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Nutritional Implications of Substituting Soybean Meal with Azolla Powder on Growth Performance, Feed Efficiency, Body Composition, Blood constituents, and Economic Aspects in Nile Tilapia Fingerlings (*Oreochromis niloticus*)

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ABSTRACT

This experiment evaluated the effects of replacing soybean meal with Azolla powder (AP) at levels of 0, 25, 50, 75, and 100% in the diet, equivalent to 0, 10, 20, 30, and 40% of the total feed formulation (D1, D2, D3, D4, and D5, respectively). A total of 150 fish with an average initial weight of 15.5 ± 0.799g were distributed into 15 aquariums (10 fish each). The results indicated that AP contained 20.01% CP, 14.32% CF, 3.24% EE, 3792 kcal/kg DM GE, and 264.71 kcal/kg DM ME. All experimental diets were iso-caloric and iso-nitrogenous. Growth parameters such as FW, TBWG, ADG, SGR, and RGR improved, particularly in the 25% and 50% AP groups. Survival was 100%, and no mortality was observed. Feed intake, FCR, protein intake, and PER increased significantly ($P<0.05$). Hematological parameters improved, while liver enzymes, glucose, and lipid profiles decreased. Fish body composition analysis showed increased moisture, CP, and ash contents, while DM, OM, EE, and energy contents decreased. Energy retention and PPV% improved significantly ($P<0.05$). Additionally, feed costs were reduced, with optimal Azolla inclusion levels between 10-20%, corresponding to the replacement of 25-50% of soybean meal.

Keywords: Azolla powder, Nile tilapia, Productive performance, Feed utilization, Fish body composition, Blood constituents, Economical evaluation.

1. Introduction

Aquaculture plays a vital role as a key source of animal protein, making it increasingly important to expand this sector for long-term food security (Ahmad *et al*., 2021; Osmundsen *et al*., 2020; Riverter *et al*., 2020). Nevertheless, in highly intensive farming environments, the yield and profitability of aquaculture remain below the threshold required for sustainable operations (Nguyen *et al*., 2021). To address this challenge, there is a growing need to adopt innovative, non-conventional technologies and optimize the use of existing resources to enhance production efficiency (Hisano *et al*., 2021). As reported by Dawood *et al*. (2019a), Nile tilapia (*Oreochromis niloticus*) is a freshwater fish species known for its ease of cultivation, disease resistance, ability to thrive in tropical developing and developed countries. Amin *et al*. (2019) and Dawood *et al*. (2019b) emphasized that improving tilapia production and quality depends largely on the availability of effective feed, which plays a vital role in growth. Sithara and Kamalaveni (2008) noted that the high cost of fish feed necessitates finding alternative ingredients that are cost-effective, accessible, and environmentally sustainable. Aquatic plants, commonly regarded as waste, have potential as feed ingredients for fish farming. According to Nekoubin *et al*. (2013), some aquatic weeds like *Lemna minor*, *Azolla pinnata*, and *Salvinia molesta* can be used as alternative feed for tilapia. Among them, Azolla, rich in plant protein, has a superior nutrient profile compared to other aquatic plants, as indicated by Sudaryono (2006), Mithraja *et al*.

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(2011), Das *et al*. (2018), and Mosha (2018). Studies have shown that 10-45% Azolla can be included in tilapia diets, depending on the species (Abou *et al*., 2007; Djissou *et al*., 2017; Youssouf, 2012). Furthermore, Maity and Patra (2008) highlighted that *Azolla pinnata*, growing in association with the blue-green algae Anabaena azollae, offers excellent nutritive value and productivity. Basak *et al*. (2002) and Magouz *et al*. (2020) also identified Azolla as a good protein source, rich in essential amino acids. Anitha *et al*. (2016) further added that Azolla is naturally high in minerals and vitamins, and contains probiotics and biopolymers. In Egypt, where feed costs represent 70% of total farming expenses, the high price of fish meal has reduced profitability in tilapia farming. Therefore, this study was conducted at the Fish Laboratory of the Animal Production Department, Biological Agriculture Research Institute, National Research Centre. The purpose of the experiment was to evaluate the effects of replacing soybean meal with Azolla powder at various levels (0%, 25%, 50%, 75%, and 100%) on the growth performance, blood parameters, energy retention (ER), protein productive value (PPV), and economic efficiency in Nile tilapia (*Oreochromis niloticus*).

2.1. Experimental Unit

150 Nile tilapia fingerlings (mono-sex) with initial weight of 15.5±0.79g, were acclimated and then randomly divided into 15 aquariums. Each aquarium (80×40×30 cm) with a 60-liter capacity housed 10 fish, resulting in three replicate tanks per experimental group.

2.2. Experimental Diets

Soybean meal in the control diet, which constituted 40% of the formulation, was replaced by Azolla powder (AP) at five levels: 0%, 25%, 50%, 75%, and 100%. These replacement levels correspond to AP inclusion rates of 0%, 10%, 20%, 30%, and 40% in the formulated diets for groups D1, D2, D3, D4, and D5, respectively, as outlined in Table 1.

Table 1: Composition of the different experimental diets.

Item	0% Control	Replace SBM by AP at different levels				Price of tone LE
		25% AP	50 % AP	75 % AP	100% AP	
	D_1	D_2	D_3	D_4	D_5	
	Composition of tested diets					
Soybean meal (44%)	40.00	30.00	20.00	10.00	00.00	23000
Azolla powder (AP)	00.00	10.00	20.00	30.00	40.00	11000
Protein concentration (56%)	17.00	22.00	27.00	32.00	37.00	16000
Yellow corn (8%)	28.00	27.00	25.00	21.00	17.00	9500
Wheat bran (13%)	10.00	6.00	3.00	2.00	1.00	11500
Vegetable oil	3.00	3.00	3.00	3.00	3.00	30000
Salt (sodium chloride)	1.00	1.00	1.00	1.00	1.00	2500
Vitamin and Minerals*	1.00	1.00	1.00	1.00	1.00	20000
Price of ton fed (LE)	16855	15900	14965	14070	13175	----
Price of kg fed (LE)	16.855	15.900	14.965	14.070	13.175	----

SBM: soy bean meal. AP: Azolla powder
** Vit. A (E672) (IU) 876.19, Vit. D3 (IU) 1141.39, Vit. E 114.30, Vit. K3 7.55, Vit. B1 13.71, Vit. B2 11.44, Vit. B6 15.33, Vit. B12 0.03, Niacin 60.96, Calpan 30.48, Folic Acid 3.04, Biotin 0.37, Vit. C 11.44, Selenium 0.27, Manganese 19.04, Iron 9.15, Iodine 0.77, Zinc 76.19, Copper 3.04, Cobalt 0.37.
Price of tone LE According to 2021.

The experimental diets were continuous for 56 days and tested diets were hand-fed for 56 days extended approximately from middle of February 2021 to middle April, 2021.

2.3. Parameters of growth performance

Body weight gain = Final weight - Initial weight.
Survival rate = Number of fish at final / Number of fish at start x100.
Specific growth rate = (In final weight (g) **-** In initial weight (g)) / Experimental days *100
Feed conversion ratio = Dry matter intake (g) / Body weight gain (g).
Protein efficiency ratio = Body weight gain(g) / Crude protein intake ((g).

Protein productive value = $[PR_1 - PR_0 / PI]$ 100.
PR_1 = Body protein at experimental end.
PR_0 = Body protein at the experimental starting.
PI = Protein intake.

Energy retention percentages = $E\text{-}E_0 / E_F$ X 100
Where: E= the energy in fish carcass (kcal) at the end of the experiment.
E_0 = Fish energy in carcass (kcal) at the experiment starting.
E_F = Energy feed intake(kcal).

2.4. Blood Sampling

Blood was collected from the caudal vein of 15 fish using a 3 ml syringe after anesthesia with clove oil (0.5 ml L^{-1}). The samples were placed in clean, dry centrifuge tubes and allowed to clot at room temperature. Afterward, they were centrifuged at 3000 rpm /15min. The serum was separated, at −20°C, and later used for biochemical analysis.

2.5. Body Composition

At the start of the experiment, 10 fish were used to determine their initial body composition, while at the end, five fish from each treatment group were randomly selected for body composition analysis.

2.6. Analytical Procedures

The chemical composition of the diets and the fish body was analysed according to AOAC (2016).

2.7. Biochemical Assays

Serum total protein was measured as per the methods of Armstrong and Carr (1964), and Cannon *et al*. (1974), while albumin was evaluated following the procedures of Doumas *et al*. (1971). Globulin was assessed following the protocols of Reitman and Frankel (1957). Hemoglobin was determined using methods from Gupta *et al*. (2008), while glucose and triglycerides were measured based on Caraway and Watts (1987) and Fossati and Prencipe (1982). Red blood cells and white blood cells were counted as per Weiss and Wardrop (2010), and alkaline phosphatase activity was measured following Beliefield and Goldberg (1971). Uric acid and creatinine were analyzed using Tietz (1990) procedures. All measurements were performed using Spectrum-diagnostics biochemical kits (Egypt) and analyzed with an Agilent Cary UV-Vis spectrophotometer.

2.8. Energy and Protein Calculations

The gross energy (kcal/kg DM) of the diets and body composition was calculated using Blaxter (1968) and MacRae and Lobley (2003) standards, assigning 5.65 kcal for each gram of protein, 9.40 kcal for fat, and 4.15 kcal for carbohydrates. Metabolizable energy (ME) was calculated based on NRC (2011).

2.9. Statistical Analysis

Data were analyzed using one-way ANOVA (SPSS 2020), and Duncan's Multiple Range Test (1955) was employed to compare the means.

3. Results

The chemical composition analysis in Table 2 shows that Azolla powder contained 20.01% CP, 14.32% CF, 3.24% EE, 3792 kcal/kg DM gross energy, 264.71 kcal/kg DM metabolizable energy, and a protein energy ratio of 75.59 mg CP/kcal ME. Chemical analysis of different experimental diets were showed in Table 3.

Table 2: Chemical analysis of Azolla powder (AP) and other feed ingredients

Item	Feed ingredients				
	Azzolla powder	Soy bean meal	Yellow corn	Wheat bran	Protein concentration
Moisture	10.00	9.50	9.77	9.96	3.05
Dry matter (DM)	90.00	90.50	90.23	90.04	96.95
Chemical analysis on DM basis					
Organic matter (OM)	80.05	93.39	98.34	94.64	93.22
Crude protein (CP)	20.01	44.00	8.00	13.00	56.00
Crude fiber (CF)	14.32	3.69	2.48	8.56	2.84
Ether extract (EE)	3.24	2.83	3.75	3.81	1.55
Nitrogen free extract (NFE)	42.48	42.87	84.11	69.27	32.93
Ash	19.95	6.61	1.66	5.36	6.68
Gross energy kcal/ kg DM	3792	4684	4398	4323	4794
Gross energy cal/ g DM	3.792	4.684	4.398	4.323	4.794
Metabolizable energy kcal/ kg DM	264.71	370.68	360.11	331.30	379.56
Protein energy ratio (mg CP/ Kcal ME)	75.59	118.70	22.22	39.24	147.54

Gross energy (kcal/ kg DM) was calculated according to Blaxter (1968); MacRae and Lobley (2003).
Metabolizable energy and protein energy ratio were Calculated according to NRC (2011).

Table 3: Chemical analysis of different experimental diets

Item	Zero% AP Control	Replace SBM by AP at different levels			
		25% AP	50% AP	75% AP	100% AP
	D1	D2	D3	D4	D5
Moisture	8.18	9.70	9.40	9.12	8.83
Dry matter	91.82	90.30	90.60	90.88	91.17
DM basis					
Organic matter	93.28	91.84	90.37	88.81	87.28
Crude protein	30.66	30.68	30.71	30.86	31.01
Crude fiber	3.51	4.34	5.25	6.27	7.29
Ether extract	5.82	5.75	5.69	5.57	5.55
Nitrogen free extract	53.29	51.07	48.72	46.11	43.43
Ash	6.72	8.16	9.63	11.19	12.72
Gross energy kcal/ kg DM	4637	4573	4510	4441	4379
Gross energy cal/ g DM	4.637	4.573	4.510	4.441	4.379
Metabolizable energy kcal/ kg DM	371.39	363.16	354.60	345.19	336.35
Protein energy ratio (mg CP/ Kcal ME)	82.55	84.48	86.60	89.40	92.20

AP: Azolla powder. SBM: Soybean meal.
Gross energy (kcal/ kg DM) was calculated according to Blaxter (1968); MacRae and Lobley (2003).
Protein energy ratio (mg CP/ Kcal ME): Calculated according to NRC (2011).

3.1. Growth Performance and Survival Rate

Table 4 reveals that incorporating Azolla powder in diets improved final weight (FW), total body weight gain (TBWG), and average daily gain (ADG). The best values ($P<0.05$) were observed in fish groups fed D2 and D3 diets (replacing 25% and 50% of soybean meal with Azolla). ADG improved by 46% and 59%, respectively, in D2 and D3, compared to the control group (D1). Specific growth rate (SGR) and relative growth rate (RGR) were also significantly higher ($P<0.05$) in D2 and D3. All groups recorded a 100% survival rate, with no mortality observed.

Table 4: Growth performance, Specific growth rate and survival ratio of different experimental groups.

Item	Zero% AP Control	Replace SBM by AP at different levels				SEM	Sign. P<0.05
		25% AP	50% AP	75% AP	100% AP		
	D_1	D_2	D_3	D_4	D_5		
Number of fish	30	30	30	30	30	-	-
Initial weight, g/ 10 fish	155	156	153	154	157	0.799	NS
Final weight, g / 10 fish	320^{b}	398^{a}	415^{a}	333^{b}	328^{b}	10.85	*
Total body weight gain, g	165^{b}	242^{a}	262^{a}	179^{b}	171^{b}	10.99	*
Experimental period				56 days			
Average daily gain, g	2.95^{b}	4.32^{a}	4.68^{a}	3.20^{b}	3.05^{b}	0.196	*
Specific growth rate	0.57^{c}	0.73^{b}	0.78^{a}	0.60^{c}	0.53^{d}	0.026	*
Relative growth rate	1.08^{c}	1.55^{b}	1.72^{a}	1.17^{c}	0.98^{d}	0.077	*
Number of fish at the starter	30	30	30	30	30	-	-
Number of fish at the end	30	30	30	30	30	-	-
Survival ratio (SR)	100	100	100	100	100	-	-

AP: Azolla powder. SBM: Soybean meal.
a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).
SEM: Standard error of mean NS: Not significant *: Significant at (P<0.05).

3.2. Feed utilization of the different experimental groups

The data in Table 5 indicates that feed intake, feed conversion ratio (FCR), crude protein intake, and protein efficiency ratio (PER) significantly increased (P<0.05) when Azolla powder replaced 25% or 50% of the soybean meal in the diet (D2 and D3). These values were notably higher compared to the control diet (D1) and other experimental diets (D4 and D5) with different levels of Azolla inclusion. This suggests that partial replacement of soybean meal with Azolla powder (specifically at 25% and 50%) enhances the fish's feed utilization efficiency.

Table 5: Feed utilization of the different experimental groups.

Item	Zero% AP Control	Replace SBM by AP at different levels				SEM	Sign. P<0.05
		25% AP	50% AP	75% AP	100% AP		
	D_1	D_2	D_3	D_4	D_5		
Total body weight gain, g	165^{b}	242^{a}	262^{a}	179^{b}	171^{b}	10.99	*
Feed intake, g	528.36^{b}	595.95^{a}	604.80^{a}	538.8^{6b}	521.64^{b}	9.790	*
Feed conversion ratio	3.20^{b}	2.46^{a}	3.31^{a}	3.01^{b}	3.05^{b}	0.102	*
Feed crude protein %	30.66	30.68	30.71	30.86	31.01	-	-
Crude protein intake, g	162.00^{b}	282.85^{a}	185.73^{a}	166.29^{b}	161.76^{b}	2.908	*
Protein efficiency ratio	1.019^{b}	1.323^{a}	1.411^{a}	1.076^{b}	1.057^{b}	0.045	*

AP: Azolla powder. SBM: Soybean meal.

3.3. Blood parameters of the different experimental groups

The results presented in Table 6 shows that replacing soybean meal with Azolla powder at varying levels (from 25% to 100%) in fish diets had a significant (P<0.05) impact on most blood parameters, with the exception of globulin levels, which were not significantly affected (P>0.05). The values of total protein, albumin, albumin-to-globulin ratio, hemoglobin, RBCs, and WBCs were significantly (P<0.05) improved compared to the control group. Additionally, the dietary treatments led to a significant (P<0.05) reduction in AST, ALT, glucose, triglycerides, cholesterol, alkaline phosphatase, uric acid, and creatinine levels.

Table 6: Blood parameters of the different experimental groups.

Item	Zero% AP Control	Replace SBM by AP at different levels 25% AP	50% AP	75% AP	100% AP	SEM	Sign. P<0.05
	D_1	D_2	D_3	D_4	D_5		
Total protein (g/dl)	5.41^{c}	5.46^{bc}	5.49^{bc}	5.56^{ab}	5.66^{a}	0.028	*
Albumin (g/dl)	2.86^{c}	2.92^{bc}	2.99^{b}	3.12^{a}	3.19^{a}	0.035	*
Globulin (g/dl)	2.55	2.54	2.50	2.44	2.47	0.017	NS
Albumin: globulin ratio	1.12^{c}	1.15^{bc}	1.20^{b}	1.28^{a}	1.29^{a}	0.020	*
AST (Unit/l)	244^{a}	231^{b}	225^{bc}	220^{c}	216^{c}	2.876	*
ALT (Unit/l)	106^{a}	99^{ab}	94^{b}	92^{b}	90^{b}	1.925	*
Hemoglobin (g/dl)	11.65^{c}	11.88^{b}	11.96^{ab}	12.03^{ab}	12.11^{a}	0.049	*
Glucose (mg/dl)	90.14^{a}	92.36^{b}	95.30^{c}	96.17^{d}	96.31^{d}	0.648	*
Triglycerides (mg/dl)	91.16^{a}	89.11^{b}	86.30^{c}	84.10^{d}	83.33^{d}	0.812	*
Cholesterol (mg/dl)	103^{a}	98^{ab}	94^{bc}	91^{c}	88^{c}	1.595	*
RBC's X10^{6} / mm^{3}	2.26^{e}	2.43^{d}	2.89^{c}	3.00^{b}	3.14^{a}	0.091	*
WBC's X10^{3} / mm^{3}	52^{d}	59^{c}	63^{c}	70^{b}	77^{a}	2.405	*
Alkaline phosphatase (U/I)	7.86^{a}	7.05^{b}	6.36^{c}	6.13^{d}	5.92^{e}	0.190	*
Uric acid (mg/dl)	9.15^{a}	8.73^{b}	8.41^{c}	7.87^{d}	7.82^{d}	0.136	*
Creatinine (mg/dl)	0.73^{a}	0.61^{b}	0.55^{b}	0.48^{c}	0.44^{c}	0.028	*

AP: Azolla powder. SBM: Soybean meal.
RBC's: Red blood cell count WBC's: White blood cell count AST: Aspartate aminotransferase.
ALT: Alanine aminotransferase.

3.4. Fish body composition of different experimental groups

The data in Table 7 demonstrate that Nile tilapia fed diets containing Azolla powder showed a significant increase in body composition parameters, including moisture, crude protein (CP), and ash content, while dry matter (DM), organic matter, ether extract, and gross energy content were significantly lower compared to control.

Table 7: Fish body composition of initial and different experimental groups that fed tested diets.

Item	Fish body composition of initial fish	Zero% AP Control	Replace SBM by AP at different levels 25% AP	50% AP	75% AP	100% AP	SEM	Sign. P<0.05
		D_1	D_2	D_3	D_4	D_5		
Moisture	80.41	68.76^{c}	78.76^{a}	78.27^{a}	74.74^{b}	74.31^{b}	0.973	*
Dry matter	19.59	31.24^{a}	21.24^{c}	21.73^{c}	25.26^{b}	25.69^{b}	0.973	*
		Chemical analysis on DM basis						
Organic matter	82.15	86.90^{a}	83.46^{b}	80.60^{c}	80.44^{c}	77.31^{d}	0.887	*
Crude protein	65.17	56.69^{c}	61.60^{ab}	62.25^{a}	59.02^{bc}	56.75^{c}	0.713	*
Ether extract	16.98	30.21^{a}	21.86^{b}	18.38^{c}	21.42^{b}	20.56^{b}	1.105	*
Ash	17.85	13.10^{d}	16.54^{c}	19.40^{b}	19.56^{b}	22.69^{a}	0.887	*
Gross energy kcal/ 100g	527.82	604.27^{a}	553.52^{b}	524.48^{c}	534.81^{c}	513.90^{d}	8.603	*
Gross energy cal/ g DM	5.2782	6.0427^{a}	5.5352^{b}	5.2448^{c}	5.3481^{c}	5.1390^{d}	0.086	*

AP:Azolla powder. SBM: Soybean meal.
Gross energy was calculated according to (**Blaxter1968; MacRae and Lobley 2003**).

3.5. Regarding energy retention (ER%) and protein productive value (PPV%)

Regarding energy retention (ER%) and protein productive value (PPV%), as illustrated in Table 8, replacing 25% or 50% of soybean meal in the control diet with Azolla powder (D2 and D3) significantly enhanced ER% compared to control (D1) and other groups (D4 and D5). Specifically, ER% improved by 11.17% and 10.16% for D2 and D3, respectively, over the control. Additionally, partial or complete replacement of soybean meal with Azolla powder resulted in a significant (P<0.05) increase in PPV%, with values rising by 58.13%, 72.09%, 16.54%, and 4.41% for D2, D3, D4, and D5, respectively, in comparison to control (D1).

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Table 8: Energy retention and protein productive value % of different experimental groups.

Item	Zero% AP Control	Replace SBM by AP at different levels				SEM	Sign. P<0.05
		25% AP	50% AP	75% AP	100% AP		
	D_1	D_2	D_3	D_4	D_5		
Initial weight (IW), g	155	156	153	154	157	0.799	NS
Final weight (FW), g	320^{b}	398^{a}	415^{a}	333^{b}	328^{b}	10.85	*
Energy retention							
Energy content in final body fish (cal / g)	6.0427^{a}	5.5352^{b}	5.2448^{c}	5.3481^{c}	5.1390^{d}	0.086	*
Total energy at the end in body fish (E)	1934^{b}	2203^{a}	2177^{a}	1781^{c}	1686^{c}	57.79	*
Energy content in initial body fish (cal / g)	5.2782						
Total energy at the start in body fish (E_0)	818	823	808	813	829	4.193	NS
Energy retained in body fish ($E-E_0$)	1116^{b}	1380^{a}	1369^{a}	968^{c}	857^{c}	58.57	*
Energy of the feed intake (Cal / g feed)	4.637	4.573	4.510	4.441	4.379	-	-
Quantity of feed intake	528.36^{b}	595.98^{a}	604.80^{a}	538.86^{b}	521.64^{b}	9.79	*
Total energy of feed intake (EF)	2450^{b}	2725^{a}	2728^{a}	2393^{b}	2284^{c}	49.59	*
Energy retention (ER) %	45.55^{ab}	50.64^{a}	50.18^{a}	40.45^{bc}	37.52^{c}	1.537	*
Productive value (PPV) %							
Crude protein % in final body fish	56.69^{c}	61.60^{ab}	62.25^{a}	59.02^{bc}	56.75^{c}	0.713	*
Total protein at the end in body fish (PR_1)	181.41^{c}	245.17^{a}	258.34^{a}	196.54^{b}	186.14^{bc}	8.687	*
Crude protein % in initial body fish	65.17						
Total protein at the start in body fish (PR_2)	101.01	101.67	99.71	100.36	102.32	0.521	NS
Protein Energy retained in body fish (PR_3) = ($PR_1 - PR_2$)	80.40^{d}	143.50^{b}	158.3^{a}	96.18^{c}	83.82^{cd}	8.761	*
Crude protein in feed intake (CP %)	30.66	30.68	30.71	30.86	31.01	-	-
Total Protein intake (PI), g	162.00^{b}	182.85^{a}	185.73^{a}	166.29^{b}	161.76^{b}	2.908	*
Protein productive value (PPV) %	49.63^{d}	78.48^{b}	85.41^{a}	57.84^{c}	51.82^{cd}	3.961	*

AP: Azolla powder. SBM: Soybean meal.

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3.6. Economical evaluation of different experimental groups

The economic evaluation results shown in Table 9 indicate that incorporating Azolla powder in place of soybean meal in the feed formulations led to a reduction in feed costs. The cost per kilogram of feed decreased from 16.855 LE in the control diet (D1) to 15.900, 14.965, 14.070, and 13.175 LE for diets D2, D3, D4, and D5, respectively. Additionally, net improvement in profitability was observed, with increases of 21.82%, 3.03%, 4.97%, and 3.68% for D2, D3, D4, and D5, respectively, compared to control, which was fed a diet without Azolla powder.

Table 9: Economical evaluation of different experimental groups.

Item	Zero% AP Control	Replace SBM by AP at different levels			
		25% AP	50% AP	75% AP	100% AP
Costing of kg feed (LE)	16.855	15.900	14.965	14.070	13.175
Relative to control (%)	100	94.33	88.79	83.48	78.17
Feed conversion ratio (FCR)	3.20	2.46	3.31	3.01	3.05
Feeding cost (LE) per (Kg weight gain)	53.94	39.11	49.53	42.35	40.18
Relative to control (%)	100	72.51	91.82	78.51	74.49
Net improving in feeding cost (%)	Zero	21.82	3.03	4.97	3.68

AP: Azolla powder. SBM: Soybean meal. LE.: Egyptian pound
Diet formulation calculated according to the local prices at year 2021as presented in (Table 1)
Feed cost (L.E) FCR×FI. Cost per Kg diet

4. Discussion

Our study revealed significant improvements in growth performance and survival rates across various experimental groups. Specifically, the values for final FW, TBWG, and ADG were notably enhanced. The most substantial improvements ($P<0.05$) were observed in groups where 25% and 50% of soybean meal in the control diet was substituted with Azolla powder. The average daily gain (ADG) increased by 46% and 59% in these groups compared to the control group (D1). Additionally, SGR and relative growth rate (RGR) also showed significant increases ($P<0.05$) in D2 and D3 compared to the control and other groups (D4 and D5). Survival ratio (SR) was uniformly high at 100%, with no recorded mortality across all tested groups. FI, FCR, PI, and PER improved significantly when Azolla powder replaced 25% or 50% of soybean meal in the diet. Magouz *et al*. (2020) similarly reported no decline in final body weight, weight gain, and SGR with up to 20% Azolla in tilapia diets. The optimal level of Azolla for maximizing growth may depend on various factors such as inclusion levels, administration periods, experimental conditions, and the species and size of fish. Magouz *et al*. (2020) noted a significant decrease in growth performance with 30% Azolla inclusion due to high fiber and anti-nutritional factors (ANFs) in Azolla, which adversely affects feed efficiency and growth (Kamali-Sanzighi *et al*., 2019; Li *et al*., 2012). Similarly, high Azolla levels may increase metabolic rate and energy expenditure while decreasing ingredient digestibility due to its ANF content (e.g., tannins, catechin monomers, caffeine) (Habib *et al*., 2014; Ahmed *et al*., 2017; Mohammadi *et al*., 2018). Sotolu *et al*. (2013) also found that Azolla affects the emulsion interface and interacts with digestive enzymes, reducing feed utilization and growth.

In terms of blood parameters, replacing soybean meal with Azolla powder at various levels (25% to 100%) significantly ($P<0.05$) affected most parameters, except for globulin levels which remained unaffected ($P>0.05$). Notably, total protein, albumin, albumin: globulin ratio, hemoglobin, red blood cells (RBCs), and white blood cells (WBCs) improved significantly ($P<0.05$) compared to the control. Conversely, values for AST, ALT, glucose, triglycerides, cholesterol, alkaline phosphatase, uric acid, and creatinine decreased significantly ($P<0.05$). As Authman *et al.,* (2021) described, biochemical parameters are valuable for evaluating fish health and the impact of dietary additives. Magouz *et al.* (2020) found that feeding Azolla to tilapia maintained normal hematological and biochemical functions, except for a significant increase in RBC count at 20% Azolla. Other studies also support the use of hematological and biochemical markers to assess nutritional effects on fish health (Burgos-Aceves *et al.,* 2019; Dawood *et al.,* 2016, 2019c, 2020a; Faggio *et al.,* 2014a,b).

Regarding body composition, diets containing Azolla powder led to significant increases in moisture, crude protein (CP), and ash contents, while decreasing dry matter (DM), organic matter (OM), ether extract (EE), and gross energy (GE) contents compared to the control. Micha *et al.* (1988) and El-

Sayed (1992) observed similar effects, noting that Azolla substitution significantly influenced body composition, with increased moisture and ash contents but decreased protein and lipid contents. Ali and El-Feky (2019) and Abo-State *et al.* (2021) found no significant differences in body moisture, ether extracts, and ash when prebiotics or oligosaccharides were used in Nile tilapia diets.

Economic evaluations showed that replacing soybean meal with Azolla powder reduced feed costs from 16.855 LE per kg in the control diet to 13.175 LE per kg in the highest Azolla inclusion diet. This resulted in net cost reductions of 21.82%, 3.03%, 4.97%, and 3.68% for the respective Azolla diets (D2, D3, D4, and D5). Tharwat (1999) highlighted that feed costs, comprising about 50% of total production costs, were significantly reduced with Azolla. Similarly, Abo-State *et al.* (2021) reported that mannan oligosaccharide and β-glucan supplements improved economic efficiency. Azolla proved to be a cost-effective alternative to soybean meal, offering substantial savings without significantly impacting fish size or growth performance up to a 25% replacement level.

5. Conclusion

In conclusion, Azolla powder shows promise as a cost-effective alternative to soybean meal in fish diets, offering significant improvements in growth performance, survival rates, and economic efficiency, while also positively influencing blood parameters and body composition. However, care should be taken to avoid high levels of Azolla that may negatively impact growth due to its high fiber and ANF content. on the results, replacing soybean meal with Azolla powder at various levels improved average daily gain, feed conversion, energy retention percentage (ER%), and protein productivity value percentage (PPV%). Additionally, it reduced feeding costs and increased cost efficiency. The optimal Azolla inclusion levels derived from the study was corresponding to a replacement of 25% of the soybean meal used in the control diet formulation.

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