Fisheries and Aquaculture Journal
ISSN: 2150-3508

Sogbesan et al., Fish Aquac J 2015, 6:4
DOI: 10.4172/2150-3508.1000141

Research Article Open Access

Utilization of Treated Duckweed Meal (*Lemna pausicostata*) as Plant Protein Supplement in African Mud Catfish (*Clarias gariepinus*) Juvenile Diets

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Abstract

The utilization of treated duckweed (*Lemna pauciscostata*) as soybean supplement in the diet of African mud catfish (*Clarias gariepinus*) was carried out for 6 weeks (42 days). Freshly harvested duckweed was given varying treatments (raw, soaked in potash (maize cob as solution), sundried and blanched (at 100°C)) were included at 50% inclusion level. The experimental set up was in triplicate. The result showed highest mean weight gain in the diet 1 (Control diet) and followed by the diet 5 (blanched diet) while diet 2 (raw duckweed) gave the lowest mean weight gain. The food conversion ratio, specific growth rate and protein efficiency ratio followed the same pattern. There was no significant difference between the net profit value of control diet (601.1) and the blanched diet (592.66) ($p>0.05$) and the blanched diet gave the best benefit cost ratio (BCF). Apart from been viable supplement for soybean meal in the diet of *Clarias gariepinus*, blanching of duckweed gave better performance than other treatments.

Keywords: Duckweed; Soybeans; Supplement; Benefit cost ratio; Blanching

Introduction

Due to the increase in world population there is need for high production of fish to supplement the catch from the wild and the success of commercial aquaculture operations depends mainly on the availability of suitable diets, which provide required nutrients for optimum growth at minimal cost [1]. The scarcity and high cost of animal protein supplement particularly fishmeal has increased interest to seek alternative protein source for feeding in aquaculture. The utilization of non-conventional protein supplements of both animal and plants origin in aquaculture has been the focal point of research in Nigeria in recent times and it is imperative for such supplement to contain optimum protein, required essential amino and fatty acids [2].

Duckweed meal has been known for its high nutritive value with as much as 40% and above crude protein depending on the culture system [3,4].

Duckweed protein has higher concentration of essential amino acids, lysine and methionine, than most plant proteins and more closely resembles animal protein in that respect. The high and fast growth rate coupled with the high protein content (41-45%) is a unique property of Duckweed plants [5], which could be used as inclusion or replacement for the more expensive plant or animal protein in fish diets. Nutritionally, duckweed plant has been reported to be an excellent substitute for soybean meal and fish meal in fish feeds [6]. Fasakin et al. [7] observed that duckweed meal can replace up to 30% of the total diet of the nile tilapia (*Orechromic niloticus*), while Hassan and Edwards [8] observed and reported greater acceptability of *Lemna perpussilla,* and *Spirodela pohyrrhiza* when fed to tilapia reared in concrete tanks. Due to the availability of this fast growing plant with high percentage protein content, this study aimed at determining the best substitution level of soybean meal (SBM) with duckweed meal with different treatment in African mud catfish (*Clarias gariepinus*) juveniles diet.

Materials and Methods

Experimental area

The experiment was carried out at the Research Laboratory of the Department of Fisheries, Federal University of Technology, Yola. The experiment lasted for a period of 12 weeks (42 days). Fresh Duckweed (*Lemna pauciscostata*) was harvested from Lake Geriyo, Yola with the aid of a bucket/plastic bowl and a sack which served as a sieve net.

Preparation of duckweed meal: Three treatments were given to the freshly collected duckweed;

Treatment with potash solution: 5 g of maize-cob ash (Potassium hydroxide) was dissolved into a solution at 5 g/l and used to soak duckweed for 24 hours following the method of Vadivel and Pugalenthi [9].

Blanching: Duckweed was boiled in water for 5 minutes at 100°C. This treatment is referred to as blanching following the methods of Akpodiete and Okagbare [10] and Sogbesan and Ugwumba [11].

Sun-dried: Also some quantity of duckweed was sun-dried.

After each treatment, duckweed given treatments (a) and (b) were also dried to constant weight. The dried duckweeds were gathered and grounded into fine particle and sieved through a mesh size of 2 mm and stored in a polyethene bag before been used for feed formulation.

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Received April 10, 2015; **Accepted** July 09, 2015; **Published** July 12, 2015

Citation: Sogbesan OA, Onoja CF, Adedeji HA, Idowu TA (2015) Utilization of Treated Duckweed Meal (*Lemna pausicostata*) as Plant Protein Supplement in African Mud Catfish (*Clarias gariepinus*) Juvenile Diets. Fish Aquac J 6: 141. doi:10.4172/2150-3508.1000141

Other feed ingredients

Other feed ingredients used for the formulation of the diets include soybeans, groundnut cake (GNC), fish meal, groundnut oil, maize, vitamin and mineral premix, salt and starch

Feed formulation

The feed formulation was done using pearson square method. The feed ingredients were grounded/drilled and weighed into a bowl with the use of a sensitive weighing balance. The maize grain and locally extracted groundnut cake (Kulikuli) were drilled separately into fine particles with the use of a milling machine.

Five (5) 40% isoproteinoic diets were formulated for the *Clarias gariepinus* catfish juveniles as shown in Table 1 with the cost of each diet. The milled feed ingredients were weighed and mixed in a bowl, then followed by a wet mixing using about 20 ml water per kilogram of feedstuff to form tough-dough. The resulted dough was extracted through an electrical powered pelleting machine into suitable pellet size. The pellets were collected and spread out to dry under the sunlight or under a fan at a room temperature (23°C) for 24 hours. The pellets were then broken into bits, packaged and stored in a cellophane bag until it was to be used.

Feed-growth response

Experimental set up: Twelve (12) Juvenile catfish of average weight, () were stocked in plastic bowls of capacity 50 L and triplicated under a laboratory condition, they were aerated with an electric aerator. The juveniles were acclimatized for a period of three days and those that survived were sorted out and stocked in each of the experimental plastic bowl in triplicate labeled according to the experimental diets. The plastic bowls were filled with water to half of their height.

Feeding rate and frequency: These juvenile were fed with 5% of their body weight for twelve weeks. The pellets were reduced to suitable size with the use of a laboratory ceramics mortar and pestle and fish were fed twice daily (morning and evening)

Ingredients	Diet 1 (100% SYB)	Diet 2 (50% SYB + 50% raw duckweed)	Diet 3 (50% SYB + 50% soaked duckweed in potash)	Diet 4 (50% SYB + 50% sundried duckweed)	Diet 5 (50% SYB + blanched duckweed)
Fish meal(g)	20	20	20	20	20
Maize (g)	24	24	24	24	24
Groundnut cake (g)	19	19	19	19	19
Soybean (g)	28	14	14	14	14
Bone meal (g)	1.5	1.5	1.5	1.5	1.5
Methionine (g)	1.5	1.5	1.5	1.5	1.5
Lysine (g)	1.5	1.5	1.5	1.5	1.5
Vitamin C (g)	1.5	1.5	1.5	1.5	1.5
Vitamin B complex (g)	1.5	1.5	1.5	1.5	1.5
Salt (g)	1.5	1.5	1.5	1.5	1.5
Duckweed meal (g)	----	14	14	14	14
Total	100	100	100	100	100
Calculated crude protein	40.02	40.01	40.02	40.00	40.01
Analysed crude protein	39.87	39.67	39.90	38.95	39.59
Cost (₦)	230.00	209.00	209.00	209.00	209.00

Table 1: Ingredient dry matter composition of the experimental diets and the cost.

Water quality management and monitoring

The water quality parameters like temperature, dissolved oxygen, pH, and ammonia were measured randomly and once in a week using Boyd standard method of measurement. Daily siphoning of uneaten feeds and draining of water when dirty (polluted) was done thereby replacing the same volume with a fresh cleaned water in order to prevent the growth of algae, fungal growth and organic load. Borehole water from the experimental laboratory was used for the experiment.

The fish were monitored for survival by removing the dead fish. The weight and length of the fish were measured and recorded weekly using sensitive weighing balance and meter rule respectively. The new weekly measurement were used to adjust the feeding ration given to the fish

Growth indices

Growth indices like specific growth rate, relative growth rate, feed conversion ratio and protein efficiency ratio using the weekly weight, length and quantity of feed.

Specific growth rate: This refers to the percentage of weight gain per days.

$$SGR = \frac{InW2 - InW1}{T2 - T1} \times 100$$

Where ln = natural logarithm

W_2 and W_1 = final and initial weight of fish respectively in grams

T_2 and T_1 = end of growth period and beginning respectively

Relative growth rate:

$$\text{RGR}=\frac{\text{Initial weight}}{\text{Mean weight gain}}\times 100$$

Feed conversion ratio (FCR): This refers to the weight gain in fish due to increase in feed taken

$$\text{FCR}=\frac{\text{Feed intake or diet fed (g)}}{\text{M Fish weight gain (g)}}$$

Protein efficiency ratio (PER): this refers to weight increase in fish mainly due to an increase in total body protein

PER = Weight gain (wet fish in g)

Weight of protein fed (crude protein fed in g)

Condition factor (k)

This expresses the health status of fish as a result of the experimental treatment and was computed at the beginning and end of the experiment using the Fulton's Condition Factor Formula as expressed by Bagenal and Tesch as:

$k=100\ W/L^{-3}$

Where W=weight of fish

L = length of fish

Proximate analysis

The proximate analysis of all the experimental diets for crude protein, crude fibre, ash and moisture content was carried out following the method of Association of Official Analytical Chemical [12] (Table 2).

Composition	Raw Duckweed	Soak in potash duckweed	Sundried duckweed	Blanched duckweed
Dry matter	97.45	97.13	97.06	97.00
Moisture Crude	2.55	2.87	2.94	3.00
Protein	29.28^{b}	36.25^{a}	28.62^{b}	30.04^{ab}
Crude Lipid	6.34	5.2	6.9	7.8
Crude Fibre	16.15	13.76	15.26	14.96
Crude Ash	15.34^{b}	17.46^{ab}	20.04^{a}	16.74^{b}
NFE	30.34	24.46	28.24	27.46

Table 2: Proximate Composition of the Treated duckweed meal. Data on the same row with different superscript are significantly different ($p<0.05$).

Parameters	Diet 1 Control	Diet 2 Raw	Diet 3 soaked in potash	Diet 4 Sundried	Diet 5 Blanched
Total Initial weight	208.80	208.80	218.70	215.60	200.30
Mean Initial weight (g/fish)	9.50	10.40	12.20	10.30	9.50
Total Final weight (g)	691.06	562.00	590.94	593.56	592.66
Mean final weight (g/fish)	43.86	35.24	41.26	35.36	36.46
Total Weight gain (g)	482.25	353.20	372.24	377.96	392.36
Mean weight gain (g/fish)	12.43^{a}	7.22^{c}	8.53^{b}	7.38^{bc}	8.73^{b}
Mean initial length (cm/fish)	9.60	9.70	9.40	10.10	9.20
Final length (cm)	44.00	41.60	42.60	43.60	40.20
Mean final length (cm)	11.00	10.40	10.70	10.90	10.10
Mean Feed Intake (g/fish)	23.00	22.10	22.70	22.00	20.70
Initial stocking Density.	12	12	12	12	12
Final stocking Density.	11	10	9	11	11
Survival (%)	92.00^{a}	83.00^{ab}	75.00^{b}	92.00^{a}	92.00^{a}
Specific growth rate (SGR)	0.86^{a}	0.54^{c}	0.55^{c}	0.56^{c}	0.67^{b}
Relative weight gain	100	59.5	41.6	23.8	36.0
Feed conversion ratio	1.85^{a}	3.06^{b}	2.66^{ab}	2.98^{b}	2.37^{a}
Protein efficiency ratio (PER)	1.1^{a}	0.6^{b}	0.4^{bc}	0.2^{c}	0.3^{bc}
Condition factor	1.65	1.57	1.69	1.37	1.77

Table 3: Growth performance and Feed Utilization of *Clarias gariepinus* Juveniles fed treated duckweed supplemented diets.

Production cost of the experimental diets and economic evaluation of feeding trials

The production cost in Naira of the experimental diets was calculated following the method of Faturoti and Lawal based on the current market price of the ingredients used for formulating the diets. Economic evaluation in terms of investment cost analysis (ICA), net profit value (NPV), gross profit (GP), profit Index (PI) and benefit cost ratio (BCR) of substituting fishmeal with the non-conventional animal feedstuffs in the culture of *Heterobranchus longifilis* was determined according to New [13] (Table 3).

Investment cost analysis (ICA): This was computed following New (1989) as:

Investment Cost Analysis (N) =Cost of feeding (N) + Cost of fingerlings stocked (N).

Net production value (NPV): This was taken as the cost of all the fish harvested at the end of the experiment as follows: NPV (N) = Total weight gain (g) x cost per kg (N/kg)

Gross profit (GP): This was taken as the difference between the net profit value and investment cost analysis in terms of the projected cost of the fish raised.

GP (N) = Net Profit Value (N) - Investment Cost Analysis (N)

Profit index (PI): This was computed using the equation below:

$$\text{Profit Index}=\frac{\text{Net Profit Value (N)}}{\text{Cost of feeding (N)}}$$

Benefit: Cost ratio (BCR)

This was determined as:

$$\text{Benefit: Cost Ratio}=\frac{\text{Net Profit Value (N)}}{\text{Investment Cost Analysis (N)}}$$

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA). Comparisons among treatment means were carried out by two-way analysis of variance followed by Tukey's multiple tests and Dunnet test. Correlation and regression analysis was carried out to determine the relationship between the treatments and some of the parameters using SPSS 12.0 and Graphpad Instat (DATASET 1) Statistical Packages Windows 2000.

Result and Discussion

Figures 1-3 showed the changes that occurred with the effect of different treatments given to duckweed meal. The green pigment was discolored as shown on Figures 2 and 3.

Table 4 shows the result of the proximate composition of the different duckweed meal. There were significant differences ($p<0.05$) between the crude protein and ash of the duckweed given different treatments.

The financial implication of the experimental diet was shown in Table 4 and it indicated that diet 5 is the most economical with highest BCR of 1.47 followed by the control and diet 3 (Potash treated) and Sun-dried.

The variation in the nutrients especially in crude protein of the duckweed with different treatment is emphasizing the effect of treatments on protein molecules. The sun-dried with lowest crude protein could have been as a result of sun radiation which would have cause yellowing of the greenish part of the leaves hence affecting the chlorophyll a molecules needed for phosphorylation and protein synthesis in the plant. The soaked duckweed for 24 hours were able to

Figure 1: Raw duckweed meal.

Figure 2: Sun-dried duckweed meal.

Figure 3: Potash treated duckweed meal.

Parameters	Diet 1 Control	Diet 2 Raw	Diet 3 soaked in potash	Diet 4 Sundried	Diet 5 Blanched
Investment Cost Analysis (ICA)	412.9	404.9	406.2	408.8	402.1
Net Profit Value (NPV)	601.1	562.0	591.4	593.56	592.66
Gross Profit (GP)	188.2	157.1	185.2	184.76	190.56
Profit Index (PI)	0.111	0.125	0.128	0.122	0.141
Benefit cost ratio (BCR)	1.46	1.39	1.46	1.45	1.47

Table 4: Economic analysis of utilizing treated duckweed meal to supplement soybean meal for *Clarias gariepinus.*

enter into the first stage of fermentation which could have resulted in the early production of single cells which are also proteinous.

The basic nutrient that cannot be compromised in the choice of ingredients for feed formulation and preparation is protein [14], and the crude protein observed in each of the experimental diet is in accordance with optimum dietary protein, 42.5% required by *Heterobranchus spp*. for better growth and development as reported by Fagbenro et al. [15]. There was an indication of adequate utilization of the experimental diets since each of them supplied the optimum required amount of protein by the fish.

The high mean weight gain observed in diet 5 (blanched duckweed + soybean) and diet 3 (soaked in potash + soybean) is an indication that the fish were able to utilize the diet than other treatments. It also implies that the blanching process and soaking in maize ash has not only reduced the anti-nutritional factors that limit growth alone but also reduce the fibre as shown in Table 1. The weight gain in Experimental treatment Diet 3 (soaked duckweed in potash) is traceable to the fact that maize cob ash solution helps to reduce the fibre content of duckweed, also maize cob ash solution improved the nutritive value of duckweed as stated by Emenalom et al. The lowest weight gain in Experimental treatment Diet 2 (raw) is traceable to the presence of anti-nutritional factors which had been reported to inhibit growth rate in fish [16].

The low weight gain in Diet 4 (sundried duckweed) could be traceable to the fact that the proteins were denatured by the heat from the sun and they become resistant to digestion due to the fermentation of the peptide bond occurring between the side chains of lysine and carboxylic acid. Fasakin [17] reported the loss of Proteins from duckweed plants through denaturation (heating process). Generally, Diet 1 (control) recorded the highest weight gain while Diet 5 (blanched) had the best weight gains if we are to compare the treatments given to duckweed.

The financial implication of the experimental diet (Table 4) showed that diet 5 is the most economical with highest BCR of 1.47 followed by the control and diet 3 (Potash treated) and Sun-dried. Most importantly, duckweed practically cost nothing as compared to the one hundred and sixty naira (N160) per module of soybean. This utilization of duckweed in *Clarias gariepinus* diets will help to reduce cost.

The irregular availability of conventional feed ingredients (like soybean) all year round coupled with their increasing cost. Thus, the search for cheaper sources of feed materials such as duckweed plants as alternatives to the conventional materials therefore stand. Duckweed which is regarded by most individuals as "weeds" which has no economic importance, but until recently has been shown to posses great potentials as a substitute in fish diet as proved in other research works and these have brought about tremendous improvement in fish feed formulation.

As a cost saving strategy, it is cheap and available all year round, but however from a nutritional point of view, it has a good amount of crude protein and varying amino acids composition such as lysine and methionine. The cost benefits (reduced feed cost) of using duckweed increased as duckweed has not been found to be sold anywhere in the world, in other words, duckweed is a cheap protein available resources with great potential in fish feed.

Acknowledgement

The authors duely appreciate the assistance of the Upper Benue River Basin, Yola Adamawa state in the collection of the duckweed.

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