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HAEMATOLOGICAL RESPONSE OF *Oreochromis niloticus (Linnaeus, 1758)* FED VARYING INCLUSION LEVELS OF FERMENTED *Tamarindus indica* L. SEED MEAL

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ABSTRACT

Soya bean meal is the most widely used feed ingredient for many aquaculture species. *Tamarindus indica* seed meal has also been reported to have a decent nutrient profile, which makes it a viable substitute for soya bean meal. The effect of replacing soya bean meal with fermented *T. indica* seed meal on some haematological indices of *Oreochromis niloticus* was assessed. *T. indica* seeds were fermented and used to compound five diets (0%, 25%, 50%, 75% and 100% inclusion levels of fermented *T. indica* seed meal) and fed to the fish for 12 weeks. Growth parameters of the fishes were taken and haematological indices were measured following standard procedures after the experiment. The results revealed that the best diet apart from the control diet was D_4 as it gave the best mean weight gain (8.58g) and standard-length gain (2.54cm). The haematological parameters of *O. niloticus* after feeding trial ranged from 40-25%, 8.33-13.34 g/dL, 28.00-35.20 x 10^6 mgl^{-1} and 110-480 x 10^3 mgl^{-1}, for packed cell volume, hemoglobin, red blood cells and white blood cells, respectively. Red blood cells and white blood cells were observed to reduce with increase in inclusion levels of fermented *T. indica* seed meal. All haematological indices varied significantly ($P<0.05$).It was concluded that fermented *T. indica* seed meal replaced soya bean successfully at 75% inclusion level and has no adverse effect on the blood indices of *O. niloticus*.

Keywords: Fermentation, Haematology, Antinutrients, *O. niloticus*, *T. indica*.

INTRODUCTION

Soya bean meal is one of the most nutritious of all plant protein sources, with a high protein content, high digestibility and relatively well-balanced amino acid profile. It is widely used as feed ingredient for many aquaculture species (Storebakken et al. 2000). Similarly, *T. indica* seed meal is an important source of proteins and has valuable amino acids and fatty acids like palmitic acid, oleic acid and linoleic acid.

Nigeria recorded fish production of 1.1 million metric tonnes between 2015-2017 (AgroNigeria, 2017). This increase in production will consequently lead to increase in demand for fish feeds. The dietary requirements of *Oreochromis niloticus* are well documented (NRC, 1993; Wilson, 1994). However, alternative feeds are very crucial for sustainable production with affordable sale prices (Ighwela et al. 2012). In view of these, strides have been made to inculcate the use of non-conventional feed stuff to reduce cost of feed production.

Haematological parameters are valuable tools for monitoring fish health and confirming maturation (Satheeshkumar et al. 2011). However, the diet composition, metabolic adaptation and variation in fish activity are the main factors responsible for the change in blood parameters of fish (Rehulka, 2003), there have been reports on fish feeds causing changes in blood parameters of fish (Ighwela et al. 2012; Dienye and Olumuji, 2014; Adewole and Olaleye, 2014). Therefore, the inclusion of plant protein sources in the ration of fish requires investigation on its limiting factors such as high crude fibre and anti-nutrient contents (Francis et al. 2001; Alegbeleye et al. 2001; Nwanna et al. 2008). *Tamarindus indica* is a multipurpose tree of which almost every part finds at least some use (Kumar and Bhattacharya, 2008), either nutritional or medicinal and is indigenous to tropical Africa. There is usually no use for the seed as they are discarded after the pulp have been removed for making kununtsamiya (Bashir and Suleiman, 2018).

"Therefore, the objective of the present

parameters of O. niloticus fed varying inclusion levels of fermented T. indica seed meal.

MATERIALS AND METHODS

Sample collection and preparation

The *O. niloticus* fingerlings used for this research were purchased from Songhai Nigeria Partnership Initiative LTD/GTE Funtua Branch, Katsina State. *T. indica* seeds were obtained from the wild around Zaria and identified at Herbarium Unit of Department of Botany, Ahmadu Bello University, Zaria.

T. indica seeds were prepared to remove dirt and bad seeds, and processed by fermentation. Fermentation was done according to the method of Shlini and Siddalinga Murthy (2015). This was done by soaking the seeds for 72 hours and then dehulled mechanically with mortar and pestle. Then the dehulled seeds were fermented by putting them into an airtight container for 72 hours. The fermented seeds were then sun dried, milled into flour and were oven dried at a temperature of 60℃ and further dried under the sun. The flour was then packaged

Anti-Nutrients in *Tamarindus indica* seed nut

Alkaloid, Saponin, Tannin and Phytate were determined using recommended methods of the Association of Official Analytical Chemist procedures (AOAC, 1980).

Experimental diet

The seeds were milled and added to replace soybean meal ingredient using various graded levels. The experimental diet for *O. niloticus* was formulated using conventional feed ingredients applying the Pearson's square method. The ingredients were mixed together in each case (each protein inclusion level) and water was added and mixed thoroughly.

A hand pelletizer was used in pelleting the feed and followed by sun drying. Each experimental feed concentrate was packed in a separate container and kept in a dried and cool condition to prevent fungi attack; the feeds were collected and kept separately in polythene bags based on various inclusion levels. Five diets including the control diet at varying inclusion levels (0%, 25%, 50%, 75% and 100%) were formulated.

Table I: Composition of Experimental Diets Used for Feeding Trial

Ingredients	Inclusion Levels of *T. indica* Fermented Seed (%)				
	0	25	50	75	100
Soya bean meal	40.90	30.68	20.45	10.23	0.00
T. indica meal	0.00	10.23	20.45	30.68	40.90
Fish meal	20.45	20.45	20.45	20.45	20.45
Yellow maize	28.64	28.64	28.64	28.64	28.64
Bone meal	1.00	1.00	1.00	1.00	1.00
Palm oil	3.50	3.50	3.50	3.50	3.50
Salt	0.80	0.80	0.80	0.80	0.80
Vitamin Premix	0.70	0.70	0.70	0.70	0.70
Methionine	2.00	2.00	2.00	2.00	2.00
Lysine	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100

Experimental setup

One hundred (100) *O. niloticus* fingerlings were collected and acclimatized within the laboratory for two weeks (14 days). The feeding and growth experiment were conducted in the Fisheries Laboratory of Department of Biology, Ahmadu Bello University, Zaria. Ten plastic aquaria with dimension of 50 × 45 × 35cm were used (the experiment was replicated twice with two aquaria/experimental diet) in a static culture system, each containing de-chlorinated water. Ten fingerlings of *O. niloticus* were randomly stocked in each aquarium.

Collection of blood samples

Blood was sampled as described by Blaxhall and Diasely (1973). Blood was collected by severance (2cm) of the caudal peduncle. Blood was collected with a 5mm syringe.

Determination of haematological parameters

Haematocrit (PCV) was determined by the Wintrobe and Westergreen method as described by Svobodova*et al*. (1991). Percentage Haemoglobin (Hb) concentration was determined as described by Mohmoh *et al.* (2012) using Drabkin's solution and with the aid of a model XF-1C haemoglobinometer. The RBC count was determined using an improved Neubauer haemocytometer under ×40 objective and calculated (Dacie and Lewis, 2001). Total white blood cell count was determined as described using the standard two slide wedge technique to make blood films and the Giemsa's staining technique, counter stained with Leishmann's stain. Total leucocytes were calculated as formulated by Campbell (1995). Erythrocyte indices which include Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin concentration (MCHC) were calculated as recommended by Miale (1982) as follows:

$$\text{MCV (Mean Corpuscular Volume)} = \frac{\text{Hct (\%)}}{\text{RBC } (10^6/\text{mm}^3)} \text{ x } 10\ (\mu\text{m}^3)$$

$$\text{MCH (Mean Corpuscular Hemoglobin)} = \frac{\text{Hb } (\frac{\text{g}}{100}\text{ ml})}{\text{RBC } (10^6/\text{mm}^2)} \text{ x } 10\ (\text{pg cell}^{-2})$$

$$\text{MCHC (Mean Corpuscular Hemoglobin Conc.)} = \frac{\text{Hb } (\frac{\text{g}}{100}\text{ ml})}{\text{RBC } (10^6/\text{mm}^2)} \text{ x } 100\ (\text{g dL})$$

During the experiment, observations were made on the significant increases in the RBCs, Hct, Hb, RBC indices (MCV, MCH, MCHC) and ESR among the three different treatment groups. Haematological parameters of fish species in the control group were compared with those of the groups fed varying inclusion levels of fermented *T. indica* seed meal. Feeding frequency recommended by Marinmuthu et al. (2010) was adopted. Feed were administered four times daily. Experimental fish were fed at 5% body weight.

Data analysis

Microsoft Office Excel (2013) statistical software package was used to run all statistical analysis. Descriptive statistics was used to summarize data obtained. Student's t-test was used to determine if there was significant difference between the anti-nutritional content of the raw and fermented seed. One-way ANOVA was adopted to determine if there was significant difference ($P \leq 0.05$) between the growth performance and haematological indices of *O. niloticus* fed varying inclusion levels of fermented *T. indica* seed meal.

RESULTS

Table 2 presents the results for anti-nutrient composition of *T. indica* seed. The values for alkaloid content of fermented and raw samples were 1.50mg/g^{-1} and 3.70 mg/g^{-1}, respectively. There was significant difference (P<0.05) between the alkaloid content of raw and fermented seeds. The values of the saponin content of fermented and raw samples (1.80mg/g^{-1} and 2.3mg/g^{-1}, respectively) also showed significant difference (P<0.05). Tannin

content of the seed also varied significantly (P<0.05). Fermentation significantly reduced the alkaloid, saponin and phytate content of the seeds by 59.46, 21.74 and 60.30%, respectively.

Table 2: Antinutrients Composition of *Tamarindus indica* Seed

Anti-nutrients (mg/g^{-1})	Raw *T. indica* Seed	Fermented *T. indica* Seed
Alkaloid	3.70 ± 0.10^{a}	1.50 ± 0.05^{b}
Saponin	2.30 ± 0.10^{a}	1.80 ± 0.12^{b}
Tannin	2.37 ± 0.02^{b}	4.22 ± 0.16^{a}
Phytate	6.75 ± 0.15^{a}	2.68 ± 0.09^{b}

Means with the same superscript along rows do not vary significantly (P>0.05)

Growth performance of *O. niloticus* fed varying inclusion levels of fermented *T. indica* seed meal is presented in Table 3. Diet D_1 recorded highest value for weight gain (12.40g) while experimental diet with 100% inclusion level of fermented *T. indica* seed meal had the least weight gain (2.25g). The highest standard-length gain (2.81cm) and total length gain (3.58cm) were observed in fish fed 0% inclusion level of fermented *T. indica* seed meal. Whereas, the lowest standard-length gain (0.70cm) and total length gain (0.72cm) were observed in those fed 25% inclusion level of fermented *T. indica* seed meal. All growth parameters showed significant difference (P<0.05).

Table 3: Growth Performance of *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindusindica* Seed Meal

Parameters	Inclusion Levels				
	0%	25%	50%	75%	100%
WG (g/fish)	12.40 ± 0.33^{a}	4.77 ± 0.92^{c}	3.19 ± 1.54^{c}	8.58 ± 0.68^{b}	2.25 ± 0.78^{c}
SLG (cm/fish)	2.81 ± 1.76^{a}	0.70 ± 0.03^{c}	1.21 ± 0.48^{b}	2.54 ± 0.24^{a}	1.24 ± 0.22^{b}
TLG (cm/fish)	3.58 ± 1.80^{a}	0.72 ± 0.00^{c}	1.88 ± 0.24^{b}	3.12 ± 0.14^{a}	1.37 ± 0.32^{b}

Means with the same superscripts along rows do not vary significantly (p>0.05)
Weight Gain (WG), Standard Length Gain (SLG) and Total Length Gain (TLG)

The results for the blood indices of fish fed varying inclusion levels of the diet are presented in Table 4. All blood indices showed significant difference (P<0.05) except for MCHC. Fishes fed 0% inclusion level of fermented *T. indica* seed meal gave the highest PCV, Hb, WBC, MCV and MCH. The highest RBC was recorded in fishes fed the 25% inclusion level of fermented *T. indica* seed meal, 35.20 x 10^{6} mgl^{-1}.The values of MCV, MCH and MCHC ranged from 7.10–12.50 μm^{3}, 2.37 – 4.17pg $cell^{-1}$ and 3.33

Table 4: Haematological Indices of *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindus indica* seed meal

Parameters	Inclusion levels of Fermented *T. indiica* seed meal (%)					P Value
	0	25	50	75	100	
PCV (%)	40.00 ± 1.00^{a}	25.00 ± 1.00^{c}	31.00 ± 2.00^{b}	31.00 ± 1.00^{b}	25.00 ± 0.00^{c}	0.001
Hb (g/dL)	13.34 ± 0.34^{a}	8.34 ± 0.34^{c}	10.34 ± 0.67^{b}	10.34 ± 0.34^{b}	8.33 ± 0.00^{c}	0.001
RBC x 10^{6} mgl^{-1}	32.00 ± 0.50^{ab}	35.20 ± 0.80^{a}	30.40 ± 1.80^{bc}	28.00 ± 0.40^{c}	29.60 ± 0.50^{bc}	0.020
WBC x 10^{3} mgl^{-1}	480.00 ± 6.00^{a}	124.90 ± 0.10^{c}	140.00 ± 0.20^{b}	110.00 ± 4.00^{d}	115.00 ± 1.00^{cd}	0.000
MCV (μm^{3})	12.50 ± 0.41^{a}	7.10 ± 0.05^{d}	10.20 ± 0.03^{b}	10.07 ± 0.00^{b}	8.45 ± 0.05^{c}	0.000
MCH (pg $cell^{-1}$)	4.17 ± 0.03^{a}	2.37 ± 0.01^{e}	3.40 ± 0.01^{c}	3.69 ± 0.11^{b}	2.81 ± 0.00^{d}	0.000
MCHC (g/dL)	3.33 ± 0.00^{a}	3.33 ± 0.00^{a}	3.33 ± 0.00^{a}	3.33 ± 0.00^{a}	3.33 ± 0.00^{a}	0.701

Means with the same superscripts along rows do not vary significantly (P>0.05)
Note: PCV = Packed cell volume, Hb = Haemoglobin count, RBC = Red blood cells, WBC = White blood cells, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration.

DISCUSSION

It is clear that the seed still contained a significant amount of Alkaloid even after pretreatment. The alkaloid content of the ferment seed is however lower than that of Soya beans reported by Okwu and Orji (2007), 1.64mg/g^{-1}. The lower level of alkaloid in the fermented sample could be due to the extended hours spent during soaking of the seed sample to remove the seed coat and fermentation process, since Kumar *et al*. (2012) have reported aqueous extraction and treatment to remove alkaloids from seed materials. Saponins have also been reported to be toxic to fish when added to water, they exhibit detergent action causing damage to the respiratory epithelium of gills and inhibits active transport of nutrients (Kumar *et al*. 2012). However, simultaneous consumption of saponin and tannin can neutralize the aforementioned condition because it results in the loss of individual toxicity of both compounds due to the formation of tannin-saponin complexes which inactivates the separate biological activity of both tannin and saponins (Bashir and Suleiman, 2018). Saponins also have the ability to precipitate and coagulate red blood cells (Sood *et al.* 2012) hence their application to heal wounds (Okwu and Josiah, 2006). Anti-nutritional effects of tannins include interference with digestion by binding to proteins or minerals. The saponins content of the fermented seed is higher than that of Soya bean reported by Banaszkiewics (2011), 0.5mg/g^{-1}. The higher level of tannin in the fermented sample can be as result of the extended hours spent during soaking of the seed sample to remove the seed coat and fermentation process which made the hydrolysable tannin content more available within the seed (Bashir and Suleiman, 2018), and it is higher than that of soya beans reported by Okwu and Orji (2011), 0.46mg/g^{-1}.

Nwaoguikpe *et al.* (2011) opined that pretreatments such as soaking and boiling, effects significant reduction of the anti-nutrients concentrations and toxicants present in *Mucuna pruriens* (Velvet Beans) seeds which is also a non-conventional legume.

The growth response recorded in this study was significantly influenced by varying inclusion levels of fermented *T. indica* seed meal. However, SLG and TLG increased with increase in inclusion level. The weight gain of *O. niloticus* are in conformity with the findings of Agbo *et al.* (2011) who reported decrease in growth rate with increase in the level of cotton seed meal in the diet of *O. niloticus*. However, SLG and TLG in this study are contrary to their findings.

The haematological parameters of fish are reported to be affected by a range of factors, which include species, size, age, physiological status, environmental conditions and dietary regime, e.g. quality and quantity of food, dietary ingredients, protein sources, vitamins, probiotics (Lim *et al*. 2000; Osuigwe *et al*. 2015). All blood indices increased with increase in inclusion levels of fermented *T. indica* seed meal. The PCV of this study are higher than that of Gbore *et al.* (2010), 19.67-39.00% and Ighwela *et al.* (2012), 12.18-13.74. White blood cell count of the fishes in the study is also greater than that reported by Kefas et al. (2015) which ranged between 108-238 x 10^3 mgl^{-1}. This result shows that fermented *T. indica* seed meal has no adverse effect on *O. niloticus* as their blood indices are not depleted.

CONCLUSION

It can be concluded that fermentation significantly decreased the anti-nutrients of the seed except for tannin content. Also, the 75% inclusion of fermented *T. indica* in the diet if *O. niloticus* gave the best weight gain compared to the rest of the diets. Fermented *T. indica* seed meal has no adverse effect on the blood indices of *O. niloticus*. Also, the little amount of anti-nutrients in the seed can serve for medicinal purposes.

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