Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(5): 559 – 578 (2024)
www.ejabf.journals.ekb.eg

Impact of Inclusion Ashwagandha (*Withania somnifera*) Root Powder as a Growth Promoter in the Diet of the Nile tilapia (*Oreochromis niloticus*) Fingerlings

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ARTICLE INFO
Article History:
Received: Aug. 19, 2024
Accepted: Sept. 7, 2024
Online: Sep. 9, 2024

Keywords:
Ashwagandha root powder,
Nile tilapia fish,
Productive performance,
Feed utilization,
Fish body composition,
Blood constituents

ABSTRACT

The ashwagandha (*Withania somnifera*) root powder (ARP) was assessed as a dietary supplement to enhance growth in the Nile tilapia (*Oreochromis niloticus*) fingerlings. The study involved incorporating ARP at levels of 0, 1.5, 3, and 4.5% into the diets, corresponding to 0, 15, 30, and 45g/ kg in diets D1, D2, D3, and D4, respectively. A total of 180 fish, each with an initial body weight of 10.36± 0.821g, were distributed across 12 aquariums, with 15 fish per aquarium. The results indicated that ARP contains 4.21% CP, 34.90% CF, 0.32% EE, with gross energy of 4032kcal/ kg DM, and metabolizable energy of 216.33kcal/ kg DM. All diets tested were isocaloric and isonitrogenous. Fish fed with ARP demonstrated improvements in final weight (FW), total body weight gain (TBWG), average daily gain (ADG), and specific growth rate (SGR), while survival rates (SR) were 100%, and mortality was zero across all groups. Feed conversion ratio (FCR) significantly improved with higher ARP inclusion. Additionally, ARP inclusion led to reduced levels of AST, ALT, cholesterol, and LDL in the blood. Fish body composition, including moisture, organic matter (OM), crude protein (CP), ether extract (EE), and gross energy content, showed significant enhancement, whereas dry matter (DM) and ash content decreased. Energy retention and protein productive value (PPV) also significantly increased. The findings suggest that ARP can be an effective growth promoter in the diets of the Nile tilapia without adverse effects on growth performance or blood parameters.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is widely recognized as a leading species in global aquaculture, primarily due to its rapid growth, adaptability to diverse farming conditions, and robust disease resistance **(Soliman & Yacout, 2016; Gabriel, 2019; Zahran *et al.*, 2020)**. However, the species is vulnerable to certain pathogens, with *Streptococcus iniae* (*S. iniae*) identified as a particularly severe bacterial threat, causing mortality rates of 30-75% in affected populations **(Shoemaker *et al.*, 2010)**.

Ashwagandha (*Withania somnifera*), often referred to as "Indian Ginseng," is a subtropical perennial shrub with significant medicinal properties. Esteemed in Ayurveda, it is known for enhancing vitality and longevity **(Singh & Kumar, 1998; Singh *et al.*, 2014; Tiwari *et al.*, 2014)**. The primary bioactive components in ashwagandha extracts include alkaloids (isopellertierine and anferine) and steroidal lactones, such as withanolides **(Gupta *et al.*, 2007; Mirjalili *et al.*, 2009)**. These compounds, along with saponins like sitoindoside VII and VIII, contribute to its wide-ranging pharmacological effects, particularly those derived from the plant's roots **(Malik *et al.*, 2007)**.

Research has shown that ashwagandha contains over 35 chemical constituents, including flavonoids, tannins, and reducing sugars, and is rich in iron. Its diverse properties include immunomodulatory, anti-inflammatory, antioxidant, and anti-stress effects **(Singh *et al.*, 1982; Sivaram *et al.*, 2004; Mirjalili *et al.*, 2009)**. These attributes make it a promising candidate for inclusion in aquaculture diets, as it may improve fish health and performance **(Girish *et al.*, 2006; Kumar *et al.*, 2015)**.

Previous studies have highlighted the importance of immunological and hematological assessments in fish as indicators of health and environmental stress. Such indices are crucial for evaluating the physiological impacts of dietary supplements **(Luskova, 1997; Shalaby & Abbas, 2005; Abbas *et al.*, 2007)**. Notably, the incorporation of medicinal plants like ashwagandha has shown positive effects on growth performance, feed efficiency, and hematological parameters in various species **(; Khobragade, 2003; Akotkar *et al.*, 2007)**. Therefire, this study aimed to assess the effects of dietary ashwagandha at varying levels (0%, 1.5%, 3%, and 4.5%) on the Nile tilapia. The research focused on its impact on growth performance, feed utilization, body composition, blood parameters, energy retention (ER), and protein productive value (PPV).

MATERIALS AND METHODS

This study was conducted at the Fish Laboratory of the Animal Production Department, Agriculture and Biological Research Institute, National Research Center. The research aimed to evaluate the effects of incorporating ashwagandha (*Withania somnifera*) root powder (ARP) at various levels on the growth performance, feed utilization, blood parameters, body composition, and energy retention (ER %) and protein productive value (PPV %) of the fingerling Nile tilapia (*Oreochromis niloticus*).

Experimental unit

A total of 180 fingerlings of the Nile tilapia, each with an initial body weight of 10.36± 0.821g, were acclimated and then randomly assigned to experimental aquariums. The fish were distributed among 12 aquariums, with 15 fish in each, averaging 155.5± 0.453g. The aquariums, each with dimensions of 80×40×30cm and a capacity of 60 liters, housed the fish in replicated groups.

Experimental diets

Ashwagandha root powder (ARP) was incorporated into the diets at four different levels: Zero, 1.50, 3.00, and 4.50%. These levels correspond to 0, 15, 30, and 45g /kg of diet for D1, D2, D3, and D4, respectively, as detailed in Table (1). The experimental diets were administered continuously for 56 days, from approximately mid-February 2024 to mid-April 2024, with the diets being hand-fed throughout this period.

Table 1. Composition of the different experimental diets

Ingredient	Experimental diets			
	Control Zero ARP*	1.5 % ARP*	3 % ARP*	4.5 % ARP*
	D1	D2	D3	D4
Composition of tested diets				
Ashwagandha root powder (ARP).	0.00	1.50	3.00	4.50
Soybean meal (SBM), (44% CP)	41.00	40.50	39.75	39.00
Protein concentration (56% CP)	16.00	16.50	17.25	18.00
Ground yellow corn (8% CP)	28.00	28.00	28.00	28.00
Wheat bran (13% CP)	10.00	8.50	7.00	5.50
Vegetable oil	3.00	3.00	3.00	3.00
Salt (sodium chloride)	1.00	1.00	1.00	1.00
Vitamins and minerals mixture**	1.00	1.00	1.00	1.00

* ARP: Ashwagandha root powder.

** Vitamins and minerals mixture: Each kg of vitamin-mineral mixture (Agrimin Forte; Virbac Animal Health India Pvt. Ltd., Mumbai, India) contained: vitamin a 700000 IU, vitamin D3 70000 IU, vitamin E 250 mg, nicotinamide 1000 mg, cobalt 150 mg, copper 1200 mg, iodine 325 mg, iron 1500 mg, magnesium 6000 mg, potassium 100 mg, sodium 5.9 mg, manganese 1500 mg, sulphur 0.72%, zinc 9600 mg, calcium 25.5%, and phosphorus 12.75%.

Parameters of growth performance

Body weight gain (BWG) = Final weight - Initial weight.

Survival rate (SR %) = Number of fish at final / Number of fish at start x100.

Specific growth rate (SGR) =

[In final weight (g) - In initial weight (g)] / Experimental days *100

Calculation of feed conversion ratio (FCR)

FCR = Total dry matter intake, (TDMI), g / Total body weight gain (TBWG), g.

Calculation of crude protein efficiency ratio (CPER)

(PER) = Total body weight gain (TBWG), g / Total crude protein intake (TCPI), g.

Feed efficiency

Feed efficiency (FE %) = [Weight gain (g) / Feed intake (g)]

Protein productive value (PPV %) = [PR_1– PR_0 / PI] 100.

Where, PR_1 = is the total fish body protein at the end of the experiment.

PR_0 = is the total fish body protein at the start of the experiment.

PI = Protein intake.

Blood sampling

Blood samples were drawn from the caudal vein of the fish using a 3ml syringe after the fish were anesthetized with clove oil (0.5ml/ L). The samples were placed in clean, dry centrifuged tubes and left at room temperature to clot. After clotting, the samples were centrifuged at 3000rpm for 15min. The serum was then separated, collected, and stored at -20°C until further biochemical analysis.

Body composition

Initially, 12 fish were used, and at the end of the study, 7 fish from each treatment group were randomly selected to assess their whole body composition.

Analytical procedures

The analysis of the tested diets and fish body composition was conducted following **AOAC (2016)** guidelines.

Biochemical assays

Serum total proteins were measured according to **Armstrong and Carr (1964)**, **Cannon *et al*. (1974)**, and **Witt and Trendelenburg (1982)**. Albumin levels were assessed using the methods described by **Doumas *et al*. (1971)**, **Tietz (1986)**, and **Tietz (1990)**. Globulin levels were calculated by subtracting albumin concentration from total protein concentration, and the albumin/globulin (A/G) ratio was determined by dividing albumin values by globulin values. Glucose levels were measured according to **Caraway and Watts (1987)**. The red blood cell (RBC) and white blood cell (WBC) counts were estimated based on the study of **Weiss and Wardrop (2010)**. Blood hemoglobin was assessed using the methods described by **Gupta *et al*. (2008)**, **Bunn (2011)** and **Elghetany and Banki (2011)**. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined following the guidelines of **Reitman and Frankel (1957)** and **Harold (1975)**. Alkaline phosphatase activity was measured according to **Beliefield and Goldberg (1971)**. Triglycerides were quantified using the method described by **Fossati and Prencipe (1982)**, while total cholesterol was measured according to **Allain *et al*. (1974)**, **Ellefson and Caraway (1976)** and **Pisani *et al*. (1995)**. High-density lipoprotein-cholesterol (HDL-C) was estimated following **Assmann (1979)**, and low-density lipoprotein-cholesterol (LDL-C) was measured according to **McNamara *et al*. (1990)**. All biochemical analyses were performed using commercial kits from Spectrum-diagnostics (Egypt), and colorimetrically analyzed with an Agilent Cary UV-Vis spectrophotometer (100/300 Series) according to the manufacturer's instructions.

Calculated data

The gross energy (kcal/kg DM) of the experimental diets and the body composition of the tested fish were calculated using the methods of **Blaxter (1968)** and **MacRae and Lobley (2003)**. Energy values used were 5.65 kcal per gram of crude protein (CP), 9.40 kcal per gram of ether extract (EE), and 4.15 kcal per gram of crude fiber (CF) and nitrogen-free extract (NFE). Metabolizable energy (ME) was determined according to **NRC (2011)** with energy values of 4.50 kcal per gram of protein, 8.15 kcal

per gram of fat, and 3.49 kcal per gram of carbohydrate. The protein energy ratio (mg CP/kcal ME) was also calculated following **NRC**'s **(2011)** guidelines.

Statistical analysis

The collected data were analyzed using the one-way analysis of variance (ANOVA) as per **SPSS (2020)**. Duncan's multiple range test **(Duncan, 1955)** was employed to differentiate between the means.

RESULTS

Chemical analysis of the ingredients and the experimental diets

The data presented in Table (2) show that ashwagandha root powder (ARP) contains 4.21% CP, 34.90% CF, 0.32% EE, 4032 kcal/kg DM gross energy (GE), 216.33kcal/ kg DM metabolizable energy (ME), and a protein energy ratio of 19.46mg CP/kcal ME.

Table (3) indicates that all experimental diets were iso-caloric and iso-nitrogenous. Specifically, CP percentages ranged from 30.27 to 30.66% across the four tested diets. Gross energy values ranged from 4465 to 4477kcal/ kg DM, while metabolizable energy values ranged from 352.15 to 357.27kcal/ kg DM. Additionally, the protein energy ratio varied from 85.82 to 850.96mg CP/kcal ME among the four diets. These values were deemed adequate and suitable to meet the nutritional needs of the Nile tilapia.

Table 2. Chemical analysis of ashwagandha root powder (ARP) and the other feed ingredients

Item	Feed ingredients				
	ARP	**SBM**	**Yellow corn**	**Wheat bran**	**Protein concentration**
Moisture	7.45	9.50	9.77	9.96	3.05
Dry matter (DM)	92.55	90.50	90.23	90.04	96.95
					Chemical analysis on DM basis
Organic matter (OM)	95.24	93.39	98.34	94.64	93.22
Crude protein (CP)	4.21	44.00	8.00	13.00	56.00
Crude fiber (CF)	34.90	3.69	2.48	8.56	2.84
Ether extract (EE)	0.32	2.83	3.75	3.81	1.55
Nitrogen free extract (NFE)	55.81	42.87	84.11	69.27	32.93
Ash	4.76	6.61	1.66	5.36	6.68
Gross energy kcal/ kg DM	4032	4684	4398	4323	4794
Gross energy cal/ g DM	4.032	4.684	4.398	4.323	4.794
Metabolizable energy kcal/ kg DM	216.33	370.68	360.11	331.30	379.56
Protein energy ratio (mg CP/ Kcal ME)	19.46	118.70	22.22	39.24	147.54

*ARP: Ashwagandha root powder. SBM: Soybean meal.

Gross energy (kcal/ kg DM) was calculated according to (Blaxter1968; MacRae and Lobley 2003). Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal. Metabolizable energy (ME): Calculated using values of 4.50, 8.15 and 3.49 Kcal for

protein, fat and carbohydrate, respectively. Calculated according to (NRC 2011). Protein energy ratio (mg CP/ Kcal ME): Calculated according to (NRC 2011).

Table 3. Chemical analysis of different experimental diets

Item	Experimental diets			
	Control Zero ARP*	**1.5 % ARP***	**3 % ARP***	**4.5 % ARP***
	D1	**D2**	**D3**	**D4**
Moisture	8.07	8.03	7.99	7.95
Dry matter (DM)	91.93	91.97	92.01	92.05
	Chemical analysis on DM basis			
Organic matter (OM)	93.24	93.25	93.26	93.28
Crude protein (CP)	30.66	30.53	30.40	30.27
Crude fiber (CF)	3.51	3.90	4.30	4.69
Ether extract (EE)	2.82	2.77	2.72	2.66
Nitrogen free extract (NFE)	56.25	56.05	55.84	55.66
Ash	6.76	6.75	6.74	6.72
Gross energy kcal/ kg DM	4477	4473	4469	4465
Gross energy cal/ g DM	4.477	4.473	4.469	4.465
Metabolizable energy kcal/ kg DM	357.27	355.58	353.85	352.15
Protein energy ratio (mg CP/ Kcal ME)	85.82	85.86	85.91	85.96

*ARP: Ashwagandha root powder. SBM: Soybean meal.

Gross energy (kcal/ kg DM) was calculated according to **Blaxter (1968)** and **MacRae and Lobley (2003)**. Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal.

Metabolizable energy (ME): Calculated using values of 4.50, 8.15 and 3.49 Kcal for protein, fat and carbohydrate, respectively. Calculated according to **NRC (2011)**. Protein energy ratio (mg CP/ Kcal ME): Calculated according to **NRC (2011)**.

Growth performance and survival ratio

Table (4) data reveal that the values for FW, TBWG, ADG, and SGR were significantly improved (P<0.05) when fish were fed diets containing 1.5, 3, and 4.5% ARP. The survival ratio (SR) was 100%, and there were no recorded mortality rates in any of the tested groups. Overall, the level of ARP inclusion in the diet significantly affected (P<0.05) the aforementioned parameters.

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

Item	Experimental diets				SEM	Sign. *P*<0.05
	Control Zero ARP*	1.5 % ARP*	3 % ARP*	4.5 % ARP*		
	D1	D2	D3	D4		
Number of fish	45	45	45	45	-	-
Initial weight, g (IW)(n=15)	153	155	156	158	0.821	NS
Final weight, g (FW)(n=15)	308^{d}	346^{c}	382^{b}	411^{a}	11.770	*
Total body weight gain, g (TBWG)	155^{d}	191^{c}	226^{b}	253^{a}	11.221	*
Duration experimental period	**56 days**					
Average daily gain, g (ADG)	2.77^{d}	3.41^{c}	4.04^{b}	4.52^{a}	0.201	*
Specific growth rate (SGR)	0.544^{d}	0.622^{c}	0.647^{b}	0.743^{a}	0.022	*
Number of fish at the starter	45	45	45	45	-	-
Number of fish at the end	45	45	45	45	-	-
Survival ratio (SR)	100	100	100	100	-	-
Number of dead fish	Zero	Zero	Zero	Zero	-	-
Mortality rate percentages	Zero	Zero	Zero	Zero	-	-

* ARP: Ashwagandha root 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).

Feed utilization of the different experimental groups

Data presented in Table (5) show that feed intake, feed conversion ratio (FCR), crude protein intake, and protein efficiency ratio (PER) significantly improved (*P*<0.05) with increasing the levels of ARP inclusion in the fish diets.

Table 5. Feed utilization of the different experimental groups

Item	Experimental diets				SEM	Sign. *P*<0.05
	Control Zero ARP*	1.5 % ARP*	3 % ARP*	4.5 % ARP*		
	D1	D2	D3	D4		
Total body weight gain, g (TBWG)	155^{d}	191^{c}	226^{b}	253^{a}	11.221	*
Feed intake (FI), g	514.50^{d}	550.62^{c}	582.12^{b}	609.42^{a}	10.863	*
Feed conversion ratio (FCR)	3.32^{d}	2.88^{c}	2.58^{b}	2.41^{a}	0.105	*
Feed crude protein %	30.66	30.53	30.40	30.27	-	-
Crude protein intake (CPI), g	157.75^{d}	168.10^{c}	176.96^{b}	184.47^{a}	3.068	*
Protein efficiency ratio (PER)	0.983^{d}	1.136^{c}	1.277^{b}	1.371^{a}	0.044	*

*ARP: Ashwagandha root 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. *: Significant at (*P*<0.05). FCR: Expressed as g of DM intake / g gain. PER: Expressed as g of g gain / g CP intake.

Blood parameters of the different experimental groups

The results presented in Table (6) indicate that incorporating ARP into fish diets significantly (P<0.05) affected all blood parameters except for albumin, which showed no significant change. Total protein, globulin, glucose, RBC count, WBC count, hemoglobin, triglycerides, and HDL-C levels were significantly higher (P<0.05) compared to the control. In contrast, dietary treatments significantly (P<0.05) reduced the albumin: globulin ratio, as well as levels of AST, ALT, alkaline phosphatase, cholesterol, and LDL-C.

Table 6. Blood parameters of the different experimental groups

Item	Experimental diets				SEM	Sign. P<0.05
	Control Zero ARP*	1.5 % ARP*	3 % ARP*	4.5 % ARP*		
	D1	D2	D3	D4		
Total protein (g/dl)	5.62^{d}	6.28^{c}	6.83^{b}	7.16^{a}	0.177	*
Albumin (g/dl)	1.24	1.23	1.22	1.21	0.006	NS
Globulin (g/dl)	4.38^{d}	5.05^{c}	5.61^{b}	5.95^{a}	0.180	*
Albumin: globulin ratio	0.28^{a}	0.24^{b}	0.22^{c}	0.20^{d}	0.009	*
Glucose (mg/dl)	46.13^{d}	51.38^{c}	57.14^{b}	62.20^{a}	1.826	*
RBC's $X10^{6}$ / mm^{3}	0.71^{b}	0.75^{ab}	0.79^{ab}	0.82^{a}	0.017	*
WBC's $X10^{3}$ / mm^{3}	35.00^{d}	48.00^{c}	57.00^{b}	65.00^{a}	3.456	*
Hemoglobin (g/dl)	1.72^{d}	2.15^{c}	3.46^{b}	4.88^{a}	0.374	*
AST (Unit/l)	293^{a}	286^{ab}	279^{b}	274^{b}	2.708	*
ALT (Unit/l)	118^{a}	113^{ab}	110^{bc}	107^{c}	1.409	*
Alkaline phosphatase (U/I)	8.81^{a}	7.16^{b}	6.33^{c}	5.12^{d}	0.405	*
Triglycerides (mg/dl)	110^{c}	119^{b}	127^{ab}	135^{a}	2.998	*
Cholesterol (mg/dl)	130^{a}	122^{ab}	116^{bc}	110^{c}	2.485	*
LDL-C (mg/dl)	65.00^{a}	61.00^{ab}	58.00^{ab}	55.00^{b}	1.420	*
HDL-C (mg/dl)	70.00^{d}	89.00^{c}	106.00^{b}	125.00^{a}	6.216	*

* ARP: Ashwagandha root 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).

RBC's: Red blood cell count WBC's: White blood cell count AST: Aspartate aminotransferase.

ALT: Alanine aminotransferase. HDL-C: High-density lipoprotein-cholesterol. LDL-C: Low-density lipoprotein-cholesterol

Fish body composition of different experimental groups

Table (7) reveales that the Nile tilapia fish fed diets containing ARP showed a significant (P<0.05) increase in the body composition parameters, including moisture, CP, OM, EE, and gross energy content. Conversely, dry matter (DM) and ash contents were significantly (P<0.05) reduced compared to the control group.

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

Item	Body composition of initial fish	Experimental diets				SEM	Sign. *P*<0.05
		Control Zero ARP*	1.5 % ARP*	3 % ARP*	4.5 % ARP*		
		D1	D2	D3	D4		
Moisture	75.12	71.39^{c}	71.48^{c}	71.65^{b}	71.81^{a}	0.175	*
Dry matter (DM)	24.88	28.61^{a}	28.52^{a}	28.35^{b}	28.19^{c}	0.175	*
Chemical analysis on DM basis							
Organic matter (OM)	84.67	86.9^{d}	87.80^{c}	89.09^{b}	89.88^{a}	1.186	*
Crude protein (CP)	61.26	62.78^{d}	63.17^{c}	63.88^{b}	64.23^{a}	0.600	*
Ether extract (EE)	23.41	24.17^{d}	24.63^{c}	25.21^{b}	25.65^{a}	0.590	*
Ash	15.33	13.05^{a}	12.20^{b}	10.91^{c}	10.12^{d}	1.186	*
Gross energy kcal/ 100g	566.17	581.91^{d}	588.43^{c}	597.90^{b}	604.01^{a}	8.909	*
Gross energy cal/ g DM	5.661	5.8191^{d}	5.8843^{c}	5.9790^{b}	6.0401^{a}	0.089	*

* ARP: Ashwagandha root 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 *: Significant at (*P*<0.05).

Gross energy (kcal/ kg DM) was calculated according to **Blaxter (1968)** and **MacRae and Lobley (2003)**. Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal.

Energy retention (ER)% and protein productive value (PPV)%

Results of ER% and PPV% that are shown in Table (8) were significantly (*P*<0.05) increased by the inclusion of different levels of ARP in the diets of the Nile tilapia fish compared to the control (D1). Specifically, ER% values were improved by 16.94, 33.95, and 45.14% for D2, D3, and D4, respectively, compared to the control. Similarly, PPV% increased significantly (*P*<0.05) by 16.43, 32.82, and 43.51% for D2, D3, and D4, respectively, relative to the control.

Table 8. Energy retention (ER) and protein productive value (PPV) % of different experimental groups

Item	Experimental diets				SEM	Sign. *P*<0.05
	Control Zero ARP*	1.5 % ARP*	3 % ARP*	4.5 % ARP*		
	D1	D2	D3	D4		
Initial weight (IW), g	153	155	156	158	0.821	NS
Final weight (FW), g	308^{d}	346^{c}	382^{b}	411^{a}	11.770	*
Calculation the energy retention						
Energy content in final body fish (cal / g)	5.8191^{d}	5.8843^{c}	5.9790^{b}	6.0401^{a}	0.026	*
Total energy at the end in body fish (E)	1792^{d}	2036^{c}	2284^{b}	2483^{a}	78.91	*
Energy content in initial body fish (cal /g)	5.6617					
Total energy at the start in body fish (E_0)	866^{b}	878^{ab}	883^{ab}	895^{a}	4.709	*
Energy retained in body fish (E-E_0)	926^{d}	1158^{c}	1401^{b}	1588^{a}	75.80	*
Energy of the feed intake (Cal / g feed)	4.477	4.473	4.469	4.465	-	-
Quantity of feed intake	514.50^{d}	550.62^{c}	582.12^{b}	609.42^{a}	10.86	*
Total energy of feed intake (EF)	2303^{d}	3463^{c}	2601^{b}	2721^{a}	47.86	*
Energy retention (ER) %	40.21^{d}	47.02^{c}	53.86^{b}	58.36^{a}	2.093	*
Calculation the protein productive value (PPV) %						
Crude protein % in final body fish	62.78^{d}	63.17^{c}	63.88^{b}	64.23^{a}	0.173	*
Total protein at the end in body fish (PR_1)	193.36^{d}	218.57^{c}	244.02^{b}	263.99^{a}	8.083	*
Crude protein % in initial body fish	61.26					
Total protein at the start in body fish (PR_2)	93.73	94.95	95.57	96.79	0.503	NS
Protein energy retained in body fish (PR_3) = (PR_1 – PR_2)	99.63^{d}	123.62^{c}	148.45^{b}	167.20^{a}	7.748	*
Crude protein in feed intake (CP %)	30.66	30.53	30.40	30.27	-	-
Total protein intake (PI), g	157.75^{d}	168.10^{c}	176.96^{b}	184.47^{a}	3.068	*
Protein productive value (PPV) %	63.16^{d}	73.54^{c}	83.89^{b}	90.64^{a}	3.171	*

* ARP: Ashwagandha root 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).

DISCUSSION

The growth performance and survival rates of the experimental groups revealed that fish fed diets with 1.50, 3.00, and 4.50% ARP showed significant improvements ($P<0.05$) in FW, TBWG, ADG, and SGR. Notably, all groups achieved a 100% survival rate (SR) with no recorded mortality. Additionally, there was a gradual and significant ($P<0.05$) enhancement in feed intake, feed conversion ratio (FCR), crude protein intake, and protein efficiency ratio (PER) with increasing the levels of ARP in the diet.

These findings are consistent with those of **Sharma *et al*. (2017)**, who observed significant growth rate increases in *L. rohita* fed with *Withania somnifera* (ashwagandha) at concentrations of 0, 0.1, 0.2, and 0.3g/ 100 g of feed over 42 days, except in the group receiving 0.3g/ 100g. Improvements in FCR were observed across all ashwagandha-supplemented groups compared to controls. Furthermore, the highest relative survival percentage (RSP) was recorded in the 0.2g/ 100g group (43.00± 0.75%) after a challenge with *A. hydrophila*, while the lowest RSP was noted in the 0.3g/ 100g group (10.00± 0.02%).

Our results also align with the work of **Sivaram *et al*. (2004)**, who reported that administering 100 and 200mg of ashwagandha per kg of feed resulted in improved weight gain, SGR, and FCR. Moreover, **Jayaprakas and Sindhu (1996)** suggested that the presence of certain steroids, such as ecdysteroids (20-hydroxyecdysone), in plant extracts may enhance growth by improving feed conversion efficiency. This mechanism may explain the enhanced growth observed in this study, as ashwagandha contains a variety of chemical constituents, primarily steroids and alkaloids, as described by **Rastogi and Mehrotra (1998)**.

The current findings align with those reported by **Kuldeep and Kumar (2020)**, who examined the effects of Ashwagandha supplementation at 0, 1, and 3% on growth rate, feed conversion ratio (FCR), condition factor, protein efficiency ratio (PER), and gross protein retention in *Labeo rohita*. They found that a diet containing 3% ashwagandha led to superior FCR, growth rate, condition factor, and protein retention compared to other treatments, with results that were significantly different ($P<0.05$). Although the diet with 1% ashwagandha also outperformed the control, it did not match the effectiveness of the 3% ashwagandha diet.

These results are consistent with those observed by **Rodehutscord and Pfeffer (1995)**, who found that the exogenous application of phytase improved FCR values. Conversely, **Forester *et al*. (1999)** reported no improvement in FCR when phytase was excluded. The ashwagandha-supplemented diet at 3% achieved the lowest FCR value (1.96/g body weight). **Liu (1997)** suggested that incorporating 3% of ashwagandha in the diet provided the best performance, potentially by mitigating anti-nutritional factors or adverse effects of phytase from plant-derived feed ingredients.

Kuldeep and Kumar (2020) also noted significant differences in PER among the different levels of ashwagandha supplementation. In the 3% Ashwagandha group, PER was recorded at 0.157 during the 8th week, whereas the 1% Ashwagandha group showed a significantly higher PER of 2.314 during the same period. The 1% Ashwagandha treatment recorded the best PER value (2.196), which was significantly different from the control. PER is a measure of how well the protein sources in the diet meet the essential amino acid requirements of the fish, and is associated with fat deposition in fish muscle. The PER values observed (0.57-2.314) suggest that this range could favor fat deposition in *Labeo rohita*, consistent with the findings of **Qamer *et al*. (2014)**.

Kuldeep and Kumar (2020) also demonstrated that enzyme supplementation in feed resulted in better protein retention compared to the control feed, which contained no enzyme. The highest protein retention (29.52%) was observed in the group fed with 3% Ashwagandha, followed by the 1% ashwagandha group (27.54%) and the control group (25.48%). The 3% Ashwagandha treatment showed significantly different results compared to the control, though there was no significant difference between the 1% and 3% ashwagandha treatments. Similarly, **Jana *et al*. (2006)** reported gross protein retention (GPR) values ranging from 28-31.05% in the milkfish (*Chanos chanos*).

Srivastava *et al*. (2020) observed the best FCR and PER values in the group that received a diet containing 2% of ashwagandha root powder (ARP) compared to other treatments and the control, indicating that ARP inclusion in feeds improves feed conversion to fish biomass. The positive effects of ashwagandha on fish growth and feeding efficiency have been documented in various species, including *L. rohita* **(Sahoo *et al*., 2006)**, the goldfish (*Carassius auratus*) **(Priyatharshini, 2008)**, the Mozambique tilapia (*O. mossambicus*) **(Immanuel *et al*., 2009)**, the common carp (*Cyprinus carpio*) **(Priyadarshini *et al*., 2012)** and the Nile tilapia (*O. niloticus*) **(Mukherjee *et al*., 2019)**. In addition to finfish, ashwagandha has also been shown to enhance the survival and growth in the shellfish such as the tiger shrimp (*Penaeus monodon*) **(Babu *et al*., 2008)**. These studies collectively support the hypothesis that dietary inclusion of ashwagandha enhances immune status and consequently improves the growth performance of both the finfish and shellfish, as reported by **Srivastava *et al*. (2020)**.

The results from the blood parameter analysis indicated that incorporating ARP into the fish diet had a significant (P<0.05) effect on nearly all blood parameters, except albumin, which was not significantly impacted. Significant (P<0.05) increases were observed in total protein, globulin, glucose, RBC count, WBC count, hemoglobin, triglycerides, and HDL-C compared to the control group. Conversely, the dietary treatment significantly (P<0.05) reduced the albumin-to-globulin ratio, AST, ALT, alkaline phosphatase, cholesterol, and LDL-C levels. Biochemical parameters serve as effective indicators of fish health and the impact of dietary additives, as noted by **Authman *et al*. (2021)**.

These findings are consistent with those of **Sharma *et al*. (2017)**, who studied the effects of ashwagandha at various concentrations (0, 0.1, 0.2, and 0.3g/ 100g of feed) on blood parameters in *Labeo rohita* over a 42-day period. Their study found that hemoglobin content and total leukocytes were significantly (P<0.05) elevated in all groups receiving ashwagandha compared to the control. Total erythrocyte count was significantly (P<0.05) higher in the group fed 0.2g of ashwagandha/100g of feed compared to the control, and hematocrit values were significantly (P<0.05) higher in the groups fed 0.2 g and 0.3g of ashwagandha/ 100g of feed. They also observed significant changes in the proportions of neutrophils, monocytes, and lymphocytes with no significant effect on thrombocytes. The study reported a higher proportion of lymphocytes in the groups fed 0.1 and 0.2g Ashwagandha/100g of feed compared to the control, but a lower proportion in the 0.3g Ashwagandha group. Additionally, the proportion of monocytes and neutrophils was significantly higher in all ashwagandha-fed groups, with the proportion of monocytes nearly doubling in these groups, except for the 0.3g ashwagandha group. **Sharma *et al*. (2017)** also noted that serum total protein content was significantly different from the control only in the 0.2g ashwagandha group, which recorded the highest protein content. All levels of ashwagandha supplementation significantly increased globulin content, while serum albumin levels showed no significant differences. The albumin-to-globulin ratio (A/G) was significantly affected by ashwagandha in the experimental groups. Blood glucose levels were significantly higher in the 0.1 and 0.2g ashwagandha groups compared to the control. There were no significant differences in serum ALT, AST activity, or alkaline phosphatase levels between the treatment groups and the control.

The findings of this study suggest that ashwagandha enhances immune response, consistent with previous studies **(Aboelhassan *et al*., 2024)**. For example, **Sivaram *et al*. (2004)** reported significant improvements in immune parameters such as serum bactericidal activity, serum globulin levels, and the albumin-to-globulin ratio in greasy grouper (*Epinephelus tauvina*) fed with dietary ashwagandha. The increase in serum total protein and globulin contents observed in this study likely reflects a strong innate immune response, as mentioned by **Wiegertjes *et al*. (1996)**. The highest protein and globulin concentrations were recorded in groups fed ashwagandha at different levels, with a notable increase in globulin and a decrease in the A/G ratio.

In this study, the significant increase in blood glucose levels with ashwagandha supplementation at various levels indicates that stress handling results in higher glucose demand by fish tissues, triggering muscle or liver glycogenolysis to release glucose for increased energy needs during and after stress, as reported by **Eslamloo *et al*. (2014)**. The assessment of enzyme activities, such as serum ALT and AST, is valuable in evaluating clinical and experimental liver damage, as mentioned by **Ali *et al*. (2021)**. The significant (P<0.05) decrease in ALT and AST levels in this study can be attributed to

ashwagandha's tendency to restore these marker enzymes to near-normal levels, as reported by **Udayakumar *et al.* (2009)**.

Additionally, **Gupta *et al.* (2008)** observed that the supplementation of ashwagandha in the diet led to an increase in erythrocytes leukocytes, hematocrit values, and hemoglobin content, indicating an improvement in fish health. **Della Porta *et al.* (2023)** supported these findings, noting that the dietary ashwagandha increased erythrocyte count, leukocyte count, hemoglobin content, and hematocrit value in humans. **Menezes *et al.* (2006)** highlighted that WBCs, as the first line of defense, play a major role in innate immunity, and their increased numbers in this study suggest ashwagandha's role in enhancing innate immunity. Ashwagandha's potential in disease prevention may be related to its impact on the immune system. **Lerose *et al.* (2024)** demonstrated that withaferin A (glycowithanolides) in ashwagandha possesses potent antioxidant properties, effectively scavenging superoxide radicals, which may offer protection against auto-toxicity and lethality. **Sharma *et al.* (2010)** further supported this by noting that Ashwagandha's effect was enhanced by the non-specific immunological defense mechanisms, such as lysosomal enzyme activity secreted by activated macrophages and phagocytic activity in *L. rohita*.

Feeding the Nile tilapia with diets enriched with ARP significantly ($P<0.05$) enhanced the fish's body composition, including increases in moisture, CP, OM, CP, EE, and gross energy content. Concurrently, there was a significant ($P<0.05$) reduction in DM and ash content compared to the control. These findings align with those of **Srivastava *et al.* (2020)**, who reported that adding ashwagandha root powder (ARP) at levels of 0, 1, 2, and 3% to the diet improved the flesh quality of *L. rohita* fingerlings. Specifically, they found the highest total protein content at 2% ARP inclusion and maximum lipid and ash content at 3% ARP inclusion. In general, ARP supplementation significantly ($P\leq0.05$) improved the flesh quality of *L. rohita*. Although data on ashwagandha's impact on flesh quality in finfish are limited, its known antioxidant properties, anti-stress effects, and ability to stimulate thyroid and protein synthesis, as demonstrated by **Tiwari *et al.* (2014)**, likely contribute to these benefits.

In contrast, **Ali and El-Feky (2019)** and **Abo-State *et al.* (2021)** observed no significant differences in whole-body moisture, ether extracts, and ash content when prebiotics or mannan oligosaccharide and β-glucan were included in commercial diets for the Nile tilapia fingerlings.

The study also revealed that different levels of ARP in the Nile tilapia diets significantly ($P<0.05$) improved ER% and PPV% compared to the control (D1). Specifically, ER% increased by 16.94, 33.95, and 45.14% for D2, D3, and D4, respectively, compared to the control. Similarly, PPV% rose significantly ($P<0.05$) by 16.43, 32.82, and 43.51% for D2, D3, and D4, respectively, over the control (D1). These findings are consistent with **Abo-State *et al.* (2021)**, who reported significant ($P<0.05$) differences in PPV and ER% among treatments, with the highest values observed in fish

fed diets supplemented with mannan oligosaccharide (MOS) and β-glucan at 2 and 4g/ kg, followed by those supplemented with 6g/ kg. They also noted no significant ($P>0.05$) differences in PPV and ER% across various MOS and β-glucan levels.

CONCLUSION

The results of this study indicate that under the experimental conditions, the inclusion of ashwagandha root powder (ARP) at different levels improved growth performance, feed utilization, and feed conversion ratio in the Nile tilapia. It also significantly ($P<0.05$) reduced blood parameters such as AST, ALT, cholesterol, and LDL. Furthermore, fish body composition, including moisture, OM, CP, EE, and gross energy content, improved, while DM and ash content decreased. Additionally, energy retention (ER%) and protein productive value (PPV%) increased.

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