ECONOMIC VIABILITY OF *Pueraria phaseoloides* LEAF MEAL INCLUSION DIETS FED TO *Clarias gariepinus* JUVENILES

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ABSTRACT
Modern aquaculture calls for the reduction of feed costs, which is partly achievable via dietary plant protein inclusion. A 70-day feeding experiment was conducted to investigate the economic viability of inclusion of Pueraria phaseoloides leaf meal (PLM) as a protein ingredient in the diets of Clarias gariepinus juveniles. Five isonitrogenous diets (D1-D5) were formulated to contain 40% crude protein with inclusion of PLM at 0%, 5%, 10%, 15% and 20% levels, respectively. D1 (0% PLM) served as the control diet. One hundred and fifty C. gariepinus juveniles of initial mean weight of 79.87 ± 5.85 g were randomly allocated to five dietary groups (G1-G5) and three replicates each, and fed with the five PLM formulated diets, respectively, at 5% biomass daily. Fifteen concrete tanks of volume 1m^3 each were used for the experiment. Each tank was stocked with ten juveniles. Results showed that the cost/benefit ratio increased with the levels of inclusion of PLM. Hence, D5 (20% PLM) gave the highest cost/benefit ratio of 1:2.38 while D1 (0% PLM) gave the least cost/benefit ratio of 1:1.87. The present findings showed that PLM can be included in C. gariepinus diet up to 20% level for profit maximization in the aquaculture industry.

Keywords: Feed, growth, nutrition, population, survival.

INTRODUCTION

Food is one of the basic necessities of life (Amadi, 2011), and of course one of humanity's top problems in 2050 (Smalley, 2003). The trends of global demographics show us that the world population is growing and will continue to grow. It is around 7.8 billion today and by 2050 it will reach 9.8 billion (Smalley, 2003): 2 billion more mouths to feed. About 90% of this growth will be in Asia and Africa (UN, 2018). Nigeria is projected to be the world's third most populous country by the year 2050 after China and India (UN, 2017). High population without a matching increase in food production and availability to the people can lead to household food insecurity (Amadi, 2011). One major problem in developing countries is the level of nutrition of the people; the bulk of the citizens are underfed and malnourished, and thus lack the physical energy and mental capacity needed to solve their problems (Olusanya, 2008).

Global demand for fish is growing due to population growth, urbanization and income growth (Delgado *et al*., 2003). However, the ocean fisheries cannot increase yields without destroying the fish stocks on which they depend. Aquaculture seems to be the only alternative means of supplying fish for consumers, to fill the growing gap between supply and demand for food fish, while maintaining wild fishery harvests at sustainable levels. However, manufactured feed is a major challenge of aquaculture because feed gulps at least 60% of the total production costs (Gabriel *et al*., 2007). Lack of locally produced, affordable and high-quality fish feed is one major cause of the low fish production in Nigeria and other parts of Africa (Gabriel *et al*., 2007). Therefore, there is urgent need to search for readily available and cheap plant protein ingredients to partially or completely replace the expensive and scarce conventional protein ingredients in fish feeds.

Clarias gariepinus is one of the most popular fish for culture in Nigeria (Adeogun *et al*., 2007). Due to increasing demand for catfish in Nigeria in recent years, fish farmers have increased production leading to increased stocking and feeding rates (Akinrotimi *et al*., 2007). Cultured *C. gariepinus* contributes about 90% of the total aquaculture production in Nigeria (Adewumi and Olaleye, 2011) and about 60% of the total aquaculture production globally (George *et al*., 2013).

Pueraria phaseoloides is a fast-growing wild legume plant used in soil conservation; the leaf is used as forage for animals especially rabbits in some African counties while the seeds are used as feed for birds in some Asian countries (Ifeanacho *et al*., 2017). Legumes are known for their high nutritive value and presence of phytochemicals which have therapeutic effects in the prevention and treatment of various diseases such as coronary heart diseases, diabetes, cancers, etc. (Rebello *et al*., 2014). Phytochemicals present in *P. phaseoloides* leaf include alkaloids, saponins, tannins, flavonoids, terpenoids, cardiac glycosides, carbohydrates, and reducing sugars (Okoye *et al*., 2020). *P. phaseoloides* is a twiner, climbs over other plants and can become an invasive species (Soria *et al*., 2002) due to its fast growth, wide seed distribution and ability to fully cover other plants. *P. phaseoloides* is found everywhere and the leaves can easily be prepared as a leaf meal.

The present study seeks to evaluate the economic viability of inclusion of *Pueraria phaseoloides* leaf meal in *Clarias gariepinus* feed for profit maximization in and sustainability of the aquaculture industry.

MATERIALS AND METHODS

Collection and preparation of *Pueraria phaseoloides* leaf meal

The *P. phaseoloides* leaf sample was sourced and collected from the premises of African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, Nigeria. The sample was identified at the Department of Plant Science and Biotechnology, University of Port Harcourt with Herbarium Number UPH/V/1460. The leaves were shade-dried at room temperature for three weeks and ground into very fine particles. The ground sample was stored for later usage.

Formulation and preparation of experimental diets

The feed ingredients used in this studywere purchased from Modern Agro Enterprises, Rumuokoro, Port Harcourt, Rivers State, Nigeria. These include: wheat bran, soya bean meal, fish meal, bone meal, commercial fish premix, lysine, methionine and vitamin C. Iodized salt, palm oil and garri (binder) were purchased from Rumuokoro market. All the feed ingredients measured out summed up to 100% as shown in Table 1. Five isonitrogenous diets (D1-D5) of 40% crude protein were formulated using Pearson Square method. The control diet (D1) contained 0% PLM, D2 contained 5% PLM, D3 contained 10% PLM, D4 contained 15% PLM, and D5 contained 20% PLM. The dough of each practical diet was pelletized separately using ARAC pelletizer through a 4mm die to produce pellets and sun-dried separately for 72 hours and more. The dry pellets were stored in air-tight plastic buckets and labeled accordingly.

Project location

The feeding experiment was done at the African Regional Aquaculture Centre (ARAC), a Department of the Nigerian Institute for Oceanography and Marine Research (NIOMR), Aluu, Rivers State, Nigeria.

Proximate analysis

All proximate analyses were determined on dry weight basis at the Plant Anatomy and Physiology Research Laboratory, University of Port Harcourt, Rivers State, Nigeria; using the Association of Official Analytical Chemists (AOAC) method (2006). The parameters analyzed for were moisture content, ash content, crude protein, crude fat, crude fibre and total carbohydrates.

Water quality analysis

Water quality analysis was done at ARAC. The water quality parameters determined include temperature, pH, dissolved oxygen, ammonia, nitrite and total hardness. Temperature was measured with mercury-in-glass thermometer, while pH, dissolved oxygen, ammonia, nitrite and total hardness were determined using LaMotte Fresh Water Aquaculture Test Kit (Code 3633-05, USA).

Source of experimental fish/acclimatization

One hundred and fifty *C. gariepinus* juveniles of the same stock and mean weight 79.87 ± 5.85 g were obtained from ARAC Catfish Hatchery. The fishes were acclimatized for two weeks and fed twice daily with ARAC catfish feed at 5% biomass.

Experimental design, rearing units and stocking of fish

Completely Randomized Design was used, with five treatment levels and three replicates each. A total of 15 concrete tanks of volume $1m^3$ each were used for the experiment. Each tank was stocked with 10 juveniles. A total of 150 juveniles were stocked.

Feeding of experimental fish

The experimental fish were handfed twice daily, morning and evening at 9:00 am and 4:00 pm. The daily ration of 5% biomass was divided into two and half fed to fish each time. The weight of feed fed was adjusted every two weeks to contain weight gain by fish. The experiment lasted for 70 days.

DATA ANALYSIS

The values of the various parameters were reported as means ± standard deviations and were analyzed statistically by one way analysis of variance (ANOVA) using SPSS Statistics version 21 software programme. LSD was used as Post Hoc Multiple Comparisons to identify which treatments were significantly different ($p < 0.05$) compared to the control.

RESULTS

Phytochemical screening of *P. phaseoloides* leaf sample

Phytochemical screening of the leaf sample revealed the presence of cardiac glycosides, flavonoids, saponins and quinones (Table 2).

Proximate analysis of *P. phaseoloides* leaf sample

Table 3 shows the proximate analysis results of the leaf sample. Moisture content was 5.23 ± 0.23%; ash was 5.35 ± 0.75%; crude protein was 18.31 ± 2.62%; crude fat was 7.00 ± 0.00%; crude fibre was 43.96 ± 4.09%; and total carbohydrate was 20.15 ± 0.57%.

Proximate analysis of the experimental diets

Table 4 describes the proximate analysis of the five experimental diets. Moisture values ranged from 7.75 ± 1.61% to 10.34 ± 1.55%; ash ranged from 9.31 ± 0.36% to 11.35 ± 0.05%; crude protein ranged from 39.21 ± 0.29% to 41.44 ± 1.21%; crude fat ranged from 7.96 ± 0.65% to 9.45 ± 0.35%; crude fibre ranged from 5.12 ± 0.65% to 11.03 ± 1.19%; and carbohydrate ranged from 19.45 ± 2.19% to 26.96 ± 0.90%.

Water quality analysis

The water quality parameters in the experimental tanks indicated that pH values ranged from 6.61 ± 0.08 to 6.68 ± 0.02. Temperature ranged from 27.27 ± 0.01 ºC to 27.29 ± 0.01 ºC. Dissolved oxygen ranged from 6.59 ± 0.02 mg/l to 6.62 ± 0.01 mg/l. Ammonia ranged from 0.10 ± 0.01 mg/l to 0.12 ± 0.02 mg/l. Nitrite ranged from 0.29 ± 0.01 mg/l to 0.30 ± 0.02 mg/l. Total hardness ranged from 43.45 ± 0.01 mg/l to 43.48 ± 0.02 mg/l (Table 5).

Survival rate

Table 6 describes the percentage survival of the experimental fish across all the dietary groups. The percentage survival ranged from 90.00 ± 10.00% to 100.00 ± 0.00%.

Cost/Benefit analysis

Table 7 describes the cost/benefit analysis of the *P. phaseoloides* leaf meal inclusion diets fed to the experimental fish. Cost of feed per kilogram ranged from ₦525.14 to ₦545.09. Feed input ranged from 2.62kg to 2.81kg. Cost of feeding ranged from ₦1428.38 to ₦1531.70. Cost of juveniles was the same at ₦1200.00 per group. Total cost ranged from ₦2628.38 to ₦2731.70. Total weight of fish harvested ranged from 15.66kg to 17.75kg. Returns ranged from ₦5098.30 to ₦6246.62. Cost/Benefit ratio ranged from 1:1.87 to 1:2.38.

DISCUSSION

Phytochemical screening of *P. phaseoloides* leaf sample

Phytochemicals are widely known to have protective effects against chemically-induced oxidative damage, by enhancing the antioxidant defence system of animal tissues and blocking of oxidative stress (Abarikwu *et al*., 2018). In the present study, we observed cardiac glycosides, flavonoids, saponins and quinones as phytochemicals present in *P. phaseoloides* leaf sample. These results are in agreement with the publication of Okoye *et al*. (2020), on the phytochemical screening and pharmacognostic properties of *Peuraria phaseoloides* leaves (roxb) benth.

Proximate composition of experimental diets

In the present study, there were no significant difference (p>0.05) in the crude protein contents of all the formulated diets. Thus, the formulated diets are isonitrogenous. Crude protein is the most important nutrient in fish feed because it helps the fish to grow to its full potentials. Moreover, all the formulated diets were accepted by *C. gariepinus* juveniles, which indicated that the levels of inclusion of PLM did not affect the palatability of the diets.

Water quality parameters

Fishes go about their activities inside water and are totally dependent on water for feeding, respiration, excretion, osmoregulation, growth and reproduction. Thus, a successful aquaculture business is dependent on the health of the fish and a good water quality management. In the present study, the water quality parameters recorded no significant difference (p>0.05) in all the experimental tanks. Hence, the basic water quality parameters measured had no effect on any observed differences in the performance of the experimental fish.

Survival Rate

The fishes in all the dietary groups recorded 100.00 ± 0.00% survival in the first 6weeks of this feeding experiment. Except for groups 4 and 5 fishes that maintained 100.00 ± 0.00% survival until the end of the 10 weeks feeding trial, the control (group 1) fishes recorded 93.33 ± 5.77% survival in week 8 and 90.00 ± 10.00% survival in week 10; group 2 recorded 96.67 ± 5.77% survival in week 8 and 93.33 ± 11.55% survival in week 10; whereas group 3 recorded 96.67 ± 5.77% survival in both week 8 and week 10. The results showed that the survival rates increased with the levels of inclusion of PLM in the last four weeks of the feeding experiment, although no significant differences (p>0.05) were recorded.

The good survival rates (90 – 100%) of *C. gariepinus* juveniles across all the dietary groups in this study were possibly achieved due to the maintenance of good water quality. The fishes also displayed good adaptability to the formulated feeds in the culture media. The handling techniques of the fishes were also good. The hardy nature of the fish (Nwadukwe and Ayinla, 2004) also contributed to fish survival.

Cost/Benefit analysis

The cost/benefit analysis of the formulated diets showed that the cost/benefit ratio increased with the levels of inclusion of PLM. Hence, D5 gave the highest cost/benefit ratio of 1:2.38, followed by D4 (1:2.30), D3 (1:2.16), D2 (1:2.00), while the control (D1) gave the least cost/benefit ratio of 1:1.87.

CONCLUSION

In the present study, the PLM was an economically viable alternative to the conventional protein ingredients in the diets of *C. gariepinus* juveniles as its inclusion drastically reduced thecosts of the formulated diets. *P. phaseoloides* leaf is readily available and cheap by virtue of the fact that there is no competition for human consumption. Results from the present study showed that the cost/benefit ratio of D5 (containing 20% PLM) was higher than that of D1 (containing 0% PLM). The fishes fed PLM inclusion diets also recorded better survival rates at the end of the feeding experiment. We therefore conclude that PLM can be included in the diets of *C. gariepinus* up to 20% level, for better survival of fish and for profit maximization in and sustainability of the aquaculture industry.

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Table 1: Percentage composition of experimental diets

Ingredients	Diet 1 (0% PLM)	Diet 2 (5% PLM)	Diet 3 (10% PLM)	Diet 4 (15% PLM)	Diet 5 (20% PLM)
PLM	0.00	5.00	10.00	15.00	20.00
Wheat Bran	19.76	15.02	10.27	5.54	0.79
Soybean Meal	33.80	33.67	33.54	33.41	33.28
Fish Meal	33.80	33.67	33.54	33.41	33.28
Palm Oil	5.00	5.00	5.00	5.00	5.00
Garri (Binder)	5.00	5.00	5.00	5.00	5.00
Bone Meal	1.50	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15
Vitamin C	0.10	0.10	0.10	0.10	0.10
Total (%)	100	100	100	100	100

Key: PLM – *Pueraria phaseoloides* leaf meal

Table 2: Phytochemical screening of *P. phaseoloides* leaf sample

S/No.	Phytochemicals	Results
1	Alkaloids	-ve
2	Cardiac glycosides	**+ve**
3	Flavonoids	**+ve**
4	Phenols	-ve
5	Phlobatanins	-ve
6	Saponins	**+ve**
7	Sterols	-ve
8	Tanins	-ve
9	Terpenoids	-ve
10	Quinones	**+ve**
11	Oxalate	-ve
12	Diterpenes	-ve

Table 3: Proximate analysis of *P. phaseoloides* leaf sample

Nutrients	% Composition
Moisture	5.23±0.23
Ash	5.35±0.75
Crude Protein	18.31±2.62
Crude Fat	7.00±0.00
Crude Fibre	43.96±4.09
Carbohydrate	20.15±0.57

Values are mean ± standard deviation of triplicate determinations.

Table 4: Proximate analysis of the experimental diets

Sample Identity	% Moisture	% Ash	% Crude Protein	% Crude Fat	% Crude Fibre	% Carbohydrate
D1 (control)	7.75 ± 1.61^{a}	11.35 ± 0.05^{a}	39.37 ± 0.87^{a}	9.45 ± 0.35^{a}	5.12 ± 0.65^{a}	26.96 ± 0.90^{a}
D2	10.34 ± 1.55^{a}	10.74 ± 0.06^{a}	39.21 ± 0.29^{a}	7.97 ± 0.15^{a}	6.48 ± 3.02^{a}	25.26 ± 1.54^{a}
D3	9.37 ± 2.72^{a}	9.91 ± 0.24^{a}	41.07 ± 1.61^{a}	7.96 ± 0.65^{a}	7.79 ± 1.54^{a}	23.90 ± 1.15^{b}
D4	9.68 ± 0.00^{a}	9.31 ± 0.36^{b}	39.69 ± 1.31^{a}	8.80 ± 0.70^{a}	9.72 ± 0.98^{b}	22.80 ± 1.13^{b}
D5	9.04 ± 0.55^{a}	10.76 ± 1.80^{a}	41.44 ± 1.21^{a}	8.28 ± 1.68^{a}	11.03 ± 1.19^{b}	19.45 ± 2.19^{b}

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different (p>0.05) compared to the control (D1).

Table 5: Water quality analysis

Parameters	Experimental Tanks				
	Tank 1 (control)	**Tank 2**	**Tank 3**	**Tank 4**	**Tank 5**
pH	6.68 ± 0.02^{a}	6.66 ± 0.01^{a}	6.61 ± 0.08^{a}	6.62 ± 0.03^{a}	6.62 ± 0.02^{a}
Temperature (°C)	27.27 ± 0.01^{b}	27.28 ± 0.01^{b}	27.28 ± 0.02^{b}	27.29 ± 0.01^{b}	27.29 ± 0.01^{b}

Dissolved Oxygen (mg/l)	6.62 ± 0.01^{c}	6.59 ± 0.02^{c}	6.60 ± 0.02^{c}	6.59 ± 0.02^{c}	6.60 ± 0.02^{c}
Ammonia (mg/l)	0.10 ± 0.01^{d}	0.11 ± 0.02^{d}	0.12 ± 0.02^{d}	0.12 ± 0.02^{d}	0.12 ± 0.02^{d}
Nitrite (mg/l)	0.29 ± 0.01^{e}	0.29 ± 0.01^{e}	0.29 ± 0.01^{e}	0.30 ± 0.02^{e}	0.29 ± 0.02^{e}
Total Hardness (mg/l)	43.46 ± 0.02^{f}	43.48 ± 0.02^{f}	43.47 ± 0.02^{f}	43.47 ± 0.03^{f}	43.45 ± 0.01^{f}

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same row are not significantly different (p>0.05) compared to the control (Tank 1).

Table 6: Changes in Survival Rate of *C. gariepinus* juveniles fed the formulated diets for 70 days

Experimental Groups	Survival Rate (%)				
	Week 2	**Week 4**	**Week 6**	**Week 8**	**Week 10**
Group 1 (control)	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	93.33±5.77^{a}	90.00±10.00^{a}
Group 2	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	96.67±5.77^{a}	93.33±11.55^{a}
Group 3	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	96.67±5.77^{a}	96.67±5.77^{a}
Group 4	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Group 5	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different (p>0.05) compared to the control (Group 1).

Table 7: Cost/Benefit analysis of *P. phaseoloides* leaf meal inclusion diets fed to *C. gariepinus* juveniles

Parameters	D1 (control)	D2	D3	D4	D5
Cost of Feed (₦/kg)	545.09	540.06	535.11	530.09	525.14
Feed Input (kg)	2.81	2.62	2.68	2.78	2.72
Cost of Feeding (₦)	1,531.70	1,414.96	1,434.09	1,473.65	1,428.38
Cost of Juveniles (₦)	1,200	1,200	1,200	1,200	1,200
Total Cost (₦)	2,731.70	2,614.96	2,634.09	2,673.65	2,628.38
Total Weight of Fish Harvested (kg)	15.66	15.71	16.67	17.66	17.75
Sales at ₦500/kg	7,830	7,855	8,335	8,830	8,875
Returns (₦)	5,098.30	5,240.04	5,700.91	6,156.35	6,246.62
Cost/Benefit Ratio	1:1.87	1:2.00	1:2.16	1:2.30	1:2.38