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Azolla pinnata as a Sustainable Protein Source: Impact on Reproductive and Biochemical Status of Female *Clarias gariepinus* Broodstock

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Abstract

The search for novel, plant-based feed ingredients as alternative protein sources to soybean meal is a priority in the aquafeed industry. This study, therefore, investigated the effects of substituting soybean meal with *Azolla pinnata* powder on the growth and reproductive performance of farmed female African catfish (*Clarias gariepinus*) broodstock. We formulated pelleted diets by replacing soybean meal with *A. pinnata* at levels of 0 (control), 15%, 30%, and 60%. Fish were fed these diets twice daily (8:00 AM and 6:00 PM) at a rate of 5% of their body weight for 90 days. After the feeding trial, we assessed parameters, including growth performance, reproductive traits, and biochemical status of the female *C. gariepinus* broodstock. Initial analysis of growth characteristics revealed no significant difference ($p > 0.05$) across all dietary treatments. However, significant differences were observed in other parameters: Reproductive Performance: The highest reproductive indices for *C. gariepinus* were recorded in the group fed the 30% *A. pinnata* diet ($p < 0.05$). Conversely, the reproductive hormone levels significantly decreased ($p < 0.05$) in fish receiving the 60% *A. pinnata* diet compared to the control. Biochemical Status: Broodstock fed the 60% *A. pinnata* diet exhibited the highest and most significant ($p < 0.05$) serum concentrations of ALP, ALT, Cholesterol, Urea, and Creatinine. No significant difference ($p > 0.05$) was found in AST or TP levels among the groups. These findings collectively suggest that 30% inclusion of *A. pinnata* powder is an adequate and optimal supplement for the fish feed, promoting better growth, reproductive performance, and overall broodstock development in *C. gariepinus*.

Keywords

Azolla pinnata Powder, Soybean Meal, Female Catfish, Reproductive Performance, Biochemical Parameters, Substitution

1. Introduction

Livestock farming is a major global concern regarding climate change, contributing significantly to greenhouse gas (GHG) emissions [1]-[3]. It accounts for 14.5% of all anthropogenic GHG emissions worldwide [4]. Meanwhile, fish remains a vital food source, supplying 22% - 50% of the animal protein in the diets of populations across sub-Saharan Africa [5] [6]. Within aquaculture, feed supply is a critical component influencing the Carbon Footprint (CF) of fish production, as the amount of feed used per kilogram of live weight gain directly affects the CF [1] [7] [8]. Looking ahead, the Food and Agriculture Organization of the United Nations (FAO) estimates that the consumption of animal products will continue its upward trend. This increase will necessitate greater production of protein-based ingredients, thereby exacerbating climate change [9] [10]. The incorporation of imported proteins in animal feed, mainly soybean meal [11] on the one hand, and deforestation for its cultivation and transport on the other [12] [13], contribute significantly to GHG emissions [12]. Finding low-climate-impact feed ingredients is a crucial mitigation strategy for the livestock sector. However, this transition must be coupled with maintaining high feed conversion efficiency (FCE). Without high FCE, increased feed use could negate the environmental benefits gained by switching to a low-impact diet [14]. Therefore, producing protein ingredients locally to reduce environmental impact compared to imported feeds is urgent.

Aquatic forage, traditionally considered waste, can be used as a feed ingredient for aquaculture [15]. *Azolla* is a floating aquatic plant Mishra *et al.* [16] [17], Ray *et al.* [15] [18]. Its bromatological composition includes: 25% - 35% crude protein [19] [20], 2.45% crude lipids; 25.50% crude ash; 11.19% crude fiber [21]; and higher quantity and quality of amino acids (lysine, methionine, cystine, threonine, tryptophan, arginine, isoleucine, leucine, phenylalanine, tyrosine, glycine, serine, and valine) [22] [23] than in soy [24]. In addition to its nutritional value, *Azolla pinnata* contains numerous phytochemical compounds, including flavonoids [25], tannins, phenols [26], alkaloids, and saponins [27] [28]. This rich composition, combined with its high protein content and rapid growth rate, has made *Azolla* the subject of extensive research in aquaculture [29] [30]. [31] reported that *Azolla* powder incorporated at 50% did not negatively affect growth parameters in tilapia, and [32]-[34] reported the same in cyprinids. However, [35] [36] reported that the incorporation rate without an adverse effect on growth parameters in tilapia and cyprinids is 25% - 30%. While numerous studies have reported positive effects of *Azolla* sp. on the growth performance of certain herbivorous fish species, very

few studies have examined its effects on the reproductive performance of omnivorous species. Hence, the general objective of this study is to contribute to research on non-conventional, locally available, inexpensive, and less polluting protein sources to improve the productivity of omnivorous species. More specifically, the aim was to evaluate the effects of substituting soybean meal with *Azolla pinnata* powder on female catfish *Clarias gariepinus*:

- Growth parameters;
- Organ weight;
- and reproductive parameters.

2. Materials and Methods

2.1. Study Area

The study was carried out at the ANTONIA Belibi Farm in Obala (LN 04˚10'00", LE 11˚32'00"). Obala is located about 1420 meters above sea level. The climate is Guinean temperate due to its altitude, with approximately 2157 mm of rainfall distributed across two seasons: mid-March to mid-June and mid-August to mid-October. The temperature ranges from 20˚C - 32˚C with an average of 26˚C, and the relative humidity generally exceeds 55%.

2.2. Plant Material and *Azolla pinnata* Powder

The plant material, *Azolla pinnata* (Figure 1), was purchased from an eFarm enterprise in the Bonaberie-Littoral Region, Cameroon. The *Azolla pinnata* was sun-dried and ground in an electric grinder. It was then passed through a 0.5 mm mesh size sieve to prepare the *Azolla* meal. Dried *Azolla* plant samples, along with the other feed ingredients, were analysed for their chemical composition using the methods described in [37]. The formulated diets and the calculated chemical analysis of these tested diets are presented in Table 1.

Table 1. Chemical composition of Dried *Azolla pinnata*.

Chemical composition of dried *Azolla pinnata* (%)				
CP	CF	EE	Ash	Digestible energy (Kcal/kg)
28.5	17.25	2.1	23.2	2410

Figure 1. *Azolla pinnata.*

2.3. Animal Material

Forty-eight (48) catfish broodstock, specifically *Clarias gariepinus* (CG) (32 females and 16 males), were purchased from reputable breeder farms in Nkoabang, Yaounde, Central Region of Cameroon. These males were used only for the actual fertility test. The fish, which had an average initial weight of 153.35 ± 6.5 g, were then stocked into concrete tanks at a density of four fish per tank, with one replicate per treatment. The broodstock were selected based on the readiness of their genitals: females were considered gravid if they displayed a swollen, reddish genital opening, while males were selected based on reddish and pointed genital papilla. The broodstocks were conditioned for two weeks in concrete tanks (100 m^2). Before the experiment commenced, the broodfish were acclimatized and maintained on a commercial diet containing 40% crude protein, which was fed twice daily.

2.4. Experimental Diet

Feed ingredients, including Fish meal, Soybeans, Yellow maize, a Vitamin mix, and a binder, were sourced from the Obala Road Fish Market. A 40% crude protein diet was formulated using Pearson's square method (Table 2).

The preparation process involved several steps:

- Processing: The soybeans were first toasted locally using a frying pan. Fish meal, cassava flour, and maize were subsequently ground into a fine powder using a grinding machine,
- Mixing and Pelleting: The powdered feedstuffs were thoroughly mixed by hand before hot water and the binder were added to form dough. This dough was then pelleted using a hand pelletizer,
- Drying and Storage: Finally, the experimental diets were sun-dried and packaged for use.

Table 2. Ingredient composition (100 Kg) and proximate composition (%DM) of basal diet.

Ingredients	Treatments			
	T1 = 0%	T2 = 15%	T3 = 30%	T4 = 60%
Fish meal	25	25	25	25
Soyabean meal	25	21.25	17.5	15
***Azolla pinnata* powder**	0	3.75	7.5	10
Granut meal	18	18	18	18
Corn starch	5	5	5	5
Cassava flour	2	2	2	2
Brewery	9	9	9	9
Wheat bran	8	8	8	8
Crude palm oil	1	1	1	1
CMAV	5	5	5	5

Continued

Shell meal	0.85	0.85	0.85	0.85
Vitamin Mix	0.15	0.15	0.15	0.15
Salt	1	1	1	1
Total	100	100	100	100
Proximate composition				
Dry matter (%MS)	88.99	89.12	89.5	90.44
Crude protein (%MS)	40.15	40.50	41.01	41.57
Crude lipids (%MS)	17.50	18.25	18.40	18.50
Ash (%MS)	30.3	31.5	32.01	32.7
Fiber (%MS)	22.1	23.14	24.45	25.57
Crude Energie (kcal/100 g)	220	219.5	219	218

Vitamin-premix-A pfizer livestock product containing the following per kg of feed: A = 4500 I.U, D = 11252 I.U, E = 71 I.U, K3 = 2m, B12 = 0.015 mg, Panthothenic acid = 5 mg, nicotinic acid = 14 mg, Folic acid = 0.4 mg, Biotin = 0.04 mg, choline = 150 mg, colbalt = 0.2 mg, copper = 4.5 mg, Iron = 21 mg, manganese = 20 mg, iodine = 0.6 mg, selenium = 2.2 mg, zinc = 20 mg, antioxidant = 2 mg.

2.5. Experimental Design

The study employed a Completely Randomized Design, where the broodfish were randomly assigned to four dietary treatments, each quadruplicated. Fish were stocked in experimental concrete tanks (1 m × 1 m × 1.25 m depth) at a sex ratio of 1 male to 2 females (1:2). The four dietary treatments were formulated as follows:

Treatment	*Azolla pinnata* inclusion Level	Diet Fed
T1	0% gr/kg	Control (without *A. pinnata* powder)
T2	15% gr/kg	Experimental Diet 1
T3	30% gr/kg	Experimental Diet 2
T4	60% gr/kg	Experimental Diet 3

For a period of 90 days, the fish received a daily ration equivalent to 5% of their body weight, administered in two feedings (08:00 and 18:00). Following this feeding trial, they were collected and artificially spawned to assess their reproductive performance.

- **Milt Extraction**

Milt was extracted from 16 sacrificed males without hormonal inducement, at rate of 4 per treatment. The milt was pooled and divided into four portions. Each portion was diluted with 2 mL of 9 gr normal saline and stored at a temperature below 7˚C.

- **Hormonal inducement**

Female broodstock, separated by age group, were moved to the hatchery. They were induced with a single injection of Ovaprim (0.5 mL/kg body weight). Following a 12-hours latency period, they were used for the evaluation of biochemical parameters.

3. Data Collection

3.1. Blood Sampling Preparation

Three non-heparinized blood samples were collected from the caudal vein at the study's end. After centrifugation (3000 rpm for 15min), the serum was stored in 1.5 mL Eppendorf tubes at −20˚C for subsequent biochemical analysis.

3.2. Biochemical Analysis

Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) and estradiol (E_2) were determined using a commercial ELISA kit (Diagnosis Automation, Inc., Calabasas, USA). Total protein (Tp); Alkaline phosphatase (ALP); Aspartate transaminase (AST); Alanine transaminase (ALT); Cholesterol (Chol); Urea (Urea) and Creatinine (Crea) were determined using commercial kits (Diamond Diagnostics, Halliston, MA, USA) and its designated protocol.

3.3. Reproductive Parameters

3.3.1. Gonadosomatic Index and Fecundity Calculation

Each female broodstock were weighed using an electronic scale (SF-400, China) to the nearest 0.1 g and, the abdomen was cut with a pair of scissors and the ovaries were extracted. The ovaries were washed in normal saline solution to remove blood and ovarian fluid. Thereafter, the ovaries were weighed using an electronic scale (METLAR 5000D) to the nearest 0.1 g. Gonadosomatic index (GSI) was calculated according to [38] as follows:

$$\textbf{Gonadosomatic index}\left(\textbf{GSI}\right)=\frac{\text{Gonad weight}}{\text{Total weight of fish}}\times 100$$

Sub-samples of the ovaries weighing 1 g were collected from each ovary from the anterior, posterior and mid part of the ovary. The sub-samples of the ovaries were fixed in Gibson fluid for 48 hours before counting the eggs. Fecundity was determined according to [38] as follows:

$$\textbf{Fecundity} = \text{Total number of eggs in 1 g of the ovary} \times \text{Ovary weight}\left(\text{g}\right).$$

3.3.2. Eggs Characteristics

100 eggs were randomly selected weighed, and preserved in 10% formalin for further egg biometric measurements. The diameter of ripped eggs was determined by using a simple ruler calibrated in millimeters scales.

3.3.3. Fertilization

2 g of eggs from each sample, containing approximately 2000 oocytes were measured into 12 separate containers. Eggs in each container were fertilized by mixing

them with diluted milt and subsequently activated with 100 mL of 0.9% saline solution. The saline was decanted after 5 minutes. Fertilized eggs were distributed for incubation across four breeding tanks (three tanks per treatment). Eggs were placed on a kakaban (shredded nylon sacks) to ensure a uniform monolayer spread and incubated in aerated indoor concrete tanks with the water level set at 30 cm^3 depth. Six hours after incubation, the color variations between the eggs were observed. Clear and transparent eggs were considered fertilized while dead/white and opaque ones were regarded as unfertilized (Dead eggs) and were siphoned out of the spawning tanks after 35 hours [39] [40].

The percentage fertilization was calculated by the average of five determinations of the fertilized eggs per 200 eggs siphoned based on color. Five different locations of the breeding tanks were marked and 200 eggs siphoned from each location and the white eggs recorded [39] [40]. The total larvae survival was determined at 10 days post-hatching. Percentage egg fertilization, hatchability and survival were determined as follows:

$$\textbf{\% Fertilization of eggs} = \frac{\text{No. of eggs fertilized}}{\text{total number of eggs}} \times 100$$

$$\textbf{Hatchability} = \frac{\text{number of hatchlings}}{\text{total number of fertilized eggs}} \times 100$$

% Hatchability was obtained by direct counting of unhatched eggs as well as the number of eggs hatched in each incubating tank,

% Survival was calculated during initial feeding according to [40] as the follows:

$$\textbf{\% Survival} = \frac{\text{Number of live larvae}}{\text{total number of larvae hatched}} \times 100$$

3.4 Statistical Analysis

Data collected were analyzed using one-way analysis of variance (ANOVA) to test for significant difference with the aid of predictive analytical software version 21.0. The probability level of ($p < 0.05$) was considered significant.

4. Results

4.1. Effects of *Azolla pinnata* Powder on Growth Performances in Female *C. gariepinus*

Table 3 shows the growth performance parameters of the experimental groups (T1-T4) subjected to different levels of *Azolla pinnata* powder substitution. Initial and final weights showed no significant differences among treatments ($p > 0.05$). Average gain weight, feed consumption, feed conversion and gonad weight rate increased with higher *Azolla pinnata* powder inclusion, peaking in T3 (30%). Food consumption varied across treatments, with the lowest intake recorded in T4. Feed conversion was high across all groups, with a slight decrease observed in T4 (0.90 ± 0.02). Table 3 shows that no significant difference was observed ($p > 0.05$) among the different growth characteristics evaluated.

Table 3. Effects of *Azolla pinnata* powder on Growth performances in Female *C. gariepinus*.

Parameters	*Azolla pinnata* powder (%)				p
	T1 = 0	T2 = 15	T3 = 30	T4 = 60	
Initial weight (g)	156.77 ± 3.74[a]	158.43 ± 1.25[a]	157.09 ± 1.80[a]	157.38 ± 2.63[a]	0.86
Final weight (g)	638.66 ± 3.21[a]	639.66 ± 1.52[a]	638.63 ± 1.70[a]	636.66 ± 3.05[a]	0.54
Gain weight (g)	481.89 ± 0.77[a]	481.23 ± 1.15[a]	481.54 ± 3.40[a]	479.28 ± 4.88[a]	0.73
Feed consumption	440.93 ± 6.65[a]	447.54 ± 1.52[a]	443.01 ± 4.72[a]	435.83 ± 5.56[a]	0.39
Feed conversion	0.91 ± 0.02[a]	0.93 ± 0.01[a]	0.92 ± 0.01[a]	0.90 ± 0.02[a]	0.43
Gonad weight (g)	33.00 ± 1.00[a]	33.03 ± 1.70[a]	33.66 ± 3.88[a]	29.43 ± 0.60[a]	0.15

The a, b and c: The means of each row marked with different letters are significantly different ($p < 0.05$): T1 (0% *Azolla pinnata* powder), T2 (15% (*Azolla pinnata* powder)), T3 (30% *Azolla pinnata* powder), T4 (60% *Azolla pinnata* powder).

4.2. Effects of *Azolla pinnata* Powder on Reproductive Performance in Female *C. gariepinus*

The effects of *Azolla pinnata* powder on Reproductive performances in Female *C. gariepinus* are shown in Table 4.

It can be deduced from this table that the fertilization and hatchability rates were significantly influenced ($p < 0.05$) by *Azolla pinnata* powder at doses of 60%. No significant difference was observed for gonadosomatic indexes, fecundity, diameters of oocytes and Survival larva rates. However, the group fed 60% *A. pinnata,* compared to other treatments, showed the lowest value for these parameters.

Table 4. Effects of *Azolla pinnata* powder on Reproductive performances in Female *C. gariepinus*.

Reproductive parameters	*Azolla pinnata* powder (%)				p
	T1 = 0	T2 = 15	T3 = 30	T4 = 60	
GSI	5.16 ± 0.13[a]	5.16 ± 0.26[a]	5.27 ± 0.60[a]	4.62 ± 0.11[a]	0.16
Fecundity (No of eggs)	7169.33 ± 391.15[a]	6919.33 ± 432.89[a]	7137.33 ± 483.86[a]	6261.67 ± 859.85[a]	0.04
Diameters Oocyte (mm)	2.06 ± 0.03[a]	2.02 ± 0.10[a]	2.03 ± 0.09[a]	1.90 ± 0.20[a]	0.03
Fertilization (%)	62.13 ± 7.23[a]	60.21 ± 1.83[ab]	61.00 ± 3.00[ab]	59.82 ± 0.72[b]	0.04
Hatchability (%)	81.61 ± 3.19[a]	79.25 ± 2.40[ab]	80.27 ± 2.98[ab]	75.19 ± 3.26[b]	0.01
Survival (14 days)	82.42 ± 2.86[a]	82.42 ± 1.05[a]	82.55 ± 2.89[a]	80.10 ± 0.37[a]	0.01

a, b and c: means of each row marked with different letters are significantly different ($p < 0.05$): T1 (0% *Azolla pinnata* powder), T2 (15% (*Azolla pinnata* powder)), T3 (30% *Azolla pinnata* powder), T4 (60% *Azolla pinnata* powder).

4.3. Effects of *Azolla pinnata* Powder on Reproductive Hormones in Female *C. gariepinus*

The effects of *Azolla pinnata* powder on reproductive hormones as shown in Table 5 reveal that, the serum levels of FSH and Estradiol decreased significantly ($p < 0.05$) in fish treated with 60% compared to the control group. The serum level of LH decreased significantly ($p < 0.05$) in fish treated with 60% compared to the control and T2 - T3 groups.

Table 5. Effects of *Azolla pinnata* powder on Reproductive hormones in Female *C. gariepinus*.

Reproductive Hormones	*Azolla pinnata* powder (%)				p
	T1 = (0)	T2 = 15	T3 = 30	T4 = 60	
FSH (mUI/ml)	1.74 ± 0.05[a]	1.60 ± 0.15[ab]	1.47 ± 0.1[ab]	1.43 ± 0.21[b]	0.05
LH (mUI/ml)	1.14 ± 0.01[a]	1.14 ± 0.08[a]	1.13 ± 0.05[a]	1.03 ± 0.06[b]	0.03
Estradiol (pg/ml)	151.81 ± 1.82[a]	150.62 ± 1.32[ab]	149.46 ± 0.55[ab]	148.58 ± 1.11[b]	0.05

a, b and c: means of each row marked with different letters are significantly different ($p < 0.05$): T1 (0% *Azolla pinnata* powder), T2 (15% (*Azolla pinnata* powder)), T3 (30% *Azolla pinnata* powder), T4 (60% *Azolla pinnata* powder).

4.4. Effects of *Azolla pinnata* Powder on Biochemical Parameters in Female fish *C. gariepinus*

Serum biochemical parameters of female fish *C gariepinus* fed diets with different levels of *Azolla pinnata* powder are shown in Table 6. The mean ALP, AST, ALT, Chol, Urea and Crea significantly ($p < 0.05$) increased with *A pinnata* powder levels in diets. Moreover, the significant ($p < 0.05$) and highest serum ALP, ALT, Chol, Urea and Crea were observed in broodstock fed with a 60% *A pinnata* powder diet. No significant difference ($p > 0.05$) was observed in the AST and TP of female *C gariepinus* fed different levels of *Azolla pinnata* powder.

Table 6. Effects of *Azolla pinnata* powder on Biochemicals parameters in Female *C. gariepinus*.

Biochemical parameters	*Azolla pinnata* powder (%)				p
	T1 = (0)	T2 = 15	T3 = 30	T4 = 60	
TP (g/l)	33.97± 0.76[a]	33.82 ± 0.90[a]	33.85 ± 0.62[a]	33.20 ± 0.55[a]	0.58
Chol (mg/dl)	153.33 ± 0.21[a]	152.80 ± 1.29[ab]	151.89± 0.35[ab]	151.21± 0.59[b]	0.01
ALP (µ/l)	13.98 ± 0.13[b]	14.26 ± 0.05[ab]	14.26 ± 0.46[ab]	14.42 ± 0.54[a]	0.05
AST(µ/l)	59.91± 0.72[a]	61.32± 0.17[a]	61.79± 0.02[a]	62.28 ± 0.19[a]	0.35
ALT(µ/l)	22.28 ± 0.59[b]	22.86 ± 1.47[ab]	23.01 ± 1.64[ab]	24.76 ±1.66[a]	0.05
Urea (mg/dl)	16.63 ± 0.59[b]	17.14 ± 1.06[ab]	17.54 ± 1.64[ab]	18.37± 2.16[a]	0.04
Crea (mg/dl)	0.23 ± 0.59[b]	0.25 ± 1.06[b]	0.30 ± 1.64[ab]	0.33 ± 2.16[a]	0.05

a, b and c: means of each row marked with different letters are significantly different ($p < 0.05$): T1 (0% *Azolla pinnata* powder), T2 (15% (*Azolla pinnata* powder), T3 (30% *Azolla pinnata* powder), T4 (60% *Azolla pinnata* powder).

5. Discussion

Due to the nutritional significance of plant meals and the unsustainable production of Soybean meal, more research has been conducted to evaluate the utility of plant meals as Soybean meal replacers in fish diets [35] [36]. Among these plant meals, only limited research has been conducted to evaluate the effects of the replacement of soybean meal with *Azolla pinnata* powder on the growth and reproductive parameters of *C. gariepinus*. The results of the present study showed that 30% of *A pinnata* can replace soybean meal in diets of *C. gariepinus* without any negative impacts on growth performance, which might be due to the presence of

high crude protein content and adequate levels of essential amino acids in *A pinnata* powder, that meets the requirements of *C. gariepinus*. Similar to the present study, [41] also reported that 50% *Azolla microphylla* can effectively substitute soybean meal in the conventional diet of *C. gariepinus*, without any adverse effects on their growth rate. These results also support the previous findings that up to 40% level of *Azolla* inclusion significantly increased the growth of carps [42] [43]. Similar to this result, a high growth performance and high survival rate in *Labeo fimbriatus*, *Labeo rohita,* and *Cirrhinus mrigala* were reported by [33] [40], Gangadhar *et al.* [44] and [32] at 40% *Azolla* meal diets. However, the variation in inclusion rates of *Azolla* powder may be due to fish species, *Azolla* quality, fibre content, and anti-nutritional factors. Analysis of *A. pinnata* established that it has trypsin inhibitors, which are well-known anti-nutritional factors [45]. In aquaculture, fish feeds are among the important factors affecting feed conversion rate, whereas others are diet composition, culture practices, fish health, genetics and feeding environment [46]. Fibre, which comprises the polymeric cellulose, forms complex inhibitors which not only affect palatability but also hinder enzymatic digestion, absorption and assimilation of nutrients into the fish's metabolism Opiyo *et al.* [47] [48]. The growth performance of a fish is controlled by its metabolism. Therefore, factors which adversely affect metabolism slow down the growth rate of fish. Fish are monogastric with simple stomachs and have reduced capacity to digest the cellulose present in the *A. pinnata* biomass [49] [50]. Opiyo *et al.* [47] indicated that lower feed intake, reduced digestibility and nutrient utilization are associated with increased dietary duckweed in O. niloticus fed on macrophytes-formulated diets.

Reproductive performance parameters, namely fecundity, egg diameters, fertilization and hatchability, declined with an increase in *A. pinnata* feed inclusion level. Similar to this result, a high reproductive performance in *O. niloticus* was reported by [51] at 20% duckweed meal diets. This is attributed to an increase the content of anti-nutritional factors as the % *A. pinnata* feed inclusion level increases. This anti-nutritional factor may have interfered with the fish's nutrition hence retarding reproductive performance. According to [52], anti-nutritional factors are natural plant compounds that can harm the health and productivity of livestock by either blocking nutrient absorption or causing toxicity. The present study showed a significant ($p < 0.05$) decrease in the serum levels of FSH, LH and Estradiol in catfish treated with 60% *A. pinnata* compared to other groups. Rinchard *et al.* [53] observed the same result in tilapia fed more than 50% of soybean meal. Decrease the serum level of FSH, LH and Estradiol in the female Cath-fish fed with more than 50% *A. pinnata* diet in this study could be attributed to anti-nutritional factors like tannins, flavonoids and alkaloids contained in this powder. Herbal extracts and plant secondary metabolites are known to alter fish fertility through changes in endocrine regulation, either by physiologically upgrading or pathologically suppressing reproductive status [45] [54]. Specifically, some phytochemicals can enhance reproductive output by down-regulating estradiol concen-

tration via the inhibition of aromatase (cyp19) or by reducing the bioconversion of testosterone to estradiol [55]. Conversely, other phytochemicals may inhibit vitellogenesis by binding to the estrogen receptor, effectively blocking the action of estradiol [56].

Serum biochemical parameters are recognized as valuable tools for monitoring the metabolic and physiological status of fish [57]. Accordingly, this study explored several key biochemical parameters to infer the health conditions of the experimental fish fed *A. pinnata* powder. In fish, proteins are major energy sources critical for maintaining blood glucose levels [58]. The observed lower total serum protein content in fish receiving the 60% *A. pinnata* diet may be attributed to the anti-nutrient load in these high-inclusion diets. This finding is consistent with reports of decreased serum total protein in *Channa punctatus* induced with stem-bark extract [59]. Furthermore, the slight elevation in serum AST in the 60% *A. pinnata* group could be a result of protein degradation and subsequent utilization for metabolic purposes. Ultimately, the higher values of ALP, ALT, Chol, Urea, and Creatinine Crea observed at the 60% *A. pinnata* inclusion level likely indicate a significant metabolic burden associated with the increased levels of anti-nutrients at this higher inclusion rate. The observation agreed with that of [60], who reported elevated ALT, AST and ALP Activities in fish fed a 30% *M. olefera* leaf meal diet. Transaminases are important enzymes for monitoring the health status of fish [61] and leak out into the bloodstream from dying or damaged liver cells. Elevated levels of transaminases in the blood serum of fish are generally linked to damaged or dying liver cells, while a decrease may suggest enzyme leakage into the serum [62] [63]. The latter effect (leakage/decrease) was observed in our study, indicating potential physiological changes at certain inclusion levels.

6. Conclusion

Based on the results obtained, *Azolla pinnata* powder can safely and economically replace 30% of the soybean meal in the diet of female African Catfish (*Clarias gariepinus*) broodstock without any negative effects on their health status or the development of their offspring. We therefore recommend replacing 30% of the soybean meal with dried *Azolla pinnata* powder in female *C. gariepinus* broodstock diets. This substitution offers a viable strategy to reduce feed production costs and increase overall profitability. Due to the anti-nutritional factors limiting *Azolla*'s use, our future studies will investigate the effect of treated *Azolla pinnata* powder on female African catfish broodstock.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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