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# Growth Performance of *Clarias gariepinus* Fed with Seaweed (*Pistia stratiotes*) Leaf Meal at Varying Inclusion Level

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**Abstract**

This study evaluated the effect of varying inclusion levels of Pistia stratiotes leaf meal on the growth performance and haematological parameters of Clarias gariepinus juveniles.15 plastics tanks (21.5x14.5x9cm) were used for the experiment each containing 10 *Clarias gariepinus* juveniles which were fed with five isonitrogenous diets containing 40% crude protein with varying inclusion of *Pistia stratiotes* (Control diet with *P. stratiotes* 0%, *P. stratiotes inclusion 25%, P. stratiotes inclusion 50%, P. stratiotes inclusion 75%, P. stratiotes inclusion, 100%).* The fish were fed 2 times a day using 5% of their body weight from their various experimental feeds for 12 weeks. The fish in tank T3 (75% inclusion of *P. stratiotes*) had the best weight gain with a mean value of 61.5±0.70g. The specific growth rate was recorded in tank T3 (75% inclusion of *P. stratiotes)* with a mean value of 0.78±0.83 recorded. The highest feed conversion ratio was recorded in tank T4 (100% inclusion of *P. stratiotes*) with a mean value of 1.91±0.70. The highest protein efficiency ratio was also recorded in T3 (75% inclusion of *P. stratiotes*) with a mean value of 0.03±0.01. The fish fed with *P. stratiotes* showed increase in haematological values of Red blood cell (RBC), (2.21±0.18), and White blood cell (WBC), (6.35±2.61) compared to the values of fish fed with control diet of Red blood cell (RBC), (1.975±0.86), and White blood cell (WBC), (4±2.96). There was a reduction in the haematological value of the fish fed with *P. stratiotes* in Haemoblogin (HB), (14.05±0.92) compared to the value of fish fed with control feed Haemoblogin, (12.05±4.59). It was concluded that using *P. stratiotes* leaves as feed for *Clarias gariepinus* enhanced the growth of the fish and had no negative impact on the health status of the fish. Seaweeds are more available and relatively cheaper, it could be recommended that partial replacement of Seaweed in the diet of *C. gariepinus* at 75% inclusion level would aid fish productivity. Therefore, partial replacement of feed seaweeds in the diet of *Clarias gariepinus* should be encouraged.

**Keywords:** Growth performance, *Clarias gariepinus*, seaweed, inclusion level.

## Introduction

Fish and fishery products remain a cheap and major source of animal protein contributing 40% of the total animal protein intake of Nigerians, particularly to the majority of our populace (WorldFish, 2018). The major importance of fish to human is majorly to serve as a source of protein, and they are being converted to different forms for different purposes (Adewunmi, 2015). Fish is widely accepted because of its high palatability, low cholesterol and tender flesh (Onyia *et al.,* 2010). However, a higher number of consumers eats fish because of its nutritional value. Fish has the highest level of easily metabolizable high-quality protein, fats, vitamins, calcium, iron, and essential amino acids when it is compared with other sources of animal protein such as poultry and beef (Ayoola, 2010). The protein deficiency in the diet can be primarily remedied through the consumption of either protein-rich plant or animal foodstuffs. According to Ayoola (2010), the demand for fish in Nigeria is increasing at the rate of 2.99% annually with a 3.9% increase in population growth. The increase in fish production in Nigeria accounted for 55 percent of the increase of its apparent total fish consumption between 1980 and 2013; the remaining 45 percent was covered by the increase in its net import (i.e. import minus export) of fish. The fish trade deficit increased from 350,000 metric tons to nearly 2 million metric tons between 2000 and 2011, before declining to 940,000 metric tons in 2013, thanks to a rapid increase in domestic fish production (WorldFish, 2018). With the increasing gap between fish supply and demand, Nigeria has embarked on fish importation to meet up for the deficit in the supply as less than 50% of the total annual fish consumed by Nigerians are produced locally (Ayoola, 2010). The African mud catfish *(Clarias gariepinus)* is an economically important fish species in Nigeria. It is considered to be one of the most important tropical catfish species for aquaculture because of its excellent biological characteristics such as its fast growth, high economic value, and popular taste. It matures and relatively easily reproduces in captivity and tolerates difficult conditions in aquaculture (Froese *et al.*, 2014). Seaweeds have great potential as feed ingredients for fish farming, though their effects on the fatty acids content and profile of fish flesh are not well established. Recent studies suggest that seaweed supplementation can increase the concentration of long-chain Omega-3 PUFA in various animal species (Toine Wilke *et al.*, 2015). Seaweed is chock-full of vitamins, minerals, and fibre. Their known medicinal effects have been legion for thousands of years. A seaweed may belong to one of several groups of multicellular algae: the red algae, green algae, and brown algae. In fish farming, the wet feed usually consists of meat waste and fish waste mixed with dry additives containing extra nutrients (FAO, 2014). The benefits of seaweed consumption are clear to see. "Seaweed is the ultimate superfood. Nothing compares, gram for gram, to the nutritional content of seaweeds". The benefits extend beyond just nutrition to salt replacement and weight management. The health attributes of seaweed are so broad, that research is showing benefit for several diseases. Seaweeds are commercially sought after. The potential for seaweed farming and all its peripheral benefits are huge (Rose, 2010).

*Pistia stratiotes* is a genus of aquatic plant in the arum family, Araceae. It is often called water cabbage or water lettuce and was first discovered from the Nile near Lake Victoria in Africa. It is now present either naturally or through human introduction, in nearly all tropical and subtropical fresh waterways (Rose, 2010). Water lettuce is among the world's most productive freshwater aquatic plants. In waters with high nutrient content, particularly those that have been contaminated with human loading of sewage or fertilizers, water lettuce can often exhibit weedy overgrowth behaviour. It may also commonly become weedy in hydrological altered systems such as canals and reservoirs (Taxon, 2011).

Research has not been carried out on the use of Seaweed as feed for *Clarias gariepinus*. This study however determined the growth performance of *Clarias gariepinus* fed with the seaweed at varying inclusion levels.

## Materials and Method

### Collection of Seaweed

Large quantities of seaweed (*Pistia stratiotes)* were collected from Waterside area, in Ogun State and it was sun-dried for three weeks at the temperature of 28±07 °C and was ground into powder using Hammer mill and stored in a dry labeled container. A small quantity was taken to Animal care feed and quality control Laboratory, Ile-Epo, Lagos State for proximate analysis.

### Feed production

Table one shows the percentage composition of the experimental feed. The experimental feeds were formulated with varying inclusion levels of seaweed at 0%, 25%, 50%, 75%, and 100%.

**Table 1:** Percentage of seaweed and other ingredients compounded together to make the fish feed per kg.

| Ingredients | T0 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| Fish meal | 24.8 | 24.8 | 24.8 | 24.8 | 24.8 |
| Soybean meal | 24.8 | 24.8 | 24.8 | 24.8 | 24.8 |
| Groundnut cake | 24.8 | 24.8 | 24.8 | 24.8 | 24.8 |
| Maize | 10.9 | 10.9 | 10.9 | 10.9 | 10.9 |
| Indomie | 10.9 | 8.17 | 5.45 | 2.72 | - |
| Premix | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| Seaweed | - | 2.72 | 5.45 | 8.17 | 10.9 |
| | 100Kg | 100Kg | 100Kg | 100Kg | 100Kg |

Tank T0: Formulated feed (Control)
Tank T1: 25% Inclusion of seaweed in fish diet
Tank T2: 50% Inclusion of seaweed in fish diet
Tank T3: 75% Inclusion of seaweed in fish diet
Tank T4: 100% Inclusion of seaweed in fish diet

**Experimental Fish**

One hundred and sixty healthy juveniles of African Catfish, *Clarias gariepinus* were purchased from Abule Odo Fish farms, Egbeda, Local Government Area in Lagos. The fish were transported to the Aquaculture Unit of the Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka.

The fishes were acclimatized for 14days, after which 10 fish with a mean weight of 21.1±1.12 were randomly distributed into each of the experimental culture tanks. The daily feeding ratio was measured at the beginning of every week using a weighing scale. Feeding response was monitored and no mortality was recorded. The water was filled to 2/3 of the volume of each tank (15 litres). The mean weight gain of the specimen in each of the labelled tanks T0, T1, T2, T3, and T4 each having triplicates was determined. The tanks labelled represent each of the feeding regimes. The water was changed daily to avoid contamination of water by the uneaten feed and faeces. The fish were fed to satiation twice daily for 12weeks. The daily feeding ratio was measured at the beginning of every week using a weighing scale. Feeding response was monitored and no mortality was recorded. The water was changed daily to avoid contamination of water by the uneaten feed and faeces.

**Feed Production**

**Feeding of specimen**

The juvenile *Clarias gariepinus* weighing (20.2±1.68) were fed 30% Coppens feed twice daily during acclimatization which lasted for two weeks and feeding to satiation during the project work which lasted for 12 weeks.

**Feeding of Fish**

The fish were fed two times a day in equal portions weighed out weekly from their various experimental feeds for 12 weeks. The daily feeding ratio was measured at the beginning of every week using an analytical Balance 0.1-5.000gweighing scale. Feeding response was monitored and the mortality rate was recorded.

**Water Quality**

The water pH was measured with a Philip metre (model pH-009 111), with a glass electrode. Temperature measurement was determined in situ using simple mercury in glass thermometer, calibrated in degrees Celsius (°C). Dissolved Oxygen (DO) was measured with DO metre (model Eutex DO 600).

**Growth and Nutrient Utilization**

The mean standard weight of the fish in each tank was determined at the beginning of the experiment weekly, and at the end of the feeding trial. The weight of fish and feed was used to calculate growth and nutrient utilization parameters as follows;

**Weight Gain**

The weight gained was calculated using the formula below.

Weight Gain $= W_f - W_i$

Where: $W_f$ = Final average weight,

$W_i$ = Initial average weight

Final weight (g) – Initial weight (g)

**Specific Growth Rate**

This is the percentage rate of change in the logarithm body weight. The SGR was calculated using the formula below.

$$SGR = \frac{Log_e W_f - Log_e W_i \times 100}{Time\ (in\ days)}$$

Where $W_f$ is final body weight

$W_i$ is the initial body weight

$Log_e$ is the natural logarithm

**Feed Conversion Ratio**

This is the amount of unit weight of feed that specimens were able to convert into unit muscle. It was determined by the formula below:

$$FCR = \frac{Feed\ intake\ (g)}{Total\ weight\ gain\ (g)} \times 100$$

**Percentage of Weight Gain**

This was calculated using the formula below:

$$PWG = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100$$

**Heamatology Determination**

The blood samples were collected from the caudal peduncle with the aid of a 2 ml plastic syringe caudal vein using 5ml ethylene diamine tetra acetate (EDTA) as anticoagulant (AOAC,1995) Blood, 2.0 ml, was decanted in heparinized bottles for determination of blood parameters. A compact Sysmex XK-21 was used to run the samples in whole blood mode and prediluted. The hematological parameters analysed include White blood cells (WBCs), Red blood cells (RBCs), Hemoglobin (HGB), Packed cell volume (PCV), Mean corpuscular hemoglobin concentration (MCHC) and Platelets count (PLT).

**Proximate Analysis:** The proximate composition for experimental diets and fish carcass were measured according to AOAC (1995) method.

**Statistical Analysis**

All values were recorded as mean standard deviation and subjected to one-way analysis of variance (ANOVA) using SPSS 15 for the window software package. Significant means were subjected to a multiple comparison test (Tukey) for post hoc comparison at $P<0.05$ level.

**Results**

**Water Quality Parameters**

The Water Quality Parameters were measured in all the experimental tanks. The results are presented in Table 2 below:

**Table 2:** Water Quality Parameters of the Experimental Tanks

| Parameters | Range | Mean and Standard Deviation |
|---|---|---|
| **PH** | 5.9-7.2 | 6.7 ±0.37 |
| **Dissolved Oxygen** | 2.34- 4.50 | 3.4±0.64 |
| **Temperature** | 24 -30$^0$C | 27±2.0 |

**Proximate Composition of the Experimental Diet**

The experimental feed was formulated with varying inclusion levels of seaweeds at 0%, 25%, 50%,75%,100%. The proximate composition of seaweed *(Pistia stratiotes)* is shown in Table 3. The crude protein percentage of seaweed is 16.76%, the fat percentage of seaweed is 2.20%, the moisture percentage of seaweed is 10.30%, the fibre percentage of seaweed is 10.97%, the ash percentage of seaweed is 17.25%, and the Energy kcal/kg of seaweed is 1489.

**Table 3:** Proximate Analysis Composition of Seaweed

| **Parameters** | **Composition (%)** |
|---|---|
| Protein | 16.76 |
| Fat | 2.20 |
| Fibre | 10.97 |
| Ash | 17.25 |
| Moisture | 10.30 |
| Energy (Kcal/kg) | 1489 |

**Proximate Content of Experimental Fish**

The proximate composition of the different experimental fishes are given in Table 4. There was a noticeable difference in their composition which was as a result of the different level of treatment.

The result of the growth performance and nutrient utilization of *C.gariepinus* fish fed with varying inclusion levels with seaweed is shown in Table 5. Throughout this experiment, no physical disease was shown by the experimental fish. There was no significant difference noticed in the initial weight of the experimental fish before the experiment commenced. However, there was a significant difference ($p<0.05$) in the final weight gain of experimental fish across all test diets. The control diet and $T_1$ had the highest values. Similarly, Specific growth rate (SGR), Feed conversion ratio (FCR) and protein efficiency ratio (PER) followed the same trend as Final weight gain (FWG), indicating a significant difference ($p<0.05$).

The highest growth rate was observed in $T_4$ as this had the highest level of seaweed concentration, proving that the fish in $T_4$ converted the nutrient in their feed very well; this, in turn, increased the immune system of the fish and reduced their susceptibility to diseases and infection. This was evident in the high count of WBC of the fish which shows that lysosome activity was very high as a result of fighting bacteria and infections. Hence, growth was significant ($p<0.05$). T3 had the highest level of WBC.

$T_3$ had the highest growth rate value; as it had the second-highest level of seaweed concentration included in the feed, which made the fish convert the feed appropriately resulting in higher immune system boost, good weight gain, and higher yield.

Control tank had the lowest level of weight gain recorded; the fish's immune system was lowered which led to the death of the fish as the highest level of mortality was recorded in this tank. White blood cell (WBC) was reduced in the fish, lysosome activity was too low and almost noticeable compared to the activities present in the cell of $T_4$ fish. The growth rate was not significant ($p>0.05$).

**Table 4:** Proximate Content of experimental fish

| Code | Ash % | Moisture % | Fat % | Crude fibre % | Protein % | Carbohydrate % |
|---|---|---|---|---|---|---|
| Tank1 | 5.47 | 68.98 | 4.83 | 16.90 | 11.32 | 10.28 |
| Tank2 | 5.06 | 71.21 | 6.46 | 15.98 | 12.16 | 5.11 |
| Tank3 | 5.51 | 67.05 | 5.88 | 16.75 | 10.66 | 10.90 |
| Tank4 | 4.79 | 62.42 | 5.81 | 16.43 | 11.20 | 15.78 |
| Control | 5.21 | 71.27 | 3.39 | 17.69 | 14.05 | 6.08 |

**Table 5:** Growth performance of *Clarias gariepinus* fed with seaweed at varying levels

| Parameters | T0 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| Final Weight(g) | $59.8\pm0.70^{ab}$ | $65.6\pm0.82^{a}$ | $81.1\pm0.70^{ab}$ | $82.1\pm2.23^{a}$ | $67.8\pm1.33^{ab}$ |
| Initial weight(g) | $20\pm0.40^{b}$ | $21.1\pm1.12^{a}$ | $21\pm0.67^{ab}$ | $20.6\pm0.70^{ab}$ | $20.9\pm0.70^{ab}$ |
| Total Feed Intake(g) | 874.05 | 996.69 | 881.88 | 1043.6 | 985.76 |
| Weight gain(g) | $41.8\pm0.70^{ab}$ | $60\pm0.70^{ab}$ | $44.6\pm0.70^{ab}$ | $61.5\pm0.70^{ab}$ | $46.9\pm0.70^{ab}$ |
| PWG | $232.22\pm31.09^{ab}$ | $284.36\pm23.40^{b}$ | $212.38\pm0.70^{b}$ | $298.54\pm56.21^{ab}$ | $224.4\pm22.70^{a}$ |
| SGR | $0.68\pm0.77^{ab}$ | $0.76\pm0.67^{ab}$ | $0.64\pm0.4^{ab}$ | $0.78\pm0.83^{a}$ | $0.66\pm0.35^{a}$ |
| FCR | $1.50\pm2.12^{b}$ | $1.51\pm0.93^{a}$ | $1.78\pm0.67^{ab}$ | $1.54\pm0.77^{b}$ | $1.91\pm0.70^{a}$ |

Means with the same superscripts in the same vertical row are not significantly different ($P>0.05$) from each other. Significant differences ($p<0.05$) were observed in all the growth parameters among all treatment groups including control. (FCR-Feed Conversion Ratio, SGR-Specific Growth Rate, PWG-Percentage Weight Gain).

$T_1$ and $T_2$ had minimal growth rate and nutrient utilization level, the fish's immune system was slightly boosted because of the seaweed concentration included in the feed resulting in reduced susceptibility to disease and infection as few mortality was recorded in these treatments.

**Haematology**

Haematological analysis of fish is as shown in Table 6. $T_3$ had the highest count of WBC and RBC and $T_3$ showed the second-highest count of WBC and RBC indicating a significant difference ($p<0.05$) while T2 showed the lowest values. No significant difference ($p>0.05$) was seen in HCT as all the values remained constant. Highest blood platelets were observed in $T_4$ and lowest in $T_2$.

**Discussion**

The fish showed good appetite to all the treatment diets, attested to by the increased in body weight. However, the greatest weight gain was achieved in the treatment containing 75% inclusion and the least in the treatment containing 0% of seaweed. The best specific growth rate, feed conversion efficiency, and protein efficiency ratio were achieved in fish fed 75% inclusion level. All the experimental diets were accepted by *Clarias gariepinus*. This might be attributed to the good processing techniques which involved drying that might have reduced the antinutrient of seaweed and increased its palatability. This observation corroborates the works of other authors (Amisah *et al.*, 2009; Olatunde *et al.*, 1989; Petterson and Mackintosh, 1994).

The growth pattern revealed that *C. gariepinus* performed better in diet T3 than all other diets. However, poor growth performance was recorded in diet T0 which had no seaweed. According to Fagbenro and Davies (2003), it is expected that fish fed similar levels of feed containing the same level of digestible protein

**Table 6:** The Heamotological analysis of *Clarias gariepinus* fed with seaweed at different inclusion levels

| Parameters | T0 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| WBC | 4±2.96 | 3.75±1.48 | 5.35±1.77 | 6.35±2.61 | 3.5±0.99 |
| RBC | 1.975±0.86 | 1.975±0.52 | 2.095±0.17 | 2.21±0.18 | 2.07±0.11 |
| HGB | 12.05±4.59 | 11.4±3.39 | 14.05±0.92 | 12.6±1.41 | 11.4±2.55 |
| HCT | 25.7±9.61 | 28.15±8.27 | 32.5±3.25 | 26.25±1.77 | 29.5±1.70 |
| MCV | $32.05±8.27^{ab}$ | $41.85±5.02^{bc}$ | $46.9±2.54^{c}$ | $25±2.12^{a}$ | $33.2±1.98^{ab}$ |
| MCH | $61.95±3.23^{ab}$ | $57.3±2.12^{a}$ | $63.6±1.13^{b}$ | $60.15±1.63^{ab}$ | $62.75±1.48^{ab}$ |
| MCHC | $46.9±4.24^{bc}$ | $40.35±0.70^{a}$ | $43.3±1.55^{ab}$ | $48.1±2.12^{c}$ | $45.7±1.56^{bc}$ |
| RDW-CV | $152±0.99^{ab}$ | $52.5±3.46^{a}$ | $70.5±1.34^{ab}$ | $164±2.82^{c}$ | $62.5±1.48^{ab}$ |
| RDW-SD | 57.55±73.54 | 82.55±7.78 | 79.7±26.16 | 55.6±125.86 | 67.8±4.95 |
| PLT | 57.55±10.11 | 82.55±12.79 | 79.7±0.28 | 79.7±13.01 | 79.7±6.50 |
| MPV | 8.55±0.21 | 8.45±0.63 | 8.25±0.35 | 8.25±0.92 | 8.1±0.14 |
| PDW | 10.7±0.14 | 10.55±1.06 | 9.55±0.49 | 10±0.0.99 | 9.15±0.78 |
| PCT | $0.125±0.63^{a}$ | $0.04±0.14^{a}$ | $0.055±0.21^{a}$ | 0.135±0.12 | 0.05±0.14 |

Values on the same row with different superscripts showed a significant difference ($p<0.05$) from each other.

**Keys:** WBC-white blood cell ($10^2/\mu L$); HGB- Hemoglobin(g/Dl); RBC-red blood cell($10^4/\mu L$); HTC-haematocrit (%), MCV-Mean Corpuscular Volume(fL); MCH-Mean Corpuscular Haemoglobin (pg); MCHC-Mean Corpuscular Haemoglobin Concentration(g/dL); RDW-CV - red blood cell distribution width (statistically expressed as coefficient of variation); RDW-SD -red blood cell distribution width (statistically expressed as standard deviation; MPV – Mean Platelet Volume; PDW -Platelet Distribution Width; PCT - platelet haematocrit ($10^4/\mu L$)

and metabolizable energy should be identical in their growth pattern. The present study carried out on *C. gariepinus* showed a significant decrease in the growth parameters at the lowest inclusion level of seaweed in the test diets.

The results of this study indicated an increase in haematological parameters of *Clarias gariepinus* fed diets containing seaweed which conforms to the similar report by Joshii *et al.*, (2002) for *Clarias gariepinus* fed on the *Citrullus lanatus* seed meal. The values recorded for haematological parameters of *Clarias gariepinus* fed diets containing *Citrullus lanatus* seed meal were all within the range of normal haematology of a healthy fish, a range of 3.61- 6.54 g/mm3 for haemoglobin; 15-31% for Haematocrit (PCV); 1.31-3.23 (106 /mm3) for RBC; 0.80-73.6 (103 /mm4 ) for WBC; 17.28-26.14 (%) for MCHC. RBC greater than $1x10^6$/mm3 is considered high and is indicative of the high oxygen-carrying capacity of the blood which is characteristic of fishes capable of aerial respiration and with high activity. This agrees with the findings of Akinwande *et al.*, (2004). The increase in other parameters of the blood of fish fed test diets with respect to that of fish fed control diets agrees with report of Adekoya *et al.*, (2001) who fed the toasted sunflower seed meal to *Clarias gariepinus* and Reddy and Bashamohideed (1999) who fed cottonseed meal to channel catfish. Rumsey *et al.*, (2004) reported that the concentration of blood plasma protein is an indicator to general health condition of fish. Although, Tacon *et al.*, (2011) reported that a reduction in plasma protein is an indicator of the effect of toxins on the kidney, spleen, and liver.

The results obtained in this study showed no significant increase in the total plasma protein of *Clarias gariepinus* test diets when compared to fish fed control diet. The decrease in protein level with higher levels of plant energy-based diets may be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis and may be due to the mobilization of protein to meet energy requirements and to sustain increased physiological activity (Rumsey *et al.*, 2004).

This is desirable because a reduction in blood glucose level might be as a result of hypoxic condition induced by feeding diets containing a higher concentration of anti-nutrients. The increase in blood glucose level recorded in this study shows an increase in the blood glucose concentration and this might have resulted from an increase in plasma catecholamine and corticosteroid hormones.

## Conclusion

In conclusion, seaweed (*Pistia stratiotes)* has the potential to make considerable contributions to the growth of catfish (*Clarias gariepinus*). Seaweed can be used to partially replace indomie at 75% inclusion level in the diet of *Clarias gariepinus,* thereby reducing the cost of feeding.

This study has demonstrated that seaweed could be included in the diet of *Clarias gariepinus* without negative effects on the growth. However, since seaweeds are more available and relatively cheaper, it could be recommended that partial replacement of seaweed in the diet of *C. gariepinus* at 75% inclusion level would aid fish productivity.

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