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Impact of *Withania somnifera* (Ashwagandha) as an exogenous growth promoter in the diet of fingerlings of *Labeo rohita* in Tarai region

Kuldeep Singh Rana and Avdhesh Kumar

Abstract
The present investigation was conducted to assess the effect of different doses of Ashwagandha supplemented feed in *Labeo rohita* on growth rate, feed conversion ratio, condition factor, protein efficiency ratio and gross protein retention. Experiment comprised of 3 treatments *viz*. Diet containing Ashwagandha @1%, 3% and control diet with no supplementation. Results revealed that diet containing 3% Ashwagandha resulted in better FCR, growth rate, condition factor and higher protein retention. The results obtained with this diet were superior to other treatments and significantly different ($P<0.05$), however the diet containing 1% Ashwagandha also yielded better results than the control diet but not as good as diet containing 3% Ashwagandha.
All the physicochemical parameters were within the optimum range as desired for fish culture practices. Any kind of adverse effects due to Ashwagandha on the water quality parameters, behavioral and feeding response of fish were not encountered during the period of investigation.

Keywords: Ashwagandha, *Labeo rohita*, Growth rate

Introduction
The present status of aquaculture industry possesses challenges and opportunities for improvement of production to meet the growing demands in coming years. This demand may be fulfilled by increasing the quality of aqua feeds through improving nutrient content as well as digestibility of low quality feed by the use of efficient feed additives.
Withania somnifera, is commonly known as Ashwagandha, Indian ginseng, Winter cherry, Ajagandha, Kanaje (Hindi), Amukkara (Tamil) and Samm Al Ferakh (Urdu), it belongs to family Solanaceae. The main constituents of ashwagandha are alkaloids and steroidal lactones. Among the various alkaloids withanine is the main constituent. The other alkaloids are somniferine, somnine, somniferinine, withananine, pseudo withanine, tropine, pseudo tropine, cuscohygrine, anferine and anhydrine. Two acyl steryl glucoside *viz*. sitoindoside VII and sitoindoside VIII have been isolated from root (Kumar A. 2000) [10]. The leaves contain steroidal lactone, which is commonly called withanolide which has C28 steroidal nucleus with C9 side chain, having six membered lactone ring**.** (Mowrey, D.1986).
The experimental fish, *Labeo rohita* (rohu) is an indigenous fish species of family Cyprinidae and it is being used as an integral member of four or six species composite culture in the country. Supplementary feed forms the major input constituting over 50 percent of the recurring expenditure in grow out. Though many of plant origin ingredients have demerits on account of presence of antinutritional factors which have an adverse impact on the digestion and nutrient utilisation of feed however, certain enzymes provide an additional powerful tool that can inactivate antinutritional factors and enhance the nutritive value of plant based protein in feeds. These provide a natural way to transform complex feed components into digestible nutrients (Alikunhi, K. H. 1957) [1]. Judicious feed management therefore requires attention in

order to enhance the profit margin.

Hence there is need to develop the environment friendly technology, which would enhance the growth of fish, thereby all aquaculture production. Keeping in mind the prime need of natural plant based growth promoter in aquaculture practices, the present study is based on the following objectives.-

1. To study physicochemical parameters of water in order to assess the impact of Ashwagandha (*Withania somnifera*) on aquatic environment.
2. To study growth of fish fingerlings in order to assess growth promoting effect of Ashwagandha (*Withania somnifera*).

Materials and Methods

135 uniform sized fingerlings of same age group 10±2g weight, 9±2cm size were collected from the fish farm of College of Fisheries, GBPUA&T, Pantnagar, Uttarakhand. Fingerlings were acclimatized in aquaria sized 120X35X38 cm^3 at the indoor aquarium unit of the Department of Aquaculture. Three test diets T1, T2 with Ashwagandha and T3 as control were prepared from the locally available ingredients. Two levels of Aswagandha 1% and 3% were used during the study and fed to two experimental diets. Dry Ashwagandha root was collected from market and grinded in the mixer till the root becomes powder. Root powder was screened out by sieving. Diet T1 was prepared with 1% Ashwagandha supplementation, diet T2 with 3% Ashwagandha supplementation and diet T3 for control diet without supplementation for experimental units. Ashwagandha root powder was mixed well with the feed ingredients in the given percentage as follows:-

Ingredients	**:**	**Inclusion rate (%)**
Mustard oil cake	:	31.00
Rice polish	:	26.23
Soybean meal	:	15.90
Fish meal	:	4.95
Wheat flour	:	19.92
Vitamin and mineral mixture	:	2.00

Nine equal sized aquaria (120X35X38cm^3) were used and stocked with 15 fingerlings per aquarium. Three sets of aquaria with fishes fed on feed containing Ashwagandha @ 1% and 3% and 0% in control were maintained as experimental set up and each set had three replicates. Feeding was done @ 3% feed / kg body weight daily in each experiment after ascertaining the total group weight weekly. The experiment was conducted for a period of 60 days February 15 to April 15, 2018. Water was replaced with fresh oxygenated water and water quality parameters such as temperature, pH, dissolved oxygen, total alkalinity and free carbon-dioxide were monitored in each aquarium weekly. Fish sampling was done fortnightly. Analysis of physicochemical parameters was done by APHA -1985**.** Growth rate (g/day), feed conversion ratio, gross protein retention (GPR), protein efficiency ratio (PER) and condition factor were calculated. Experimental data was subjected to statistical analysis following the CRD and the variation among the treatments means was tested by significance of analysis of variance techniques as described by Gomez (1984) [6]. Level of significance used for F and T test were P=0.05 from the table given by Fisher. The critical difference of treatment means has been worked out.

Results and Discussion

1. FCR: Perusal of data (Table 1, Figure1) on FCR revealed significant differences among the treatments on FCR. Lowest FCR (1.69) was found in T2 during the 8th week. FCR has shown a decreasing trend right from the beginning of 2nd week up to end of 8th week. In the treatment means although better FCR (2.15) was achieved with T1 as compared to FCR recorded with control T3 (2.34) but T2 has shown the best FCR (1.96) which is significantly different. Exogenous application of enzyme resulted in improvement in FCR as compared to control. The reason could be fast metabolism which in turn resulted in better FCR. The present findings are similar with those of Rodehutscord and Pfeffer (1995) [12] who found better FCR values by the exogenous application of Phytase. Forester *et al.* (1999) [5] found that non inclusion of Phytase does not show any improvement in the FCR value. In T2 where Ashwagandha was supplemented @ 3% showed lowest FCR value (1.96/g body weight). This suggests that inclusion of Ashwagandha @ 3 % gave the best performance.

Table 1: Effect of Ashwagandha supplementation on FCR

Weeks Treatments		**2nd week**	**4th week**	**6th week**	**8th week**	**Mean**
T1	Feed supplemented with 1% Ashwagandha	2.41	2.23	2.05	1.90	2.15
T2	Feed supplemented with 3% Ashwagandha	2.25	2.08	1.82	1.69	1.96
T3	Control feed with no Ashwagandha	2.56	2.36	2.32	2.13	2.34
		Treatments				
	CD (P = 0.05)	0.27				

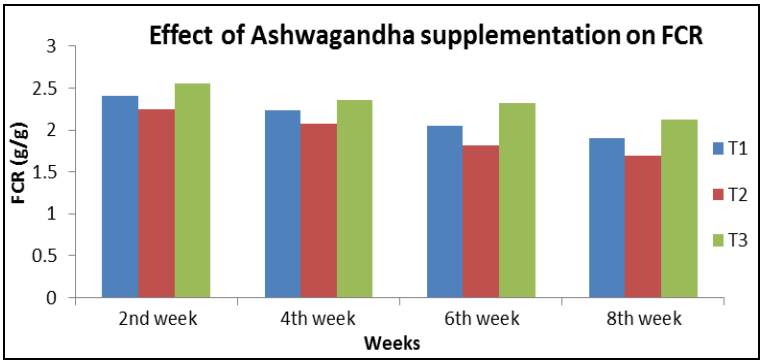

Fig 1: Effect of Ashwagandha supplementation on FCR

2. *Growth rate*

Different levels of Ashwagandha supplementation revealed significant difference among the various treatment means on growth. The highest growth rate (2.10g/day) was recorded in T2 which was significantly different from other treatments ($P<0.05$). However T1 and T3 were not significantly different from each other. All the above results are summarized in Table 2, Figure2.

The reduced growth rate in control treatment could be due to the presence of anti-nutritional factors in feed (Kakade *et al.* 1973) [9] which in turn have an adverse impact on growth performance and availability of various dietary compounds (Spinelli *et al.* 1983, Richardson *et al.*, 1985, Satoh *et al.* 1989) [14]. This suggests that Ashwagandha supplementation in the diet may be effective in reducing either anti-nutritional factors or adverse consequences of phytase from plant origin ingredients of feed which is supported by the findings of Liu (1997).

Table 2: Effect of Ashwagandha supplementation on Growth rate

Weeks Treatments		2nd week	4th week	6th week	8th week	Mean
T1	Feed supplemented with 1% Ashwagandha	1.4	1.47	1.51	1.65	1.51
T2	Feed supplemented with 3% Ashwagandha	1.82	1.92	1.98	2.10	1.96
T3	Control feed with no Ashwagandha	1.25	1.32	1.41	1.51	1.37
		Treatments				
	CD (P = 0.05)	0.41				

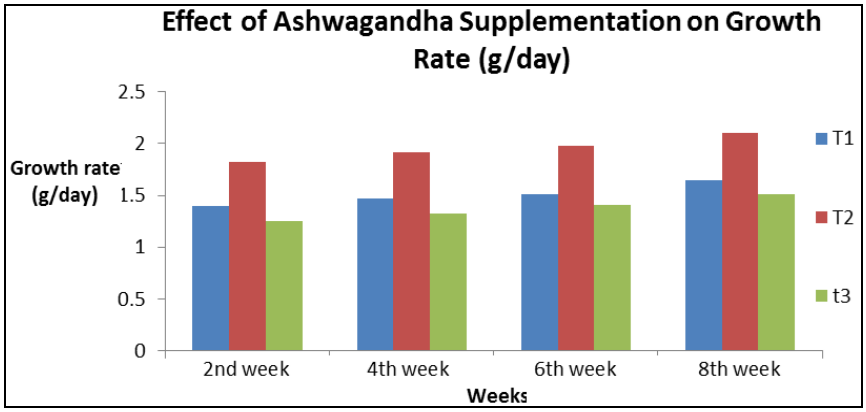

Fig 2: Effect of Ashwagandha Supplementation on Growth Rate (g/day)

3. *Protein efficiency ratio-*

As evident from Table 3 and Figure 3 with different levels of ashwagandha supplementation PER differed significantly. In treatment T3 PER 0.157 was recorded during 8th week whereas treatment T2 showed a significantly higher ($P<0.05$) PER 2.314 in the same period. The treatment T2 showed best PER (2.196) which is significantly different from the control.

Protein efficiency ratio is a parameter which shows as to how well the protein sources in the diet could provide essential amino acid requirement of the fish. Furthermore, that this index has been associated with fat deposition in fish muscle. Hence the PER in the range of 0.57-2.314 could favour fat deposition in *Labeo rohita* and this finding is as evident with the work done by Desilva and Anderson (1995) [4].

Table 3: Effect of Ashwagandha supplementation on PER

Weeks Treatments		2nd week	4th week	6th week	8th week	Mean
T1	Feed supplemented with 1% Ashwagandha	1.025	1.075	1.103	1.124	1.081
T2	Feed supplemented with 3% Ashwagandha	2.074	2.184	2.214	2.314	2.196
T3	Control feed with no Ashwagandha	0.092	0.113	0.124	0.157	0.121
		Treatments				
	CD (P = 0.05)	1.67				

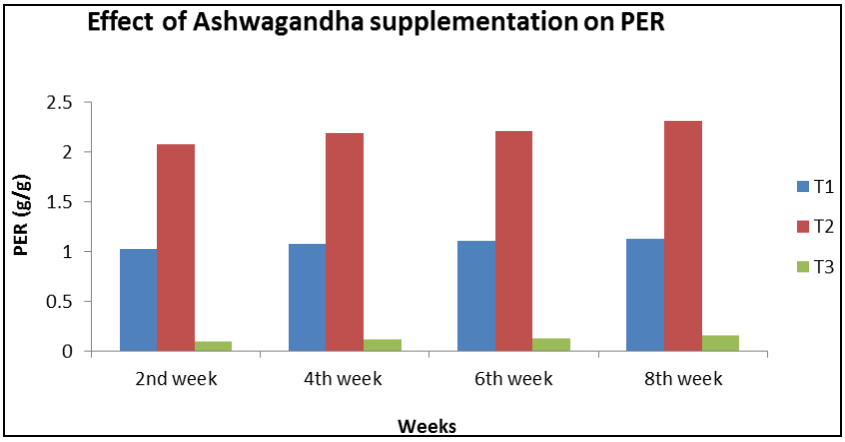

Fig 3: Effect of Ashwagandha supplementation on PER

4. *Gross protein retention*

Enzyme supplementation in feed resulted in better protein retention when compared to control feed where no enzyme was supplemented. A maximum protein retention (29.52%) was obtained in feed T2 followed by T1 (27.54) and T3 (25.48%). The treatment T2 showed significantly different results from control however T1 and T3 were not significantly different (table 4, figure 4). The Gross Protein Retention values recorded in the present study are in agreement with those of Jana *et al.* (2006) [7] who has recorded a GPR value in the range of 28-31.05 % in milk fish *Chanos chanos*.

Table 4: Effect of Ashwagandha supplementation on GPR

Weeks Treatments		2nd week	4th week	6th week	8th week	Mean
T1	Feed supplemented with 1% Ashwagandha	22.36	24.51	26.57	27.54	25.25
T2	Feed supplemented with 3% Ashwagandha	24.35	25.64	27.23	29.52	26.69
T3	Control feed with no Ashwagandha	21.17	22.50	23.41	25.48	23.14
		Treatments				
	CD (P = 0.05)	2.24				

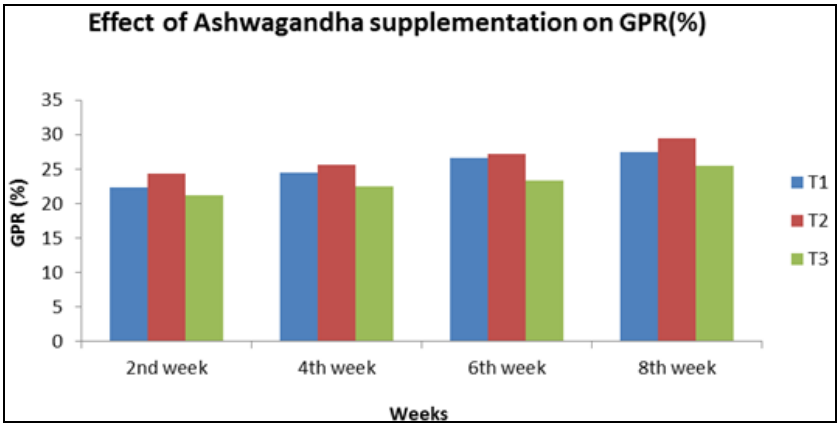

Fig 4: Effect of Ashwagandha supplementation on GPR (%)

5. *Condition factor*

T1 and T2 showed better results then control T3. The treatment T2 showed best CF (1.24) which is significantly different from control. However T1 and T3 were not significantly different. (Table 5, Figure 5)

CF increases as weight of fish increases for its length. Fish with a high CF are relatively heavy for their length while fish with a low CF are light for their length (Chellappa *et al.* 1995). Several scientists have calculated Ponderal index or condition factor of different fishes *viz.* 0.73 to 0.95 in *Tor putitora* (Pathani and Das, 1980), 1.03 -1.31 in *Salmo trutta fario* (Kumar *et al.* 1979) [10].

Table 5: Effect of Ashwagandha supplementation on Condition factor

Weeks Treatments		2nd week	4th week	6th week	8th week	Mean
T1	Feed supplemented with 1% Ashwagandha	1.53	1.42	1.34	1.24	1.38
T2	Feed supplemented with 3% Ashwagandha	1.32	1.25	1.21	1.18	1.24
T3	Control feed with no Ashwagandha	1.91	1.81	1.71	1.61	1.76
		Treatments				
	CD (P = 0.05)	0.42				

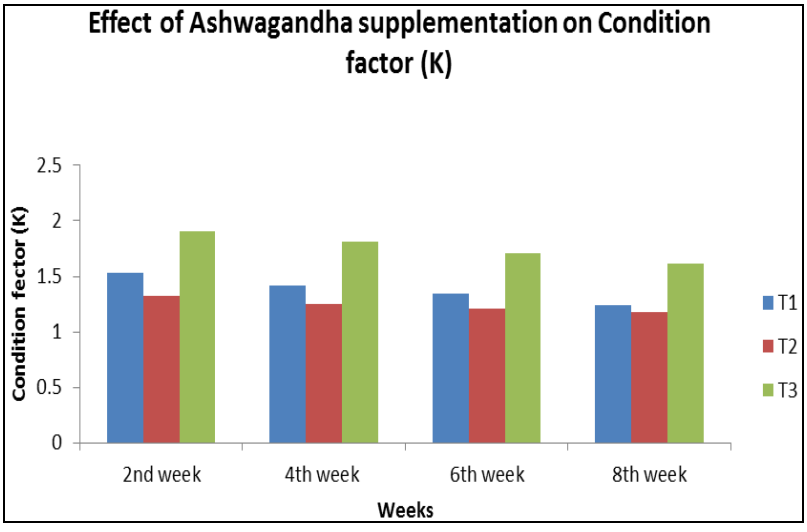

Fig 5: Effect of Ashwagandha supplementation on Condition factor (K)

6. *Water quality parameters*

Almost similar status of all physico chemical parameters (Temperature, pH, Dissolved oxygen, free carbon dioxide and alkalinity) was found without any adverse effect of the use of ashwagandha supplementation on the water quality. (Table 6-10, Figure 6-10)

Table 6: Variations in water temperature during experimental period.

Weeks	Treatments	2nd week	4th week	6th week	8th week	Mean
T1	Feed supplemented with 1% Ashwagandha	17.9	19.3	20.9	22.3	20.1
T2	Feed supplemented with 3% Ashwagandha	17.8	19.5	20.5	22.5	20.1
T3	Control feed with no Ashwagandha	18.1	19.2	20.7	22.8	20.2
		Treatments				
	CD (P = 0.05)	0.23				

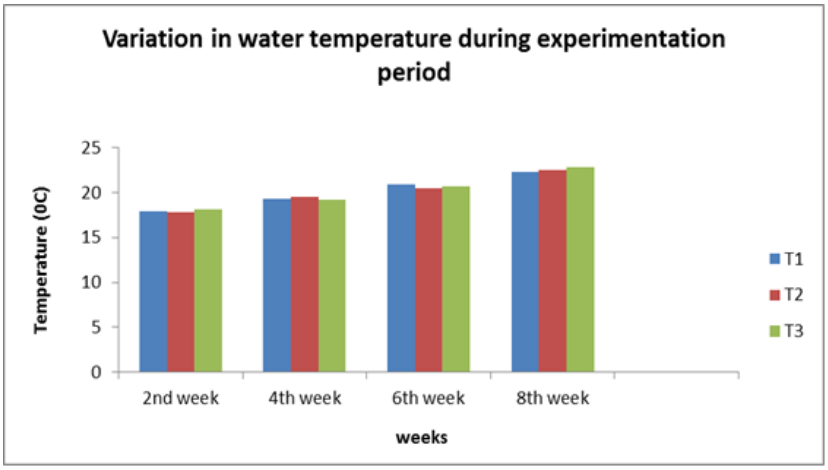

Fig 6: Variation in water temperature during experimentation period

Table 7: Variations in DO during experimental period

Weeks	Treatments	2nd week	4th week	6th week	8th week	Mean
T1	Feed supplemented with 1% Ashwagandha	9.8	7.8	9.6	8.2	8.9
T2	Feed supplemented with 3% Ashwagandha	8.9	9.2	7.8	10.1	9.0
T3	Control feed with no Ashwagandha	8.5	7.5	8.9	9.6	8.6
		Treatments				
	CD (P = 0.05)	0.63				

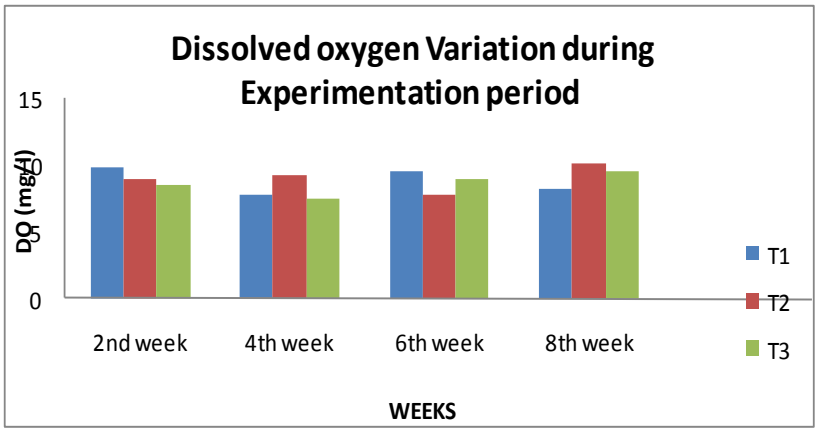

Fig 7: Dissolved oxygen Variation during Experimentation period

Table 8: Variations in Free CO_2 during experimental period.

Weeks	Treatments	2nd week	4th week	6th week	8th week	Mean
T1	Feed supplemented with 1% Ashwagandha	1.1	0.9	1.2	0.6	0.95
T2	Feed supplemented with 3% Ashwagandha	0.9	1.3	0.9	0.9	1.00
T3	Control feed with no Ashwagandha	0.7	0.7	0.5	1.1	0.75
		Treatments				
	CD (P = 0.05)	0.45				

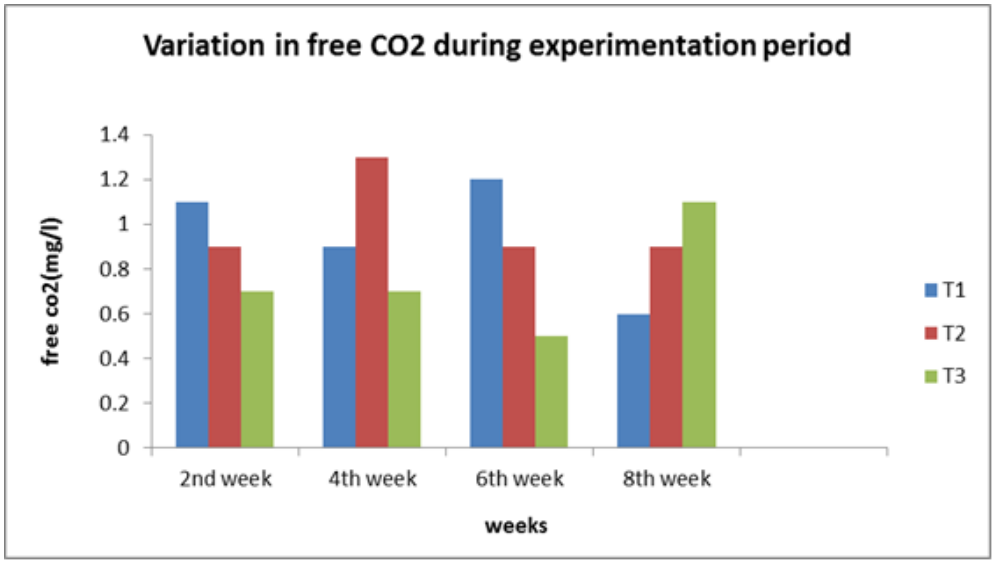

Fig 8: Variation in free CO2 during experimentation period

Table 9: Variations in water Alkalinity during experimental period.

Weeks Treatments		2nd week	4th week	6th week	8th week	Mean
T1	Feed supplemented with 1% Ashwagandha	115	118	129	130	123.0
T2	Feed supplemented with 3% Ashwagandha	125	115	125	125	122.5
T3	Control feed with no Ashwagandha	110	119	110	122	115.3
		Treatments				
	CD (P = 0.05)	3.45				

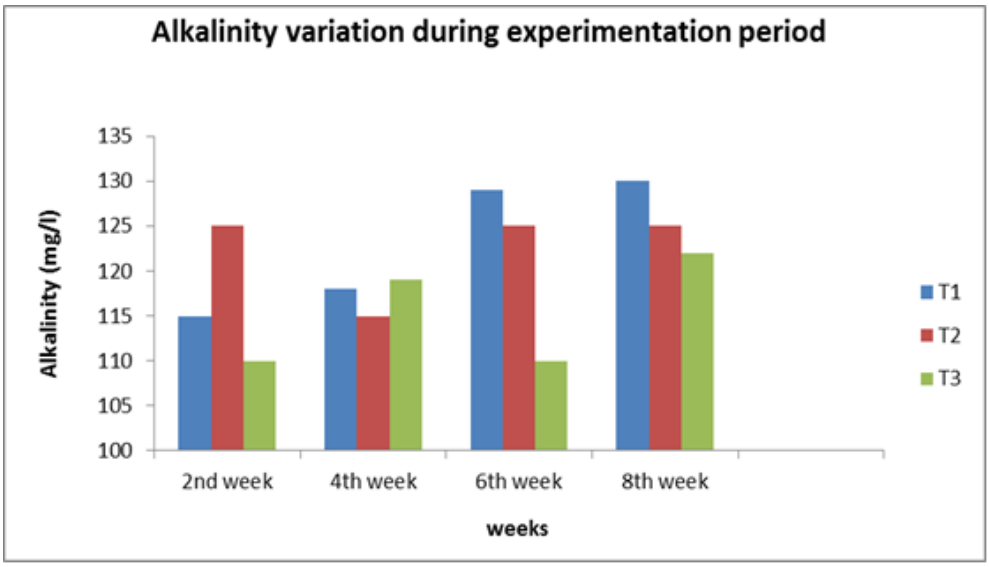

Fig 9: Alkalinity variation during experimentation period

Table 10: Variations in water pH during experimental period

Weeks Treatments		2nd week	4th week	6th week	8th week	Mean
T1	Feed supplemented with 1% Ashwagandha	6.7	7.3	7.5	7.3	7.2
T2	Feed supplemented with 3% Ashwagandha	7.1	6.9	7.8	7.5	7.3
T3	Control feed with no Ashwagandha	7.2	7.1	7.6	7.7	7.4
		Treatments				
	CD (P = 0.05)	0.26				

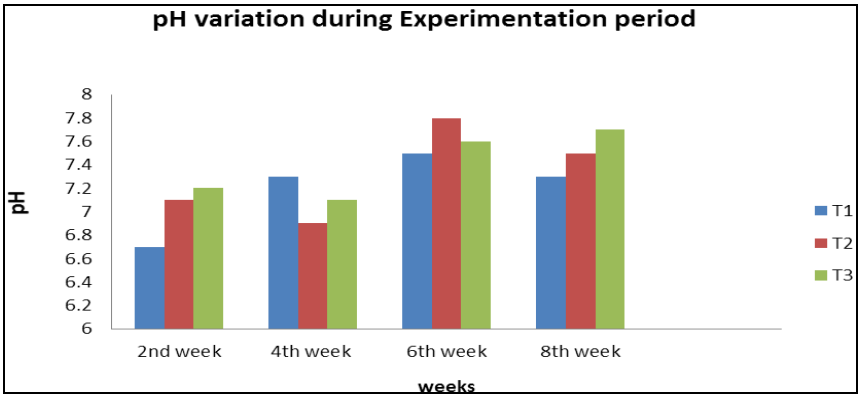

Fig 10: pH variation during Experimentation period

Conclusion

The findings of the above study resulted in better FCR, growth rate, condition factor and higher protein retention. All the physicochemical parameters were within the optimum range as desired for fish culture practices. Any kind of adverse effects due to Ashwagandha on the water quality parameters, behavioral and feeding response of fish were not encountered during the whole course of investigation.

Thus it is concluded from the results of this study that the supplementation of Ashwagandha, *Withania somnifera* as exogenous growth promoter may be practiced as a tool for formulation of diets for fingerlings of *Labeo rohita* to obtain better growth and it will be more efficient if it is used at 3% supplementation.

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