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Efficacy of *Aloe vera* as a growth promoting additive in carp (*Labeo rohita* Ham.) grow out feed

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Abstract

The present study was carried out to evaluate efficacy of *Aloe vera* powder (AVP) as a herbal feed additive, for improving survival, growth and flesh quality of rohu (*Labeo rohita* Ham.). Fishes were fed @ 2% of total fish biomass daily with feeds supplemented with AVP @ 0% (T₀), 1%(T₁), 2%(T₂) and 3%(T₃), respectively. Feed without AVP (T₀) served as control. Physico-chemical parameters of water in all the treatments remained within the recommended range for carp culture, with adequate plankton production. AVP supplementation improved fish survival and enhanced fish growth (net weight gain, specific growth rate and feed conversion ratio) and enhanced flesh quality significantly ($P \leq 0.05$) at all inclusion levels (1-3%). However, best results were obtained at 3% AVP inclusion level, which resulted in 48.19, 21.37 and 24.59%, higher NWG, SGR and protein content over control with better feed conversion ratio and protein efficiency ratio.

Keywords: *Aloe vera*, growth, additive, carp, *Labeo rohita* Ham

Introduction

With intensification of aquaculture systems, prophylactic and therapeutic use of antibiotics and other chemicals have increased in aquaculture for health management to achieve high production targets. Unlike terrestrial animals, application of antibiotics and other chemicals in an aquaculture system is quite expensive besides being associated with negative impacts including development of antibiotic resistance, residues in cultured species and accumulation in environment. It not only affects the non target species, but also poses serious health hazards for the consumers, which has attracted more attention to formulate eco-friendly alternatives (organic immuno-stimulants, vaccines and probiotics) for health management in aquaculture in the recent past [1]. In this direction, more attention is now being diverted towards use of organic or herbal innovations for growth enhancement and health management in aquaculture. Application of herbal dietary supplements has emerged as a promising alternative due to presence of various useful bio-active compounds, which not only improves the health of aquatic organisms (fish/shellfish), but has also been reported to enhance survival, growth and quality of many aquatic species reared for food purpose [2]. Many medicinal herbs possess growth promoting immuno-stimulatory potential, due to presence of several bio-active compounds (phenols, polyphenols, alkaloids, flavanoids, quinones, sulphur, lectins and polypeptides) and serve as very effective alternatives to antibiotics, vaccines, chemicals and other synthetic therapeutics [3, 4].

In aquaculture, a wide range of herbal medicinal plants like ginger, turmeric, garlic, onion, tulsi, ashwagandha, *Aloe*, amla and neem, have been reported to improve survival and growth; strengthen immune system; and promote maturation in aquatic species due to their antioxidant, antimicrobial and anti-stress properties [3]. These herbs, which are being used for treatment of many infectious diseases, since ancient times, can serve as an inexpensive biodegradable alternative for health management without any consumer safety and environmental issues.

Among various known herbal plants, *Aloe vera* is a perennial plant of the Aloeaceae family, which grows in hot dry climate. It is one of the known immuno-stimulant used to control pathogenic bacteria in animals, including fish. *Aloe* gel consists mainly of water (>98%) and polysaccharides like pectins, cellulose, hemicellulose, glucomannan, acemannan and mannose derivatives [5]. *Aloe* is an amazing mixture of 200 constituents, including active compounds like galacto-mannan, beta-mannan, glycoproteins, lectins, salicylic acid, phenols, sulphur,

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amino acids, sterols, tannins and enzymes [6, 7]. It possesses antibacterial property against various pathogenic bacteria, particularly gram positive bacteria. Auxins and gibberellins are hormones in *Aloe* that help in wound healing and have anti-inflammatory action. It also provides 12 anthraquinones (phenolic compounds), known as laxatives.

Although, *A. vera* has been found to eliminate principal pathogenic bacteria, strengthen immunity, enhance growth and improve flesh quality in freshwater fish, including common carp (*Cyprinus carpio*), African catfish (*Clarias gariepinus*), Nile tilapia (*Oreochromis niloticus*) and rainbow trout (*Oncorhynchus mykiss*) [8-11], but information regarding efficacy of *A. vera* on survival, growth and proximate composition (flesh quality) of freshwater carps, is inadequate with special reference to one of the priced Indian major carp, rohu (*Labeo rohita*).

Carp fish, contributing major share of total production from freshwater aquaculture sector in India, is a low value but practically indispensable fish due to non-availability of any strong alternative species to contribute significantly towards aquaculture diversification across the nation. Hence, there is need to formulate innovative cost effective health management strategies for carps for sustainable development. *A. vera* is a wonderful medicinal plant, which can be easily cultivated without much cost and labour, hence making it an economically viable option for utilization in carp culture. In this context, the present study was designed to evaluate the possibility of utilizing *A. vera* powder (AVP) as a feed additive for improving survival, growth and flesh quality of rohu (*L. rohita* Ham.).

Materials and Methods

Experimental layout

The present study was carried out in outdoor 80m² cemented tanks (in duplicate) at the fish farm of College of Fisheries (COF), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab, India for the culture period of 180 days.

Preparation of experimental feeds

For preparing *Aloe vera* powder (AVP), fresh *A. vera* leaves were collected from the herbal garden of COF, GADVASU. After cleaning, it was cut into small pieces, dried (oven drying at 40°C/sun drying) and ground to obtain a fine powder, with dry matter (DM) recovery of 5.4%. Four pellet experimental feeds were prepared with the help of hand pelletizer, as per composition presented in Table 1. The proximate composition (crude protein, ether extract, ash, crude fibre and nitrogen free extract) of the feed ingredients and the formulated experimental feeds (Table 2) was analyzed (Table 2) as per standard methods of AOAC [12].

Table 1: Details of feed formulation in different treatments

Ingredients	Treatments			
	T ₀ (Control)	T ₁	T ₂	T ₃
¹ Basal diet (%)	100	99	98	97
² <i>Aloe vera</i> powder (AVP) (%)	-	1	2	3

¹ Basal diets – 49% Rice bran (de-oiled) + 49% Mustard meal (de-oiled) + 1.5% Vitamin- mineral mixture + 0.5% Common salt

² AVP incorporated in T₁, T₂ and T₃ treatments @ 10g, 20g and 30g kg⁻¹, respectively.

Table 2: Proximate composition (DM basis) of feed ingredients and experimental feeds

Ingredients/ diet	Crude Protein	Ether Extract	Crude Fibre	Total Ash	Nitrogen Free Extract
Rice Bran*	12.37	1.29	15.65	11.53	59.14
Mustard Meal*	38.93	1.92	11.55	8.17	39.54
<i>A. vera</i> powder (AVP)	11.56	1.96	13.24	18.7	54.54
Control Feed (T ₀)	25.35	1.40	13.85	9.48	49.73
1% AVP Feed (T ₁)	25.28	2.05	14.82	10.42	47.43
2% AVP Feed (T ₂)	25.34	2.25	14.94	10.54	46.93
3% AVP Feed (T ₃)	25.37	2.50	15.01	10.59	46.53

*Solvent extracted/De-oiled

Preparation of experimental tanks

The experimental tanks were layered with soil (10cm), lined with lime stone @ 250kg ha⁻¹(2kg tank⁻¹), filled with bore well water (1 meter) and manured with farm yard manure (FYM) @ 5000 kg ha⁻¹ (40 kg tank⁻¹).

Stocking and feeding of fish

L. rohita fingerlings were stocked in the experimental tanks @ 10,000 fingerlings ha⁻¹ (80 fingerlings tank⁻¹) after 10 days of manuring. Fish was fed daily after sun rise with control (T₀) and AVP included feeds (T₁-T₃) @ 2% body weight (BW) for 6 months and feeding ration was adjusted every month in accordance to weight gain of the fish. All the tanks were manured with FYM throughout the culture period @ 500 kg ha⁻¹ (4 kg tank⁻¹) at monthly interval for consistent plankton production.

Observations

At monthly interval, the water quality parameters (water temperature, pH, dissolved oxygen, total alkalinity, total hardness, ammonical-nitrogen, nitrate nitrogen and orthophosphate content) and plankton population [13]

(phytoplankton and zooplankton) were estimated following standard methods [13]. Fish growth in terms of total body length (TBL) and BW was recorded at monthly intervals, by random sampling of 10 fish from each tank. At the end of the experimental period, full stock from each tank was harvested to record survival percentage (%), final average (Av.) TBL and final Av. BW to evaluate growth performance of fish, in terms of total body length gain (TBLG), net weight gain (NWG), specific growth rate (SGR), condition factor (K-value), feed conversion ratio (FCR), protein efficiency ratio (PER), as per following formulae [14, 15].

TBLG = Av. final TBL (cm) – Av. initial TBL (cm)

NWG = Av. final BW (g) – Av. initial BW (g)

$$\text{SGR (\% increase BW day}^{-1}\text{)} = \frac{\text{Log}_e \text{ final BW} - \text{log}_e \text{ initial BW}}{\text{Culture days}} \times 100$$

$$\text{Condition Factor (K-value): } K = \frac{W}{L^3} \times 100$$

Where, W = Weight of Fish (g), L= Length of Fish (cm)

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed given (dry weight) (g)}}{\text{NWG gain (wet weight) (g)}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{NWG(g)}}{\text{Protein Intake (g)}}$$

Proximate composition (flesh quality) analysis of harvested fish, in terms of total protein, total lipids, total carbohydrates, moisture and ash content, was done as per standard methods [12, 16, 17].

Statistical analysis

Analysis of data was done using one way ANOVA and Duncan's multiple range test using SPSS 20.0 software

($P \leq 0.05$) to study significant differences among different treatments with respect to water quality, plankton population, growth and proximate composition of fish.

Results and Discussions

Although, water quality in different treatments differed significantly ($P \leq 0.05$) (Table 3), with respect to culture period mean pH, dissolved oxygen, total hardness, nitrate nitrogen and orthophosphate, but all the parameters were well within the recommended range [18-20], which indicates that inclusion of *A. vera* powder in *L. rohita* fingerling feeds did not alter the water quality beyond optimum / permissible limit in any of the treatments.

Table 3: Mean culture period water quality parameters in different treatments

Parameters	Treatments			
	T ₀	T ₁	T ₂	T ₃
Water temperature (°C)	28.30 ^a ±0.56	28.32 ^a ±0.57	28.33 ^a ±0.58	28.09 ^a ±0.60
pH	8.32 ^c ±0.04	8.68 ^{ab} ±0.03	8.72 ^a ±0.03	8.57 ^b ±0.06
Dissolved oxygen (mg l ⁻¹)	9.12 ^c ±0.11	10.57 ^b ±0.08	10.78 ^a ±0.08	10.74 ^a ±0.04
Total hardness(mg l ⁻¹)	196.19 ^b ±6.06	241.50 ^a ±4.04	240.12 ^a ±2.79	238.50 ^a ±4.40
Total alkalinity(mg l ⁻¹)	170.52 ^a ±5.10	177.59 ^a ±5.00	182.17 ^a ±5.12	176.31 ^a ±4.3
Ammonical nitrogen (mg l ⁻¹)	0.060 ^a ±0.002	0.062 ^a ±0.002	0.072 ^a ±0.010	0.058 ^a ±0.003
Nitrate nitrogen (mg l ⁻¹)	0.543 ^a ±0.013	0.504 ^b ±0.015	0.527 ^{ab} ±0.017	0.486 ^c ±0.161
Orthophosphate (mg l ⁻¹)	0.093 ^b ±0.007	0.219 ^a ±0.011	0.246 ^a ±0.015	0.241 ^a ±0.014

Values (mean ± S.E.) with same superscripts in a row do not differ significantly ($P \leq 0.05$)

As compared to control (T₀), culture period mean total phytoplankton population was significantly ($P \leq 0.05$) lower in all AVP fed treatments T₁-T₃ (Table 4), which can be attributed to bigger size of fish (Table 5) and hence, higher consumption of plankton in all the AVP fed treatments. However, differences among all the treatments (T₀-T₃) in respect to culture period mean total zooplankton population were insignificant (Table 4), which actually indicates healthy plankton production supporting more fish biomass in AVP fed treatments (T₁-T₃) as compared to control (T₀). Supportive findings in this regard are however lacking. Among

phytoplankton, chlorophyceae (green algae) was the most dominant group in all the treatments (T₀-T₃), constituting 51.1, 47.0, 49.34 and 49.35% of the population, respectively with predominance order chlorophyceae > cyanophyceae > bacillariophyceae > euglenophyceae. The results indicate that, AVP supplementation did not alter relative predominance order of phytoplankton groups in any of the treatments. While, among zooplankton, relative predominance of different groups varied in different treatment, with copepoda being the most dominant group and ostracoda being least abundant group

Table 4: Mean culture period plankton (pytoplanktons and zooplanktons) population in different treatments

Parameters	Treatments			
	T ₀	T ₁	T ₂	T ₃
Phytoplankton population (no. x 10⁶l⁻¹)				
Chlorophyceae	137.2 ^a ±4.96 (51.1%)	110.07 ^b ±1.84 (47%)	113.71 ^b ±2.08 (49.34%)	114.12 ^b ±2.12 (49.35%)
Cyanophyceae	64.41 ^a ±2.92 (23.88%)	63.98 ^{ab} ±2.46 (27.31%)	54.67 ^c ±2.04 (23.91%)	57.45 ^{bc} ±1.85 (25%)
Bacillariophyceae	49.81 ^a ±1.44 (18.65%)	48.05 ^a ±0.80 (20.51%)	49.48 ^a ±0.88 (21.39%)	48.40 ^a ±0.81 (20.78%)
Euglenophyceae	15.14 ^a ±0.69 (5.59%)	12.39 ^b ±0.41 (5.29%)	11.64 ^b ±0.35 (5.08%)	11.76 ^b ±0.43 (5.09%)
Total phytoplankton population	268.00 ^a ±7.38	234.43 ^b ±2.77	229.50 ^b ±3.03	231.74 ^b ±3.17
Zooplankton population (no.l⁻¹)				
Copepoda	216.00 ^b ±5.14 (29.19%)	225.10 ^{ab} ±4.08 (31.73%)	230.88 ^a ±5.57 (31.81%)	230.14 ^{ab} ±4.61 (32.26%)
Cladocera	219.19 ^a ±7.33 (29.59%)	211.71 ^a ±2.83 (29.76%)	219.07 ^a ±4.97 (30.29%)	219.17 ^a ±3.16 (30.71%)
Rotifera	240.71 ^a ±5.02 (32.43%)	210.67 ^b ±3.38 (29.62%)	210.64 ^b ±4.05 (29.04%)	205.86 ^b ±5.78 (28.75%)
Ostracoda	64.52 ^a ±2.16 (8.64%)	61.83 ^b ±1.78 (8.60%)	63.31 ^{ab} ±1.45 (8.76%)	58.43 ^b ±1.60 (8.19%)
Total zooplankton population	740.43 ^a ±14.99	709.31 ^a ±16.08	723.90 ^a ±11.81	713.60 ^a ±11.04

Values (mean ± S.E.) with different subscripts in a row differ significantly ($P \leq 0.05$)

Values in parentheses indicates per cent contribution of each group in total phytoplankton/zooplankton population

AVP supplementation improved fish growth significantly ($P \leq 0.05$) at all inclusion levels (1-3%) with best results at 3% inclusion level. As compared to control (T_0), 23.13, 40.23 and 48.19% higher NWG and 13.67, 18.80 and 21.37% higher SGR was recorded in fish fed with 1 (T_1), 2 (T_2) and 3% (T_3) AVP supplemented feeds, respectively (Table 5), with

improved fish survival of 93.75 - 95.60%. Condition factor (k-value) of fish remained above 1.0 and was comparable with control, indicating healthy condition of fish. Both FCR and PER also improved with increase in AVP inclusion level from 1-3%, indicating better feed conversion efficiency in AVP fed fish.

Table 5: Growth parameters of rohu, *L. rohita* in different treatments

Parameters	Treatments			
	T ₀	T ₁	T ₂	T ₃
Initial TBL(cm)	9.67 ^a ±0.09	9.49 ^a ±0.11	9.39 ^a ±0.045	9.60 ^a ±0.12
Final TBL (cm)	19.68 ^d ±0.16	20.59 ^c ±0.12	21.69 ^b ±0.11	22.36 ^a ±0.06
TBLG (cm)	10.01	11.10 (+9.80%)	12.5 (+19.92)	12.7 (+23.30)
Initial BW (g)	9.74 ^a ±0.092	9.70 ^a ±0.41	9.76 ^a ±0.24	9.95 ^a ±0.32
Final BW (g)	89.44 ^d ±0.56	107.84 ^c ±0.70	119.99 ^b ±1.26	128.07 ^a ±0.91
NWG (g)	79.70	98.14 (+23.13%)	111.77 (+40.23%)	118.11 (+48.19%)
SGR (%)	1.17 ^d ±0.01	1.33 ^c ±0.02 (13.67%)	1.39 ^b ±0.01 (18.80%)	1.42 ^a ±0.00 (21.37%)
FCR	2.06	2.06	2.00	2.00
K-value	1.18 ^{ab} ±0.02	1.24 ^a ±0.02	1.18 ^{ab} ±0.03	1.15 ^b ±0.01
PER	1.92	1.93	1.97	1.97
Survival (%)	92.50	93.75	95.00	95.60
Initial total fish biomass stocked (g tank ⁻¹)	779.20	776.00	780.8	796.00
Final total fish biomass harvested (g tank ⁻¹)	6618.56	8088.00	9119.24	9733.32
Net fish biomass harvested (g tank ⁻¹)	5839.36	7312.00 (25.22%)	8338.44 (42.80%)	8937.32 (53.05%)

Values (mean ±S.E.) with same superscripts in a row do not differ significantly ($P \leq 0.05$)

Values in parentheses indicates per cent change over control

The enhanced growth performance of rohu fingerlings fed with AVP supplemented feeds is attributed to presence of growth promoting bio-active compounds like polysaccharides (galacto-mannans, acemannans) anthraquinones, lectins, salicylic acids, phenols and sulphur) in *A. vera*, possessing antioxidant, anti-toxicity and immuno-stimulatory characteristics [21, 22], along with many other useful amino acids, lipids, sterols, tannins and enzymes [6] Major benefits of *A. vera* are attributable to its polysaccharide (65% of DM) content in its gel [23]. Polysaccharide acemannan has immuno-modulatory, anti-microbial, and anti-tumor effects and has also been reported to improve intestinal microflora in broilers [24].

Although, some previous research reports with respect to growth promoting effect of *A. vera* in Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*), African catfish (*Clarias gariepinus*) and starlet sturgeon (*Acipenser ruthenus*) [8-11] are available, but information with respect to growth promoting efficacy of *A. vera* supplemented feeds in carps, with special references to rohu, is inadequate. Improved NWG, SGR, FCR and FCE was observed in common carp, *C. carpio* fed with diets

containing *A. vera* ethanolic extract @ 2.5% (25g kg⁻¹) [10, 22]. As per a recent study, both *A. vera* gel and *A. vera* extract can be incorporated up to 2% level (20 g Kg⁻¹) in common carp feed for 60% and 100% higher fish production, respectively [25]. Similar results have been reported in rainbow trout, (*O. mykiss*) and African catfish (*C. gariepinus*) fed with 1% *Aloe* (*A. barbadensis*) supplemented feeds [26, 27]. However, in case of Nile tilapia (*O. niloticus*), addition of rosemary extract (@ 0.1-0.5%) and *A. vera* extract (@ 0.5-2.5%) in the feed did not change the growth parameters, biometric indexes or chemical composition of fish [28]. All the available reports indicates growth promoting efficacy of *A. vera* in fish. However, the differences, regarding optimum incorporation level of *A. vera* is attributed to variations among studies in respect to species, fish size, *A. vera* form (powder, gel or extract) and experimental setup (duration and method of feeding).

The proximate composition (flesh quality) of *L. rohita* fingerlings, in terms of total protein, total lipids, total carbohydrates, ash and moisture content (Table 6), also improved significantly ($P \leq 0.05$) with AVP supplementation at all inclusion levels (1-3%).

Table 6: Proximate composition of *L. rohita* flesh (% wet weight basis) in different treatments

Parameters	Treatments			
	T ₀	T ₁	T ₂	T ₃
Total protein (g 100g ⁻¹)	14.92 ^d ±0.15	15.38 ^c ±0.14	16.54 ^b ±0.07	18.59 ^a ±0.09
Total lipid (g 100g ⁻¹)	1.26 ^d ±0.016	1.45 ^c ±0.016	1.77 ^b ±0.031	1.92 ^a ±0.027
Total carbohydrates (g 100g ⁻¹)	3.27 ^a ±0.032	2.24 ^b ±0.033	1.90 ^c ±0.041	1.78 ^d ±0.027
Ash (g 100g ⁻¹)	1.05 ^c ±0.031	1.31 ^b ±0.044	1.54 ^a ±0.029	1.38 ^b ±0.024
Moisture (%)	78.92 ^b ±0.17	79.61 ^a ±0.14	78.24 ^c ±0.05	76.32 ^d ±0.07

Values (mean ± S.E.) with same superscripts in a row do not differ significantly ($P \leq 0.05$)

Total protein and total lipid content increased significantly with increase in AVP inclusion level of (1-3%), whereas, total carbohydrates and moisture content decreased with increase in inclusion level ($P \leq 0.05$). While, ash content was significantly high in all the AVP treatments as compared to

control. Overall results reveal that *A. vera* incorporation in the feed improved the flesh quality of rohu, fingerlings by enhancing the protein content by 3.08, 10.85, 24.59% at 1% (T_1), 2% (T_2) and 3% (T_3) inclusion levels, respectively. Although, many scientists have investigated efficacy of *A.*

vera in feed of aquatic species, but information in respect to its effect on flesh quality is lacking. Improved flesh quality of fish in the present study can be attributed to growth promoting, immuno-stimulating properties of *A. vera* due to its antioxidant and antimicrobial bio-active compounds [21, 22], subsequently leading to enhanced protein uptake and assimilation of protein in the flesh.

Feed cost decreased with increase in inclusion level of AVP in experimental diets as *A. vera* was procured from the herbal garden of COF, GADVASU, where it has been propagated

without any significant expenditure and farmers can also cultivate these plants on the dykes of ponds without much cost and labor (Table 7). *A. vera* powder inclusion in fish feed @ 1, 2 and 3% resulted in 25.17, 42.81 and 53.08% higher fish biomass production, with an additional earning of 25.69, 45.59 and 56.78% over control, indicating that *A. vera* can be incorporated in rohu, fingerlings feed @ 3% (30g kg⁻¹ feed) for enhanced growth, better flesh quality, higher production and profitability without affecting survival of fish.

Table 7: Comparative economics of different treatments with respect to feed cost and production

Parameters	Treatments			
	T ₀	T ₁	T ₂	T ₃
Fish production (kg tank ⁻¹)*	5.84	7.31 (25.17%)**	8.34 (42.81%)	8.94 (53.08%)
Feed cost kg ⁻¹ feed (Rs.)	17.10	16.93	16.76	16.59
Total feed cost tank ⁻¹ (Rs.)	205.74	254.97	279.57	296.63
Total income (Fish sale @ Rs.120 kg ⁻¹)	700.8	877.2	1000.8	1072.8
Net income with respect to feed (Rs.)	495.06	622.23	721.23	776.17
Additional net income over control (%)	--	25.69	45.59	56.78

* Tank size 80 m²

**Values in parenthesis indicate per cent change over control

Conclusion

Overall results revealed that dried *A. vera* powder improved growth (NWG, SGR, K-value, FCR and PER) and flesh quality of *L. rohita* fingerlings significantly ($P \leq 0.05$) at all inclusion levels (1-3%), but best results were recorded at 3% inclusion level, resulting in 53.08% higher biomass production, 24.59% higher flesh protein content and 56.78% higher net profit over control in respect to feed cost.

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