2.26±0.19^{bc}
Crude Fibre (%)	0.00±0.00^{d}	5.12±0.24^{c}	5.68±0.02^{b}	5.61±0.32^{b}	5.83±0.18^{ab}	6.17±0.24^{a}
Ash Content (%)	7.28±0.01^{a}	1.55±1.27^{c}	1.16±1.22^{d}	1.73±0.43^{b}	1.53±0.54^{c}	0.72±1.25^{e}

**Means in the same row with the same superscript are not significantly different from each other.*

Table 5. Haematological parameters of *O. niloticus* juveniles fed MSKM diets.

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
PCV (%)	21.33±1.53^{b}	21.67±1.53^{b}	23.33±2.89^{ab}	26.33±1.53^{a}	23.00±2.65^{ab}
Hb (gdL^{-1})	6.80±0.30^{c}	6.90±0.52^{b}	7.77±1.19^{ab}	8.43±0.40^{a}	7.63±0.96^{ab}
RBC (10^{6}µl^{-1})	1.71±0.44^{c}	1.49±0.15^{d}	1.96±0.57^{ab}	2.61±0.49^{a}	1.80±0.41^{ab}
WBC (µl^{-3})	153.33±4.51^{b}	161.83±21.71^{a}	144.83±12.00^{c}	142.67±7.22^{d}	146.50±4.50^{c}
MCV	128.46±21.53^{a}	146.39±21.25^{a}	124.07±25.81^{a}	102.40±12.17^{a}	130.40±21.45^{a}
MCH	41.13±7.92^{a}	46.69±7.63^{a}	41.02±7.18^{a}	32.85±4.35^{a}	43.15±6.16^{a}
MCHC	31.92±0.91^{a}	31.84±0.77^{a}	33.20±1.20^{a}	32.04±0.63^{a}	33.17±0.76^{a}
Platelet (µl^{-3})	146.33±115.90^{de}	171.00±209.52^{b}	161.67±283.08^{c}	182.00±131.15^{a}	137.67±187.71^{e}
Lymphocyte	58.00±2.65^{b}	65.33±3.06^{a}	62.67±5.51^{a}	63.00±2.65^{a}	59.67±5.03^{b}
Heterophil	34.67±3.51^{a}	26.33±3.21^{d}	31.33±4.93^{b}	29.67±3.06^{c}	32.67±5.13^{ab}
Monocyte	3.00±1.00^{c}	3.00±0.00^{c}	3.67±0.58^{ab}	4.00±1.00^{a}	2.33±0.58^{d}
Eosinophil	3.33±1.53^{c}	5.00±0.00^{b}	2.00±2.00^{d}	2.67±1.15^{cd}	5.33±1.15^{a}
Basophils	0.33±0.58^{ab}	0.33±0.58^{ab}	0.33±0.58^{ab}	0.67±0.58^{a}	0.00±0.00^{c}

**Means in the same row with the same superscript are not significantly different from each other.*

Table 6. Water quality parameters of *O. niloticus* fish tanks fed MSKM diets.

	Initial	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
DO (mg/L)	4.71±0.08[a]	4.68±0.33[a]	4.67±0.35[a]	4.50±0.49[a]	4.85±0.30[a]	4.89±0.42[a]	4.67±0.39[a]
pH	7.60±0.00[a]	7.63±0.17[a]	7.56±0.09[a]	7.64±0.22[a]	7.77±0.20[a]	7.63±0.21[a]	7.64±0.09[a]
NH_3 (mg/L)	0.00±0.00[a]	0.25±0.00[b]	0.28±0.07[b]	0.47±0.32[a]	0.30±0.11[b]	0.30±0.11[b]	0.43±0.24[a]
NO_{3-} (mg/L)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
NO_{2-} (mg/L)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Temp. (^{0}C)	27.00±0.00[a]	25.99±0.99[b]	26.07±0.74[ab]	26.45±0.54[ab]	25.78±0.40[b]	26.03±0.33[ab]	25.93±0.15[b]

**Means in the same row with the same superscript are not significantly different from each other*

In Table 5 blood samples analysed indicated that there was significant differences (p<0.05) for PCV, Heterophil, Platelet, Monocytes and Eosinophil among all treatments. PCV was highest in T4 (26.33±1.53), and lowest in T1 (21.33±1.53). Values for Hb ranged between 6.80±0.30 in T1 and 8.43±0.40 in T4. RBC and WBC were significantly different among all treatments. Platelets and monocytes were highest in T4 and lowest in T5 with significant difference observed among all treatments. Basophils varied between 0.00±0.00 in T5 and 0.67±0.58 in T4. Lymphocytes in T2 (65.33±3.06); T4 (63.00±2.65) and T3 (62.67±5.51) were not significantly different (P>0.05).

The forth-night physico-chemical water quality parameter (Table 6) in fish tanks were within the limits for fresh water fish culture. Dissolved oxygen (DO) and pH were not significantly different among all culture media while ammonia and temperature showed slight significant difference in treatment tanks when compared to the initial parameters.

Discussion

The range of water quality parameters experiments were within the acceptable ranges for fish culture (Beveridge and McAndrew, 2000). Temperature 25.78^{0}C -27.00^{0}C and pH 7.56-7.77 similar to the reports of Omojowo *et al.* (2010); Omoregie (2001); Azaza *et al.* (2007) respectively, who stated that digestion and utilisation of ingested feed is rapid and better in higher temperature (26-30^{0}C) with improved performance of fish. Dissolved oxygen and nitrite are in line with *O. niloticus* culture (Gabriel *et al.* 2011) but lower than the range as reported in Soyinka *et al.* (2015).

MSKM has been identified to contain phytochemicals capable of inhibiting its consumption by man and livestock at various degree of toxicity hence the need to subject it to processing before utilisation in diet formulation. The method of autoclaving adopted in this study is well documented in El Boushy *et al.* (2000). The compositional analysis of MSKM in this study was similar to Patil *et al.* (1982) and El-Kholy *et al.* (2008); fat and fibre were higher in values while ash content was within the range of 1.97-4.10 reported in El Boushy *et al.* (2000). Dakare *et al.* (2014) reported the nutritional importance of MSKM and maize.

Protein in the formulated diet is within the recommended dietary protein requirement of 35% for *Oreochromis species* (Falaye *et al.* 2010). The slight variation in nutritional analysis of diet is not statistically different and is in agreement with the observation of Omojowo *et al.* (2010) and Obasa *et al.* (2013) for *O. niloticus* fingerlings fed with mango by-product diet. Increase in dietary fibre corroborates Falaye and Omoike (2012) when maize bran was fed to *O. niloticus*. High crude fat in all dietary treatments of MSKM corroborates the observation reported by Obasa *et al.* (2013).

The response of fed fish to all treatments indicated that MSKM could be utilized by the fish as food and in-line with previous investigations by Omoregie (2001); Omoregie and Ogbemudia, (1993); Omoregie *et al.* (1991) on mango seeds and palm kernel meal in fish diets. The improved MWG observed in fish fed T2 was similar to the observation of Obasa *et al.* (2013) who reported same inclusion level of MSKM as best for *O. niloticus*. The FCR is lower than that earlier reported by Ojukannaiye *et al.* (2014).

Ofojekwu *et al.* (2003) reported a decrease in weight gain of *O. niloticus* with an increase in levels of palm kernel meal. Dada and Abiodun (2014) noticed that growth was stalled at inclusion greater than 5.0g/kg when *T. occidentalis* extract was incorporated in tilapia diet. Aderolu *et al.* (2015) also noted similar growth depression impact on tilapia fed rice husk above 7.5% replacement level for maize. Omojowo *et al.* (2010) reported decrease MWG and PWG with increase in replacement level of mango peel meal in *O. niloticus* diets. This was also observed in specific SGR.

The MWG in this study was similar to Ejidike and Falaye (1997) when soyabean hull was fed as energy source to *O. niloticus* fingerlings. FCR and MWG in control similar to control fish of Falaye *et al.* (1999b) when cocoa pod husk was fed to *O. niloticus*, also growth depression was observed at higher inclusion which could be as a result of nutritional toxins in seed meal. FCR lower than Obasa *et al.* (2013). FCR is in consonance with Dada and Abiodun (2014) when *T. occidentalis* extract diet was fed to fish. Feed intake T1-T5 significantly compared with TM1, TM2, TM3 of El-Kholy *et al.* (2008) while PER of both experiment were similar across all treatment. Elevated carcass protein of fish similar to the reports of Ejidike and Falaye (1997); Falaye and Omoike (2012) that stated the sparing action of energy feedstuff leads to protein increase in fish carcass. Reduced fish crude protein of T5 was similar to final fish D5 of Falaye *et al.* (1999a) when cassava leaf meal was fed to catfish and to Obasa *et al.* (2013) up to 75% MSKM. The carcass protein in fish fed MSKM was similar to the trend observed by Obasa *et al.* (2013). The body composition values obtained in this study was contrary to those reported by Diab *et al.* (2002); Lara-Flores *et al.* (2003); Hamid and Mohamed (2008) and Fallanpour *et al.* (2014).

The poor performance of fish fed with Diet 5 which contains 100% MSKM without maize meal could be attributed to its high crude fibre levels. This was similar to the findings of Omojowo *et al.* (2010); Obasa *et al.* (2013); Akegbejo-Samson and Omoniyi (2006) thus indicating the inability of fish to digest high fibre content feed. Anti-nutritional factors could have been responsible for low feed intake with increase in inclusion level as observed by Patil *et al.* (1982) when MSKM was fed to poultry.

El-Kholy *et al.* (2008) observed an optimum level of inclusion at 15%-35% for MSK while Omojowo *et al.* (2010) reported 25% mango peel meal as optimum for *O. niloticus* fingerlings. A lower replacement for maize of 20% MSKM in rabbit diets was reported by Shittu *et al.* (2013); Souza *et al.* (2013) 33% mango meal with peel optimal in tilapia diet; Obasa *et al.* (2013) and Bezerra *et al.* (2014) reported MSKM 50% replacement for tilapia and tambaqui; while findings of Falaye and Omoike (2012) of 66%-100% replacement of maize bran as energy is in line with this study of up to 75% compared to the control.

Comparison of haematological samples with reference value ranges need to take consideration of different factors before meaningful comparison can be achieved (Clauss *et al.* 2008). Haematological parameters were within the range reported by Falaye *et al.* (1999c) and Ajani *et al.* (2016). WBC and erythrocytes indices higher in this study while PCV, Hb, RBC (T1, T2, T3, T4) lower to Obaroh *et al.* (2014). Hrubec *et al.* (2000) reference values were considered to be high which might be due to the increased stocking densities and body mean weight in the culture media which is opposed to this study. Increase in T3 and T4 Hb could have been responsible for growth increase up to 75% inclusion in line with Antache *et al.* (2014) who stated that Hb is an interface for fish to maximise feed utilisation under controlled management. The value of PCV and haemoglobin were observed to increase with increase in inclusion of mango seed meal in the diets and this is similar to the observation of Bbole *et al.* (2016). PCV and Hb were observed to increase with increased dietary inclusion MSKM similar to the observation of Bbole *et al.* (2016). While the decrease observed in WBC may be due to stress stimulus in the digestive process with increased MSKM in diets (Rehulka, 2002; Chem *et al.* 2004; Martins *et al.* 2004) as a result of dietary fibre increase; adventitious toxins which were not fully ameliorated during autoclaving and composition of feed (Secombes *et al.* 1994). This reduction in WBC is an indication of a good health status of a fish as they were not subjected to too much stress from disease and infection compared to the control treatment

Conclusions

Incorporating mango seed kernel meal had neither significant depression on growth nor deleterious effect on health of the fish. Hence its substitution for maize improved the growth performance, nutrient utilization and haematological parameters in *Oreochromis niloticus* up to 75% above this inclusion level, growth and feed utilization apparently reduce.

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