International Journal of Agricultural Research and Review
Abbreviated Key Title: Int. J. Agric. Res Rev.
ISSN: 2360-7971 (Print) & Open Access
DOI: 10.54978/ijarr.2025. Vol. 13, Issue 1, Pp.: 12-18, Jan. 2025.

Effect of Replacing Ascorbic acid with Spinach (Spinacia Oleracea) Leaf Meal in the Diet of *Clarias Gariepinus* (Burchell 1822)

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Abstract

Researchers wanted to find out what happened to the fish's growth, weight, and feed conversion rate when spinach leaf was fed instead of ascorbic acid to young Clarias gariepinus. It was experimental research conducted in the nutrition laboratory of the Department of Biological Sciences of the Nigerian Police Academy, Wudil, Kano State. Data obtained from the experiment was subjected to one-way analysis of variance (ANOVA). Results revealed that the final weight for fish is higher in diet 4, with a mean value of 274.20 g, followed by diet 1 (251.10 g). There is a significant difference between diet four and diet three. Mean weight gain is higher in diet (4) 257.47 g and lowest in diet (3) 189.87 g. There is a significant difference between the mean value in diet (4) and (3). Mean daily weight gain is higher in diet (4) 4.29g, followed by diet (1) 3.90g, and lowest in diet (3) 3.16g. Specific growth rate is higher in diet (4) with the mean value of 1.98 g, followed by diet (1) 1.97 g, and lowest in diet (2) 1.67 g. Percentage weight gain is higher in diet (4) 1536.1 g and followed by diet (1) 1384.1 g. This means that feed with a high dose of spinach has a significant effect on the growth, weight, and feed conversion of Clarias gariepinus. It is therefore recommended that fish farmers should go for spinach leaves as a source of vitamin C in their diet since it is cheaper and available any time of the season always have even those in the rural areas will always access to spinach leaves rather than vitamin C.

Keywords: African Catfish, Growth Indices, Spinach Leaf Meal, Feed Conversion

INTRODUCTION

Catfish (C*larias gariepinus) is* one of the most popular aquaculture species and is farmed in more than 20 countries and reregions. rican catfish (C*larias gariegariepinus) has* bome popular in agricultural business in N centreseria. This is because this species species cano aide range of temperatures and to low oxygen and low salinity levels (Agupugo, Nsofor, Ezewudo, & Edeh, 2022). Moreover, the fish matures in a space of months, aof six is of most importance in Nigeria.

Nigeria is now the largest producer of catfish in the world (Adetola, 2023). Likewise, fish consumption was reported be hireported toy in Nigeria, this due to the rapidly increasing human population. In 2020-2022, the aquaculture industry about 178industry recordedonnes of production, generating a revenue of USD 265 billion (FAO, 2022). However, the high cost of fish feed and inconsistent supply of feed have become major challenges to fish production (Food and Organisation of the United Nations, 2020).

Fish feeds required large doses of nutrients for fish survival, which include carbohydrates, minerals, and vitamins. Among these vitamins is ascorbic acid, or vitamin C. The importance of this ascorbic acid cannot be overemphasized.Voveremphasized. c acid) is an important part of a fish's diet because it helps the body absorb ions and nutrients. If a fish doesn't get enough of it, it can damage its structure, leading to problems like curved spines, broken skulls, and bleeding. Hence, a high dose of vitamin C is recommended in fish diets; vitamin C is a major mineral needed for fish growth. Therefore, it is necessary for fish feed to be prepared with required doses of Vit C supplement for proper functioning of the body, and it should be noted that Vit C can be oxidated during processing and storage of feed (Adeyemi-Doro & Iyiola, 2018). The use of plant-based ingredients, such as in fish diets, can reduce the cost of feed production, leading to increased fish production.

It is worth noting that global gross fish production from aquaculture operations has increased steadily within the last few years at a rate of 9.2% per annum (FAO, 2022). However, it has been noticed that aquaculture has not yet been able to reach the expected large-scale global food replacement for the teeming populace, especially the rural poor of the world, including Nigeria. In aquaculture, fish diet is often considered the single largest cost item and can represent over 50% of the operating cost in intensive aquaculture (El-Sayed as cited in Abdel-Warith, 2018). The high cost of fish diet makes commercial production of catfish highly capital intensive, as it accounts for between 30 and 60% of variable operating cost (De Silva & Anderson, as cited in Nadir, Eyup, Yahya, & Nilgun, 2007). The general approach adopted to reduce diet cost has been to develop low-cost diets that can favourably replace the costly diets containing cheaper plant sources, such as spinach leaf, without compromising the health and growth or productivity of cultured organisms (Ugwumba & Ugwumba, 2003).

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family of (*Amaranthaceae*). It is native to central and southwestern Asia. It is an annual plant (rarely biennial), which grows to a height of up to 30 cm. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, and ovate to triangular-based, very variable in size from about 2–30 cm long and 1–15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3–4 mm in diameter, maturing into a small, hard, dry, lumpy fruit cluster 5–10 mm across containing several seeds.

Fresh spinach leaves are a rich source of several vital antioxidant vitamins like vitamin A and vitamin C, as well as flavonoid polyphenolic antioxidants such as lutein, zeaxanthin, and beta-carotene. All of these chemicals work together to protect cells from oxygen-based free radicals and reactive oxygen species (ROS), which help the body heal as it ages and gets sick. *Zea-xanthin is a carotenoid that is found in many foods. It is selectively absorbed into the retinal macula lutea in the eyes, where it is thought to protect cells from damage and act as an antioxidant.* It thus helps protect from "age-related macular disease" (ARMD), especially in the elderly.

Spinach leaves are an excellent source of vitamin K. 100 g of fresh greens provides 402% of daily vitamin K requirements. Vitamin K plays a vital role in strengthening the bone mass by promoting osteotrophic (bone-building) activity in the bone. Additionally, it also has an established role in patients with *Alzheimer's disease* by limiting neuronal damage in the brain. This green leafy vegetable also contains significant amounts of various B-complex vitamins, including vitamin-B6, thiavitamin B-6 (pyridoxine), vitamin B-1, riboflavin, folates, and niacin. Folates help prevent neural tube defects in the offspring.

100 g of farm-fresh spinach has 47% of daily recommended levels of *vitamin C*. Vitamin C is a powerful antioxidant, which helps the body develop resistance against infectious agents and scavenge harmful oxygen-free radicals. Its leaves also contain a beneficial amount of minerals like *potassium*, manganese, magnesium, copper, and zinc. Potassium is an important component of cell and body fluids that helps control heart rate and blood pressure. Manganese and copper are used by the body as co-factors for the antioxidant enzyme *superoxide dismutase.* Copper is required in the production of red blood cells. Zinc is a cofactor in many enzymes that regulate growth and development, sperm generation, digestion, and nucleic acid synthesis. Vitamin C plays a crucial role in the body's defence against infectious agents and its ability to scavenge harmful oxygen-free radicals. When a fish's diet is deficient in vitamin C, it typically exhibits observable signs of defective collagen formation. So, we saw overgrowth of collagen and cartilage tissue, kyphosis, scoliosis, lordosis, resorbed opercula, deformities, and overgrowth of the jaw. The wounds also didn't heal well. Lim et al. (2000) investigated the interaction between vitamin C and iron if they are supplemented in the diet of catfish (Ictalurus punctatus). If the vitamin C was supplemented in high doses (3000 mg/kg), there was a significant effect on survival. Also, there are reports that mention the important role of the combination of vitamins D and C in the ossification process in fishes and in the formation of skeletal deformations (Darias et al. 2011). Therefore, a high dose of ascorbic acid is beneficial for fish farming and product production, particularly for the species Clarias gariepinus.

In Africa, there are various species of *clarias gariepinus*. David, in Bruton (2024), recognised five species within this subgenus. The author looked at things like the shape of the vomerine teeth, the number of gill rakers, and the ratio of the vomerine teeth bands to the premaxillary teeth bands. The five species were *Clarias anguilarus, Clarias senegalensis, Clarias lazera, Clarias mossambicus, and Clarias gariepinus*.

Clarias gariepinus, which is widely considered one of the most important tropical catfish species for aquaculture, has an almost pan-African distribution, from the Nile to West Africa and from Algeria to Southern Africa. They also occur in Minor Asia (Israel, Syria, and South of Turkey). Clarias anguillaris has a more restricted distribution and is found in Mauritania, in most West African basins, and in the Nile. In general, *Clarias gariepinus* lives in most river basins.

Clarias spp. inhabit calm waters from lakes, streams, rivers, swamps, and floodplains, some of which are subject to seasonal drying. The most common habitats frequented are floodplain swamps and pools in which the catfish can survive during the dry seasons due to the presence of the accessory air-breathing organs (Bruton, 1979a; Clay, 1979).

Figure 1: A Sample of claria gariepinus

Fish is a cheap and important source of protein that also contains calcium, lipids, minerals, vitamins, and oils with desirable low cholesterol levels in the diet of fish lovers. Economically, it is a source of income, and it has continued to be the most affordable source of animal protein for an average Nigerian family (Haruna, 2006). The by-product of fish can be incorporated into feeds of livestock and poultry. Fish accounts for more than 40% of the protein diet of two-thirds of glol poof thelation. Fish interact with the various levels of the food chain and influence the structures of lakes, streams, and estuaries since they are usually restricted to particular modes of life related to their food sources and reproductive requirements (Ashade, Osineye, & Kumoeye, 2013).

C. Gariepinus Gariepinus holds a significant position in fish farming in Africa due to its wide geographical spread, high price, and high demand from both fish farmers and consumers in Nigeria, whether in smoked or fresh form. It is found throughout Africa, Nigeria included, and lives in fresh water and human-made habitats, such as earthen ponds or concrete ponds. The fish was introduced all over the world in the early 1980s for aquaculture purposes. *Clarias gariepinus* has a more restricted distribution and is found in Mauritania, in most West African basins, and in the Nile. In general, *Clarias gariepinus* lives in most river basins. This is mostly reared by farmers in sub-Saharan Africa and has become a good source of food and nutrients for the people. *Clarias gariepinus* (Burchell, 1822), also called the mud catfish or the African sharptooth catfish, is an omnivorous fish that eats fruits, seeds, and different kinds of aquatic organisms, such as plankton, invertebrates, and vertebrates (Skelton 2001; Odongo et al. 2019). It is widely adopted as a culturable species in Nigeria because of its hardy nature and having a good feed conversion rate (Sotolu & Faturoti 2011). The fish is in high demand among fish consumers due to the tasty nature of the flesh (Idodo-Umeh, 2003). However, a hike in the cost of fish meal (a major protein source in fish diets) has increased the cost of production, leading to a low supply of fish to consumers. Therefore, we must boost the production of fish by incorporating more ascorbic acid.

However, the rapid increase in the high cost of ascorbic acid ingredients on the market has been of significant concern to fish farmers in terms of fish nutrition. This necessitates the substitution of ascorbic acid with spinach. Spinach leaf (Spinacia oleracea) is a cheap and easy-to-obtain source of ascorbic acid that can be utilised for fish feed formulation. Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamin A (and especially high in lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, betaine, iron, vitamin B2, calcium, potassium, vitamin B6, folic acid, copper, protein, phosphorus, zinc, niacin, selenium, and omega-3 fatty acids. Recently, opioid peptides called rubiscolins have also been found in spinach.

Despite the availability of the spinach, its utility as a vegetable, its feed formulation has not been fully exploited. The importance of Spinacia oleracea as a source of vitamin C in fish feed formulation (Spinacia oleracea) will go a long way in reducing the cost of ascorbic acid for fish feed and providing a high dose of vitamin C for increasing fish production in Nigeria.

Many scientists have worked on different natural products and the use of leaf meal to increase fish production, such as onion bulb (Bello et al., 2012), (Ny & Aus2012), garlic (009), turmeric (Muniruzzaman & Chowdhury, 2004), and mistletoe (Park & Choi, 2012), Leucaena leucocephala (Lam.) de Wit leaf meal (Agupugo, Nsofor, Ezewudo, & Edeh, 2022), water spinach (Yousif et al., 2019), and Ipomoea aquatica (Fabusoro, Odulate, Idowu & Odebiyi, 2014), but there is a dearth of information and research on the use of spinach leaves to replace amino acids for C. gariepinus. This study therefore investigates the effect of replacing spinach with ascorbic acid in fish meal of Clarias gariepus. This was guided by three *specific objectives are:*

1. To determine the effect of spinach leave meal on the average body weight (ABW), juvenile of *clarias gariepinus*
2. To evaluate the effect of spinach on specific growth rate (SGR),of *clarias gariepinus*
3. Ascertian the effect of spinach on survival rate and feed conversion ratio (FCR) of *clarias gariepinus.*

MATERIALS AND METHODS

Study Area

This study was conducted in the nutrition laboratory of Department of Biological Sciences of Nigerian Police Academy, Wudil Kano State, Nigeria.

Collection and Preparation of *Spinacia oleracea*

Spinach leaf was procured in Wudil market freshly, it was washed with running water, cleaned, dried under shade, and grind into powder by the use of manual miller. The grounded powder will be kept in air tight container until required.

Experimental design

Spinacia oleracea leaf meal powder was used instead of vitamin C at 0% (control), 5%, 10%, and 15% levels. These levels were tracked as D1, D2, D3, and DD4. The experimental diet was allocated to four treatments in replicates (3 each).

Fifteen (15) fingerlings were stocked in a 12, 50-litre capacity plastic basin in a randomised block design. The fish were fed with the experimental diet for 58 days, 2 times daily, morning and afternoon.

A 42% crude protein (42% CP) *Clarias gariepinus* fingerling diet was formulated using the following fish feed ingredients: GNC, fish meal, wheat bran, lysine, methionine, vegetable oil, table salt, binder (cassava starch), and ascorbic acid (Pal Pharmaceutical Industries Ltd)GNCLtd) sh meal Wheat bran was ground into powder separately and kept in an airtight container until required. The Pearson square method was used to formulate the 42% CP of *Clarias gariepinus* fingerlings. Proximate composition of the formulated diet was carried out following the method described by AOAC (1990).

Table 1: Experimental diet

Feed Ingredients (%)	**Inclusion Level of *Spinacia Pleracea* (%)**			
	D_1	D_2	D_3	D_4
Fish meal	41.45	40.40	39	38.7
GNC	41.45	40.40	39	38.7
Wheat bran	14.50	14.10	13.9	13.6
Salt	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5
Starch	0.5	0.5	0.5	0.5
Spinacia oleracea0	0	2.35	4.7	7.05
Total	**100**	**100**	**100**	**100**

Proximate Composition

Composition of the experimental diet was analyzed following (AOAC, 1990).

The fish were weighed bi-weekly to adjust their feeding rates using sensitive Ohaus LS 2000 analytical weighing balance. The uneaten feed was siphoned every morning before feeding. Dirty water was replaced whenever it is observed. The length and weight of the fish were recorded in cm and mm respectively. Quantity of feed consumed by each treatment and quantity of feed dispensed were also recorded.

Fish performance during the experiment was determined using the following formula:

Mean Weight Gain (MWG): Mean Final Weight – Initial Weight,

Total percentage Weight Gain (TPWG %) =Total Weight Gain/Initial Weight x 100

Specific Growth Rate (SGR), $\log_e W_2$ - $\mathrm{Log}_e W_1$/tx100

Where W_2 = Final Weight (g), W_1 = Initial Weight (g) and t = rearing periods (days), $\log_e$ = logarithms

Feed conversion (FCR) = (dry feed consumed weight (g) /fish weight (g)

Protein efficiency ratio (PER = (Fish weight gain (g) / protein fed (g))

Feed efficiency = Live weight gain (g) /feed supplied (g)

Data Analysis

Data obtained from the experiment was subjected to one way analysis of variance (ANOVA).The mean between the treatment was determined using Duncanss multiple range test (DMRT).

RESULTS

Results from the tables revealed that there is an increase in growth performance of fish fed spinach leaves with an increasing level of spinach leaves. Table 1 shows the average weight gain (MWG), average daily weight gain (MDWG), specific growth ratio (SGR), percentage weight gain (PWG), protein efficiency ratio (PER), feed conversion ratio (FCR), and condition factor (K).

The final weight for fish is higher in diet 4, with a mean value of 274.20 g, followed by diet 1 (251.10 g). There is a significant difference between diet (4) and (3). Mean weight gain The mean weight gain is higher in diet (4) at 257.47 g and lower in diet (3) at 189.87 g. There is a significant difference between the mean value in diet (4) and (3). Mean daily weight gain is higher in diet (4) 4.29g, followed by diet (1) 3.90g, and lowest in diet (3) 3.16g. Specific growth rate is higher in diet (4) with the mean value of 1.98 g, followed by diet (1) 1.97 g, and lowest in diet (2) 1.67 g. Percentage weight gain is higher in diet (4) 1536.1 g and followed by diet (1) 1384.1 g. There are significant differences between diet (4) and other diets; the protein efficiency ratio is higher in diet (4) 17.5 g lower in diet (2) 5.91 g. There are significant differences between diet (4) and (2), but there is no significant difference between diet (4) and (1). The condition factor is higher in (4) 2.98, followed by diet (2) 2.78; the survival percentage is higher in diet (4) 100%, followed by diet (1) 90%. There is no significant difference between diet (2) and (3). Final length is higher in diet (4) 10.45 cm and lowest in diet (2) 9.58 cm; there is no significant difference in diet (2) and (3). Therefore, diet (4) had better growth performance in all the parameters and on the growth and survival of *Clarias gariepinus* fingerlings.

Table 2: mean growth and survival of *clarias gariepinus* fingerlings fed different level of spinach leave. Levels of inclusion g/kg of feed

Growth Parameter	$D_{1\ (0)}$	$D_{2\ (5)}$	$D_{3\ (10)}$	$D_{4(15)}$
Initial weight (g)	16.90	16.87	16.87	16.73
Final weight (g)	251.10	226.73	205.73	274.20
Initial length (cm)	4.00	3.83	3.93	4.47
Final length (cm)	9.99	9.98	9.93	10.45
Mean weight gain (g)	234.20	209.87	189.87	257.47
Mean daily weight gain (g)	3.90	3.50	3.16	4.29
Specific growth rate (g)	1.97	1.67	1.79	1.98
Percentage weight gain (g)	1384.1	1246.1	1124.0	1536.1
Protein efficiency ratio (g)	11.5	13.91	12.8	17.5
Feed conversion ratio (g)	6.13	9.91	6.58	9.45
Condition factor (k)	2.07	2.78	2.05	2.98
Survival (%)	9.00	63.33	63.33	100.0

Table 3: Mean value of water quality parameter during the experiment

Treatment	Temperature(Oc)	PH(m)	DO(cm)
1	**25.5**	**7.4**	**3.8**
2	**23.8**	**7.2**	**3.5**
3	24.6	7.2	4.1
4	24.2	7.2	3.9

DISCUSSION

Based on how the fish responded to this experiment, the growth and survival of *Clarias gariepinus* fingerlings show that they do better at growing and using nutrients when they are fed diets with 15% spinach leaves. Fresh spinach leaves are full of many important antioxidants, such as vitamin C, which makes up about 47% of spinach leaves and helps fight oxygen-based free radicals, also known as reactive oxygen species (ROS), which damage cells and cause ageing and many diseases.

Conclusion and Recommendation

Fish-fed diets (4) containing 15% spinach leaves had better growth performance and nutrient utilisation than the other diets, which contained various levels of spinach leaf inclusion. Thus, this study shows that *Clarias gariepinus* fingerlings could eat up to 15% spinach leaves without any negative effects on their growth or ability to stay alive. Other diets' reduced growth performance may not be due to palatability issues but to diet imbalance, since subjects did not reject feed during the experiment. The findings from the study on *Clarias gariepinus* fingerlings underscore the significant impact of diet composition on their growth and nutrient utilization. The exceptional results achieved with a 15% spinach leaf diet not only promote enhanced growth rates but also indicate an efficient use of nutrients, highlighting the potential for plant-based diets in aquaculture. Importantly, this dietary approach did not lead to any adverse effects on either growth or survival rates, suggesting that spinach can be a viable alternative in fish nutrition. Additionally, while some variations in growth performance were observed, they appear to stem from dietary imbalances rather than palatability concerns. This research opens new avenues for optimising feeding strategies, which could benefit fish health and aquaculture sustainability. Therefore, it

Therefore, it is recommended that fish farmers go for spinach leaves as a source of vitamin C in the diet of fish, since it is cheaper and available at any time of the season, and those in the rural areas will also have access to spinach leaves rather than vitamin C.

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Citation: Azubuike A.C: ***Effect of Replacing Ascorbic acid with Spinach (Spinacia Oleracea) Leaf Meal in the Diet of Clarias Gariepinus (Burchell 1822)*** Int. J. Res. Rev. 13(1) Pp.12-18, 2025