



Research Article

**GROWTH RESPONSE, NUTRIENT UTILIZATION AND
APPARENT NUTRIENT DIGESTIBILITY OF NILE TILAPIA
(*OREOCHROMIS NILOTICUS*) FINGERLINGS FED VARYING INCLUSION
LEVELS OF GERMINATED TROPICAL KUDZU
(*PUERARIA PHASEOLOIDES*) SEED MEAL**

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ABSTRACT

This study investigates the use of germinated *Pueraria phaseoloides* (Tropical kudzu seed): GPPSM in practical diets for Nile tilapia *Oreochromis niloticus* fingerlings. Fish (mean initial weight 1.4±0.1 g) were fed five isonitrogenous and isolipidic diets formulated, at 35% crude protein and 9% lipid-containing different levels of inclusion of GPPSM and designated as D1 (0 % inclusion), D2 (10 % inclusion), D3 (20 % inclusion), D4 (30 % inclusion) and D5 (40 % inclusion) for 56 days. 20 fish per hapa were accommodated in Fifteen net hapa (0.5×0.5×1m³) suspended in two outdoor concrete ponds (8m × 5m × 1.5m) with the aid of kuralon twine tied to plastic poles, the concrete ponds were filled to 5/6 of its volume (40m³) with filtered and dechlorinated tap water. The fish were fed at 5% body weight three times daily. The results showed that fish fed D3 and D4 had the highest significantly values in all the growth and nutrient utilization values measured and were significantly different ($P<0.05$) from fish fed other experimental diets, while fish fed D5 had the lowest value, however, was not significantly different ($P>0.05$) from fish fed D1 and D2. There was no significant difference in the percentage survival and apparent nutrient digestibility among all the fish fed the experimental diets. The general assessment shows that fish fed all the experimental diets did well in all the growth parameters measured and D4 had a better feed utilization than the other fish fed the experimental diets. In conclusion, the inclusion of GPPSM up to 30% is suitable and had no negative impact on the growth, survival and nutrient utilization of *O. niloticus* fingerlings. This study also shows that GPPSM can be a potential ingredient for the aquafeed industry.

Keywords: *Oreochromis niloticus*, Growth performance, Tropical kudzu seed meal, Hapas.

INTRODUCTION

With the growing population and high demand for animal protein, the need to increase fish production in Nigeria has become most desirable (Bake *et al.*, 2013). However, the

exorbitant cost of imported fish feeds has been reported to be as high as 60-70% of production cost (Eyo, 2003; Fagbenro & Adebayo, 2005). This has made fish production economically unattractive for the small-scale

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fish farmers in the developing nations especially in Nigeria. It's been reported that in early 2002 aquaculture industry was using only about 34% of the total global fishmeal produced (Hardy, 2006) but by 2010, this value had tremendously increased to 45% (Ayinla, 2007), and the demand for fishmeal is still expected to continue to rise not only in aquafeed production but also in other animal feed production (Aniebo *et al.*, 2009). However, its increase in aquafeed production is expected to be at the expense of other animal feeds production indicating that technically, the impact of aquaculture expansion on the availability of fishmeal may be marginal and is expected to soar to 70% in the nearest future (Tacon & Metian, 2015). The high-quality protein content and concentration of essential nutrients, especially of the well-balanced amino acid profile, high digestibility, essential n-3 polyenoic fatty acids, and energy content make fishmeal an indispensable ingredient in the diets of the most aquaculture species (Hardy & Tacon, 2002; Miles & Chapman, 2006). Consequently, the sustainability of feed-based production systems may be threatened by unpredictable availability, shortages and price rises of fishmeal and thus steps must be taken to reduce its inclusion levels in aquafeeds (Fagbenro & Adebayo, 2005).

Tilapia species are the second most cultured freshwater fish in the world and their production is still on the increase (FAO, 2015). This increase in the production of tilapia has been attributed to their tolerance of stressors imposed in routine aquaculture practices, marketability, and their ability to utilize nutrients from a wide variety of sources, including plant feedstuffs (Bake *et al.*, 2009; Gonzales Jr *et al.*, 2007). While the diversity of acceptable feedstuffs is an asset in culture, several challenges still remain in feeding tilapia. Plant proteins as an alternative protein source in fish feed have been extensively studied for use in aquafeed formulations for aquaculture species (Gatlin *et al.*, 2007); these include various pulses, legumes and lupins seeds even for carnivorous fishes such as rainbow trout *Oncorhynchus mykiss*. Ordinarily, plants provide nearly two-thirds of the world supply of food proteins for humans and animals in which 10-15% comes from legumes. Plant proteins examined have included soya bean meal (Quartararo *et al.*, 1998), Cacao husks (Poumogne *et al.*, 1997) various cereals (Al-Ogaily *et al.*, 1996). Despite their usefulness, most of these conventional ingredients are scarce and expensive due to their high demand for livestock production and other industrial production sectors. Moreover, their cultivation generally requires a high use of inputs and energy subsidies (Fasakin *et al.*, 1999; Francis *et al.*, 2002). This makes them unaffordable, unsustainable and sometimes even conflicts with food security interests, particularly among resource-poor farmers of developing nations Nigeria inclusive.

Recently, research focus has shifted and giving priorities to the analysis of the nutritional value of wild plant materials i.e. unconventional ingredients and they have shown to contain a significant amount of essential

nutrients, (proteins, amino acids, vitamins, minerals, oils, and carbohydrates) that can be used for the formulation of animal feeds. The proximate composition of seeds of some wild plants of Nigerian origin reveals that they could be adequately used in the formulation of animal feeds provided the levels of their toxic substance i.e. antinutritional factors are reduced or eliminated (Eromosele & Eromosele, 1993). The need, therefore, arises to look into these nutritional potentials of various wild and unconventional fruits and seeds abundantly present and available in our environments.

Tropical kudzu is a vigorous twining and climbing perennial legume. It is deeply rooted with hairy, slender stems. The stems may root from the nodes and then develop many branches (Chin *et al.*, 1997; Cook *et al.*, 2005; FAO, 2015). Tropical Kudzu forms swards of tangled branches that may reach 60-75cm in height. The pods are 4-11cm long and 3-5mm in diameter. They contain 10 to 25 black, oblong 3mm long seeds. The pods are thinly hairy, green when young, and they turn black when maturing. They are commonly found on riverbanks, roadsides, fallow fields, and secondary forest (Chin *et al.*, 1997). The pods easily split open after sun-dry (Acevedo-Rodríguez, 2005; Chin *et al.*, 1997; Cook *et al.*, 2005). Tonnes of these seeds are wasted every year since they are neither consumed by animals nor utilized for any medicinal propose. Laboratory analysis of *Pueraria* seeds shows it contains about 28% crude protein and relatively low in lipid content (Ezeagu *et al.*, 2000), its relative abundance has remained largely unexploited and underutilized for animal nutrition. *Pueraria phaseoloides* seeds, like other legumes, contain the anti-nutrient factors such as trypsin inhibitor, lectins, phytates, tannin and saponin, which negatively affect the nutritive value of *Pueraria* through direct and indirect reactions such as induce pathological changes in the intestine and liver tissue thus affecting metabolism (Bressani, 1993). These effects limit the use of the raw seed meal, although various processing techniques like cooking and heating (toasting) and other food processing techniques tend to help in reducing the anti-nutritional compounds (Soetan & Oyewole, 2009). (Akinmutimi, 2001; Emenalom & Udedibie, 2005; Sørensen *et al.*, 2009), reported that there are anti-nutritional factors associated with plant proteins hence, plant-derived ingredients still have limited application in aquafeed production, this is because of the complexity of nutrients and anti-nutritional compounds commonly present in typical plant-derived ingredients, both of which reduce nutrient availability to fish (Fagbenro *et al.*, 2003). Development of plant-derived ingredients that are very digestible and with less negative factors affecting digestion and metabolism in fish is of paramount importance to aqua feed researchers and producers. Most of these plant-derived ingredients can better be used when properly processed. In aquaculture, various processing techniques can be applied to allow better and proper plant-derived ingredients utilization among this is the germination processing method. The germination processing method is an effective

food processing method, a unique process which usually improves the nutritional quality of the feed ingredients and has the ability to reduce anti-nutritive compounds, boosting the level and digestibility of free amino acids and available carbohydrates, increasing mineral bioavailability, and improving the functional properties of cereal and pulses (Echendu *et al.*, 2009). Due to the significant effects of germination, sprouted grains and pulses have become popular and widely accepted as functional foods and functional food ingredients. It is a food processing technique that can provide a promising future for plant-derived ingredients for sustainable aquaculture.

Although *Pueraria phaseoloides* seed is in abundant and produced tonnes of pods especially during the dry season and not much work has been done on its utilization as an ingredient in the diet of *O. niloticus* fingerlings and its subsequent impact on the digestibility of the diet by the fish. It is in the view that this study was carried out with the main objective to evaluate, among other things the growth response, nutrient utilization, and nutrient digestibility of *O. niloticus* fingerlings fed varying inclusion levels of germinated *P. phaseoloides* seed meal (GPPSM).

MATERIALS AND METHODS

Ingredients and diet formulation Fishmeal (FM) and Soybean Meal (SMB)

The fishmeal used in this experiment was obtained from the Musgola Fish Farm, along Bosso Estate road Minna Niger State Nigeria. The crude protein and lipid content of fishmeal were 65.34 % and 11.36% respectively as shown in Table 1. Raw soybean was purchased from the Bosso market Minna (Niger State). The soybean was processed by toasting the soybean in frying pan at 80°C for 60 minutes until the colour changes to golden brown and allowed to cool before milling with the aid of a grinding machine. Crude protein and lipid contents of SMB were 43.63% and 7.00%, respectively as shown in Table 1.

Germinated *Pueraria phaseoloides* seed meal (GPPSM)

P. phaseoloides seed pods were collected manually during the dry season from Rafin Yashi Area of Bosso Local Government, Niger State. The pods were manually crushed with aid of a pestle and mortar to get the seed. 5kg of the seed was pre-soaked inside a bowl; the pre-soaked seeds were carried out by mixing the *P.ueraria phaseoloides* seed with water in the ratio 3:1 (1 part of *P. phaseoloides* seed to 3 parts of water). This was done to separate the viable seed from the not viable once, the not viable seeds floated to the water surface while the viable seeds remain at the bottom of the bowl. The viable seeds were then soaked inside warm water (80°C) overnight for 8 hours in a firmly sealed transparent plastic container. After 8hrs the soaked seeds were removed and placed in a new bowl and covered with wet cotton wool at room temperature (29-30°C) for 72 hrs. The cotton wool was moistened with clean water at regular intervals of 12 hrs. Seeds that had failed sign of

germination were discarded. The seed coats of the germinated seeds were removed and the seed cotyledons were washed and spread on a polythene sheet in a room and dried for six (6) days up to about 90% of the dry matter. The seeds were grounded into powder using a hammer mill. Crude protein and lipid contents of GPPSM were 33.44% and 5.45%, while RFSM was 26.85% and 4.94% respectively as shown in Table 1. All the ingredients were separately milled and mixed with warm water to form consistent dough, which was then pelleted, sun-dried, packed in polyethylene bags and stored. The feed composition table is shown in Table 2.

Experimental diets

Based on the nutritional requirements of *O.niloticus* fingerlings (NRC, 2011), five isonitrogenous and isolipidic diets were formulated at 35 % protein and 9.0 % lipids, containing 10 – 40% GPPSM at different levels of inclusion.

Experimental conditions and fish rearing

The experimental fish, pure-bred *O. niloticus* fingerlings, with an initial mean weight of (1.42-1.44g) were purchased from National Institute for Freshwater Fisheries Research (NIFFR) New Bussa, Niger State. The fish were transported in a well-oxygenated water plastic container to the old University Departmental Fish Farm Bosso Campus. Upon arrival, they were acclimatized in a transitional tank at the farm for a week before the commencement of the experiment and were fed a conditioning diet (Vital-feed) at 40% crude protein once a day. Twenty fish were randomly distributed into 15 net hapas (0.5 m × 0.5 m × 1 m) suspended in two outdoor concrete tanks (8 m×5 m×1.5 m) with the aid of a kuralon twine tied to plastic poles. The concrete tanks were filled to 5/6 of their volume (40 cm³) with filtered and de-chlorinated tap water. The fish were subsequently fed the experimental diets containing different inclusion levels of GPPSM, designated as D1 (0% inclusion), D2 (10% inclusion), D3 (20% inclusion), D4 (30% inclusion) and D5 (40% inclusion) for 56 days. Each treatment was randomly distributed in triplicate hapas. Photoperiod was dependent on the natural light and the water quality parameters in the system were monitored weekly. The temperature ranged between 25°C-29°C while the concentration of dissolved oxygen ranged between 5.85-7.64 mg/L and the pH values of the treatments ranged from 6.45-7.80. No critical values were detected for nitrite and nitrate. The feed was manually administered until apparent satiation three times daily at 09:00 am, 12:00 pm and 16:00 pm. The uneaten feed and faecal matters were siphoned out of the hapas every morning before feeding, and 45 minutes after the fish had been fed. Faecal samples were collected for digestibility analysis from each hapa daily for 7 days (8 h after feeding) by siphoning with a rubber tube and were oven-dried at 60°C. The fish were denied feed 24 h prior to sampling. Five fish were randomly sampled on a weekly basis, and weights were

measured using a digital electronic balance (CITIZEN MP-300 model).

Biochemical Analysis

The major ingredients, experimental diets and fish samples were subjected to chemical analysis with proximate composition determined according to (AOAC, 2000) procedures. A total of 10 initial fish and 5 fish of final samples from each hapa were pooled separately and then homogenized using a laboratory mortar and pestle. Moisture content was determined by drying samples at $105\pm 2^{\circ}\text{C}$ until a constant weight was obtained. Dried samples were used for determination of crude fat, protein and ash contents. Crude fat was measured by a solvent extraction method in a Soxhlet system using n-hexane. Crude protein content was calculated by measuring nitrogen content determined by the Kjeldahl method. A conversion factor of 6.25 was used for calculation of crude protein content according to (AOAC, 2000). Anti-nutritional factors of the seeds; tannins and trypsin inhibitor activity (TIA) were analyzed by modifying the procedures of (AOAC, 1984). Phytic acid was determined by the method of (Latta & Eskin, 1980).

Acid insoluble ash (AIA) analysis

Analysis of AIA was carried out on the diets and faeces to estimate nutrient digestibility. The AIA was obtained by adding 25 ml of 10% HCl to the weighed ash content of each sample. The sample was covered with a water-glass and boiled gently over a low flame for 5 minutes. Then the sample was filtered using ashless filters and washed with hot distilled water. The residue from the filter was returned to the crucible and ignited until it was carbon-free after which it was weighed. Percentage

AIA was calculated as:

$$\% \text{ AIA} = \frac{\text{Weight of AIA}}{\text{Weight of Ash}} \times 100$$

Determination of the digestibility coefficient

The determination of the protein and lipid digestibility coefficient was done according to Jimoh *et al.* (2010) which were calculated based on the percentage of AIA in feed and in faeces and the percentage of nutrient on diets and faeces.

$$\text{Apparent protein digestibility (\%)} = 100 - \left(\left(\frac{\text{AIA in diet (\%)}}{\text{AIA in faeces (\%)}} \right) \times \left(\frac{\text{N in faeces (\%)}}{\text{N in diet (\%)}} \right) \right) \times 100$$

Evaluation of Growth and Nutrient Utilization Parameters

Growth and nutrient utilization were evaluated using weight gain (%) (WG), Feed Efficiency (FE), Specific Growth Rate (SGR), Feed Intake (FI), Protein Efficiency Ratio (PER) and Protein Retention (PR). The following formulas were used:

$$\text{Weight gain (\%)} = \frac{(\text{Final weight (g)} - \text{initial weight (g)})}{\text{initial weight (g)}} \times 100$$

$$\text{Feed efficiency (\%)} = \left(\frac{\text{weight gained (g)}}{\text{feed fed (g)}} \right) \times 100$$

$$\text{Specific growth rate (\%)} =$$

$$\left(\frac{\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}}{\text{feeding period (day)}} \right) \times 100$$

$$\text{Feed intake (mg/fish/day)} = \frac{\text{dry feed (mg) given / number of fish}}{\text{feeding period (day)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{weight gain}}{\text{protein intake (g)}}$$

$$\text{Protein retention (\%)} = \frac{\text{protein gain}}{\text{protein fed}} \times 100$$

Statistical analyses

Data were analyzed using one-way analysis of variance (ANOVA) using Minitab 8.0 (Stat-Soft, Inc., Oklahoma, USA). Differences between treatments were compared by Tukey's test. Level of significance was tested at $P < 0.05$.

RESULTS AND DISCUSSION

Over the 8-weeks feeding period, no significant differences were observed in the water-quality indices between the experimental treatments. The water temperature ranges from $25.2\text{--}29.6^{\circ}\text{C}$, Dissolved oxygen from $5.94\text{--}7.82$ mg/l, pH from $6.18\text{--}7.92$ and ammonia from $0.23\text{--}0.29$ mg/L. Table 1 shows the proximate composition of the major ingredients used in formulating the experimental diets. Fish meal has the highest crude protein and lipid content (65.34% and 10.09%) followed by soybean meal (43.07% and 7.00%), while the crude protein and lipid content of both the raw (RPPSM) and germinated *Pueraria phaseoloides* seed meal (GPPSM) was ($26.85\text{--}34.44\%$ and $4.94\text{--}5.45\%$) respectively. Table 2 showed the anti-nutritional factor composition of both the untreated raw *Pueraria phaseoloides* seed meal (RPPSM) and the treated germinated *Pueraria phaseoloides* seed meal (GPPSM). More than 60% concentration of all the anti-nutritive factors parameters measured was lower in the treated GPPSM ingredient as compared to RPPSM. The proximate composition of the experimental diets is shown in Table 3. There were no much variations in the protein and crude lipid content among the experimental diets. The result of growth and the nutrient utilization parameters measured is presented in Table 4. Fish fed D3 and D4 had the highest values in all the growth parameters measured (FBW, WG and SGR) and were significantly different ($P < 0.05$) from fish fed other experimental diets. Fish fed with D5 had the lowest value in all the growth parameter indices measured however, was not significantly different ($P > 0.05$) from the fish fed D1 and D2. There was no significant difference ($P > 0.05$) in the percentage survival among all the fish fed the experimental diets. The nutrient utilization of the fish fed experimental diets follow the same trend as the growth parameters measured. Fish fed D3 and D4 had the highest TFI, FE, PER and PR value among all the fish fed the

experimental diets, and was significantly different from other fish fed other experimental diets. Fish fed D5 had the lowest TFI FE, PER and PR value but was not significantly different from fish fed D1 and D2 ($P>0.05$).

The apparent digestibility coefficient of the experimental diets is displayed in Table 5. Except for the crude fibre which was not significantly different ($P>0.05$) in all the treatments. Fish fed D4 diet had the highest ADC in crude protein, and lipid value and was significantly higher than fish fed other experimental diets ($P<0.05$), while fish fed D5 diet were significantly lower than fish fed other experimental diets ($P<0.05$), however, there was no significant difference between fish fed D1, D2 and D3 ($P>0.05$). Table 6 showed the proximate composition of the fish fed the experimental diets. There was increased in accumulation of protein, lipid and ash in the final carcass of the experimental fish fed the formulated experimental diets as compared to the initial value. The final carcass moisture decreases with the inclusion level of the GPPSM in the diet, hence D1 has the highest moisture carcass concentration and was significantly ($P<0.05$) different from those fed other experimental diet, while fish fed D5 diet had the lowest carcass moisture value although not significantly ($P>0.05$) different from those fed D4. The final carcass lipid accumulation was inversely proportional to the moisture; hence, the lipid accumulation increases with the inclusion level of GPPSM in the diet. There was no significant difference ($P>0.05$) in carcass protein and ash among all the fish fed with experimental diets. The results of this present study demonstrated the suitability of germinated *Pueraria phaseoloides* seed meal inclusion in the diet of *O. niloticus* fingerlings. The crude protein and lipid content of the raw *P. phaseoloides* in this study was lower than those reported by Ezeagu *et al.*, (2000); Gulizia & Downs, (2019). This could be attributed to differences in environmental conditions such as soil types, harvesting time and is in line with the (FAO, 2004) report that factors such as the geographical location of the plant, climatic and soil conditions of the cultured environment could directly affect the composition plant chemical and physiological structures. However, in this study, there was a significant increase in both the crude protein and the lipid values of the GPPSM (33.44% and 5.42%) when compared to the values of raw PPSM (26.85% and 4.94%). This increase can be attributed to the germination processing technique used to process the PPSM. This agrees with the findings of (Echendu *et al.*, 2009; King & Puwastien, 1987), that processing of plant ingredients improves and boost the nutrients component in legumes and other plant products. The physicochemical parameters of the water used in this experiment were within the acceptable and optimum range for the normal physiological functioning of *O. niloticus* fingerlings (Balarin & Hatton, 1979; Boyd, 1982; Li *et al.*, 2006).

There was a significant reduction in contents of the anti-nutritional factors, phytic, oxalate, tannin, trypsin, and saponin values in the GPPSM as compared to the RPPSM an indication that germination process reduced to a large

extent the levels of anti-nutrients present in the seed. The result of this study showed that the phytic, oxalate, tannin, trypsin, and saponin values were reduced drastically by 78.58%; 97.06%; 66.67%; 98.67% and 86.34 % respectively in GPPSM has compared to the values in the RPPSM. This result agrees with the finding of (Bichi & Ahmad, 2010; Echendu *et al.*, 2009) they reported that adequate processing can reduce significantly the antinutritional compounds in seeds. These anti-nutritional factors are growth inhibitors and are common in almost all plant materials. In most cases they form a shield in the protein molecule of the ingredient, thus preventing the proteases (digestive enzyme) from getting to the protein molecule, making them unavailable for digestion, absorption and hence impair the growth of fish. This usually results in wastage in protein contents of the diets as the crude proteins are passed out along with faeces (Eyo, 2003). Therefore, the decrease in the level of anti-nutritional factors in the seeds portends to make the seeds more digestible, absorbable and provide the nutritional element of the seed to the fish. (Jobling, 1993) reported that growth is not constant and that food consumption and growth rate may be influenced by numerous environmental factors (temperature, fish size, stocking density, access to an acceptable quality of food, water exchange and salinity). In this study, there was high acceptability of all the experimental diet during the feeding period, however, the total feed intake, growth and utilization of the diets varies significantly among the treatment. Source and inclusion level of alternative ingredients especially plant protein-based ingredient in aquafeed production directly affects the palatability, acceptability and digestibility thereby having a significant impact on the growth performance and nutrient utilization of the fed fish (Keremah *et al.*, 2017). The growth parameters measured in this study showed that the fish fed the experimental diet (D3) had a higher weight gain although not significantly different from those fed D4. However, beyond this level of inclusion, a slate decline in weight gain was recorded. (Adewole & Olaleye, 2014) reported that weight gain of fish fingerlings is usually a reliable indicator of nutritional adequacy of the diet. (Akbulut *et al.*, 2002) reported that the growth rate is considered a trait of great economic importance for all fish species used in aquaculture. The specific growth rates recorded are higher than the one recorded in the control diet. However, the SGR is considered to vary depending on the size of the fish; though (Sumpter, 1992) reported that larger fish do not grow faster as compared to the smaller ones, the result from this study showed that SGR was high signifying that the fishes used in this experiment were fingerlings still in their growing phase. The protein efficiency ratio recorded is comparable with the control diet. However, (Hei & Sarojnalini, 2012) reported that protein efficiency ratio is a measure of protein quality because the estimation of the nutritional value obtained depends upon the amount of food consumed and the protein content of the diet. The survival rate was splendid; this may

be attributed to good processing, proper handling of the experimental ingredients and diet palatability. In this present study, the result of the feed utilization revealed that all the fish fed the experimental diets effectively utilized the nutrients in the diets as evident in the high FE, PER and

PR recorded. This result is in line with the findings of (Alegbeleye *et al.*, 2004; Bake *et al.*, 2013; Bake *et al.*, 2014; Eyo, 2003) they reported that removal of undesirable components is essential for the enhancement and effective utilization of plant nutrients in fish feed.

Table 1. Proximate composition (expressed on a dry-matter basis) of the major ingredients used for the experimental diets including germinated *Pueraria phaseoloides* meal (GPPSM).

Ingredients Proximate composition	Fishmeal	Soybean meal	Maize meal	Millet meal	RPPSM	GPPSM
Moisture (%)	5.86	3.10	4.66	3.22	4.59	5.74
Crude protein (% d.b. ^{*1})	69.34	43.07	9.32	12.86	26.85	33.44
Crude lipid (% d.b. ^{*1})	10.09	7.00	4.20	4.36	4.94	5.42
Ash (% d.b. ^{*1})	14.34	8.15	3.22	2.33	3.65	3.48
Crude fibre (% d.b. ^{*1})	0.06	5.00	3.40	2.60	7.24	6.92

^a Means of two replicate analyses.

Table 2. Effect of fermentation treatment on anti-nutritional factors in Raw and Germinated *Pueraria phaseoloides* seed meal (GPPSM).

Anti-nutritive factors (%)	RPPSM	FPPSM	(%) decrease of anti-nutritive factors after Germination
Phytic	0.28	0.06	78.57
Oxalate	2.72	0.08	97.06
Tannin	0.15	0.05	66.67
Trypsin	1.5	0.02	98.67
Saponin	4.76	0.65	86.34

^a Means of two replicate analyses.

Table 3. Formulation profile and proximate composition of experimental diets (g/kg).

Ingredients	D1	D2	D3	D4	D5
Fishmeal (Clupeid) ^{*1}	459.00	406.40	353.90	301.40	248.90
Soybean meal	50.00	50.00	50.00	50.00	50.00
GPPSM	0.00	100.00	200.00	300.00	400.00
Yellow Maize	50.00	50.00	50.00	50.00	50.00
Millet meal	50.00	50.00	50.00	50.00	50.00
Starch	50.00	55.00	50.00	50.00	50.00
Cellulose	270.60	217.50	174.40	126.20	78.00
**Vitamin premix	20.00	20.00	20.00	20.00	20.00
Soybean oil	30.40	31.10	31.70	32.40	33.10
**Mineral	20.00	20.00	20.00	20.00	20.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00
Moisture (%)	6.7	6.6	7.0	6.7	6.7
Crude protein (% d.b. ^{*1})	33.3	33.1	33.2	33.2	33.2
Crude lipid (% d.b. ^{*1})	8.6	8.7	8.7	8.7	8.4
Ash (% d.b. ^{*1})	12.3	12.5	12.3	12.1	12.3
Crude fibre (% d.b. ^{*1})	5.0	5.1	5.2	5.2	5.3
AIA (% d.b. ^{*1})	4.7	4.8	4.9	4.3	5.0

*FM= Fishmeal; SBM= Soybean meal; GPPSM= Germinated *Pueraria phaseoloides*; MM= Yellow Maize; SBO= Shea butter oil, *d.b. = dry bases, AIA = Ash insoluble ash.

** : Premix composition: vitamin and mineral premix (IU or mg / kg of premix). Vitamin A: 4800 IU; Cholecalciferol (vitamin D): 2400 IU; Vitamin E: 4000 mg; Vitamin K: 800 mg; Vitamin B1: 400mg; Riboflavin: 1600 mg; Vitamin B6: 600 mg, Vitamin B12: 4 mg; Pantothenic acid: 4000 mg; Nicotinic acid: 8000mg; Folic acid: 400 mg; Biotin: 20 mg, Manganese: 22000 mg; Zinc: 22000 mg; Iron: 12000 mg; Copper: 4000 mg; Iodine: 400 mg; Selenium: 400mg; cobalt: 4.8 mg.

Table 4. Growth performances and nutrient utilization of *O. niloticus* fingerling fed experimental diets for 56 days.

Diet code	Body weight (g)		Weight gain (%)	Survival rate (%)	Specific growth rate (%)	Total feed intake (g)	Feed efficiency	Protein efficiency ratio	Protein retention (%)
	Initial	Final							
D1	1.43±0.22	11.93±0.31 ^b	734.0±16.4 ^b	98.2±2.2 ^a	3.03±0.83 ^b	13.4±1.2 ^b	0.79±0.48 ^b	2.36±0.15 ^b	38.5±0.8 ^b
D2	1.42±0.16	12.14±0.41 ^b	755.2±15.5 ^b	98.3±2.2 ^a	3.07±0.36 ^b	13.7±0.4 ^b	0.79±0.32 ^b	2.37±0.18 ^b	38.6±0.5 ^b
D3	1.42±0.24	13.89±0.31 ^a	878.2±18.5 ^a	98.6±2.5 ^a	3.26±0.52 ^a	15.3±1.3 ^a	0.81±0.25 ^a	2.45±0.16 ^a	40.0±0.4 ^a
D4	1.42±0.18	13.56±0.32 ^a	855.2±26.7 ^a	98.3±2.3 ^a	3.22±0.66 ^a	15.0±1.7 ^a	0.81±0.44 ^a	2.44±0.11 ^a	40.0±0.5 ^a
D5	1.44±0.28	11.65±0.34 ^b	708.8±18.4 ^b	98.5±2.5 ^a	2.99±0.57 ^b	13.3±1.5 ^b	0.77±1.34 ^b	2.32±0.14 ^b	37.8±0.9 ^b

Values in the same column with different superscript letters are significantly different ($p<0.05$) from each other.

Table 5. Apparent diet digestibility coefficients of experimental diets fed to *O. niloticus* fingerlings for 56 days.

Diet code	ADC of crude protein (%)	ADC of crude lipid (%)	ADC of crude fibre (%)
D1	86.6±1.5 ^b	83.3±1.2 ^b	57.1±1.2 ^a
D2	86.2±1.5 ^b	83.1±1.4 ^b	57.5±1.1 ^a
D3	86.7±1.4 ^b	83.2±2.2 ^b	57.6±1.5 ^a
D4	87.9±1.6 ^a	84.8±1.2 ^a	57.4±1.3 ^a
D5	85.4±2.8 ^c	82.2±1.5 ^c	57.6±1.6 ^a

Values in the same column with different superscript letters are significantly different ($p<0.05$) from each other.

Table 6. Proximate composition analyses of whole-body *O. niloticus* fingerlings (wet basis) fed experimental diets for 56 days.

Component (%)	Initial	Final ^{*1}				
		D1	D2	D3	D4	D5
Moisture	75.42	74.5±1.2 ^a	73.4±0.8 ^b	73.1±1.4 ^b	71.6±1.5 ^c	71.2±1.4 ^c
Protein	13.18	15.4±1.5 ^a	15.6±1.3 ^a	15.7±1.1 ^a	15.4±1.2 ^a	15.4±1.5 ^a
Lipid	3.66	4.1±0.5 ^c	4.6±0.4 ^b	4.7±0.6 ^b	5.2±0.4 ^a	5.7±0.4 ^a
Ash	3.26	4.8±0.2 ^a	4.7±0.5 ^a	4.6±0.3 ^a	4.8±0.3 ^a	4.9±0.3 ^a

*1 Values in the same row with different superscript letters are significantly different ($p<0.05$) from each other (n=3).

Fish fed the experimental diets showed that the crude protein content in all the fish was significantly higher ($p<0.05$) at the end of the experiment than that of the fish at the beginning. The accumulation and deposition of the protein, lipid and ash in the carcass of the fish fed the experimental diets increased compared to the initial value. However, the carcass compositions vary among treatment. Proximate composition of the whole body of the fish fed experimental diets in this study revealed that except for protein and ash, moisture and lipid content of carcass of fish fed the experimental diets were influenced by the

varying inclusion levels of GPPSM. The protein content recorded in this study is an indication of the protein-rich nature of the experimental fish. Although, there was no significant variation amongst all the treatment. Furthermore, the result showed that the carcass lipid in this study was closely and inversely related to the carcass moisture content of the fish fed the experimental diets. The carcass lipid increased with the inclusion level of GPPSM in the diet while the moisture decreased. This result in agreement with the findings of (Bake et al., 2013; Bake et al., 2012; Luo et al., 2005; Yildirim et al., 2003) these

authors reported that lipid content is directly related to the nutrition of the fish.

CONCLUSION

In conclusion, the results of this study demonstrate that 10–30% of GPPSM can be included in the fish diet, and can also be used effectively as an ingredient especially in the practical diet of *O. niloticus* fingerlings. Furthermore, amino acid and essential lipid of GPPSM should be investigated.

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