Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 955-959

Received: 18-01-2019
Accepted: 30-04-2019

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Effect of amla (*Phyllanthus emblica*) fruit powder supplemented feed on growth performance and proximate composition of an Indian major carp, *Labeo rohita* (Ham.) fingerlings

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Abstract
Efficacy of amla (*Phyllanthus emblica*) supplemented feeds, on survival, growth and flesh quality of an Indian major carp, *Labeo rohita* (Ham.) fingerlings, was evaluated through an outdoor study in cemented tanks (80m^2) with stocking density of 1 fingerling/m^2 (80 fingerlings/tank). Fingerlings were fed with feeds containing 0 (T_0), 1 (T_1), 2 (T_2) and 3 (T_3) % of amla fruit powder (AFP) for 180 days @ 2% of total fish biomass. Feed T_0 (without AFP) served as control. In all the treatments, the water quality parameters remained well within the recommended range. In AFP fed treatments (T_1- T_3), 95.00 to 96.88% fish survival was recorded compared to 92.50% in control (T_0). Net weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio improved significantly ($P \leq 0.05$) in all the AFP fed treatments and was best at 3% inclusion level (122.08 g, 1.44%, 1.65 and 2.46, respectively). AFP supplementation also improved condition factor of fish significantly ($P \leq 0.05$) at 2 (T_2) and 3% (T_3) inclusion levels. Further, dietary inclusion of AFP also improved flesh quality significantly ($P \leq 0.05$). Total protein, carbohydrate and ash content was recorded maximum (13.79, 3.57 and 1.51g/100g, respectively) and moisture content was minimum (78.34%) in fish fed with feed containing 3% AFP (T_3), while total lipid content was recorded maximum (2.69 g/100g, respectively) with 2% AFP feed. Among all AFP treatments, 3% AFP inclusion level was found best in terms of growth enhancement and flesh quality improvement in *L. rohita* fingerlings.

Keywords: Amla, flesh quality, growth, rohu, survival

Introduction
Nutritional innovations are one of the technological interventions required for achieving production targets in livestock sector, including aquaculture. India's total fish production in 2015-16 was 10.76 mmt, out of which aquaculture food fish production was 5.70 mmt [1] and the domestic demand is expected to cross 16 mmt mark in 2025 [2]. As the capture resources are already overexploited, there is limited scope of any significant increase in production from these resources. Hence, aquaculture sector is expected to fill the gap to achieve the said demand. To improve fish productivity, various chemical and artificial feed additives are being used in aquaculture but due to its negative effect on environment, fish and consumer, focus on use of natural feed additive such as herb and herbal products is increasing day by day [3, 4].
Among traditional Indian herbs, amla or Indian gooseberry (*Phyllanthus emblica* Linn. or *Emblica officinalis* Gaertn.), belonging to family Euphorbiaceae [5, 6], is a highly nutritive and richest source of ascorbic acid/vitamin C among all fruits after barbados cherry [7]. It contains very important phytochemicals like phyllaemblic compounds, alkaloids (Phyllantidine and phyllantine), gallic acid, ellagic acid, flavonoids (kaempferol), hydrolysable tannins (Emblicanin A and B), pectin, quercetin, vitamin C and various polyphenolic compounds, which are responsible for its antioxidant, immunostimulatory, anticarcinogenic, antitumour, antigenotoxic and anti-inflammatory activities [8,5] and has been used as one of the important constituents in 'Rasayana' (an Ayurvedic preparation which promote health and longevity) and triphla [9, 5]. It strengthens digestion, absorption, liver function, assimilation of food, enhances protein synthesis and supports the reproductive system of both male and female [8].
Due to its high nutritive value, use of amla and its products as dietary supplementation in fish feed has been studied by several researchers and has obtained significant results with respect to growth, survival, immunity and health in different fin fish species [10-14],

but there is inadequacy of literature regarding comprehensive study of effect of amla on survival, growth and proximate composition (flesh quality) of fresh water carps in general and rohu (*Labeo rohita*) in particular, which is one of the most priced carp species.

Indian major carps contribute about 85% of total fish production from fresh water aquaculture sector in India [15], and they will remain to contribute the major share, till alternate species for large scale adoption are available. In this context, the present study was designed to evaluate the possibility of incorporating amla fruit powder (AFP) as feed additive to evaluate its efficacy in enhancing survival, growth and flesh quality of Indian major carp, *L. rohita* (rohu).

2. Materials and Methods

The experiment was conducted in outdoor rectangular cemented tanks (80 m^2), in duplicate, at fish farm of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, India for a period of 180 days from May to October, 2017.

For AFP preparation, fresh good quality amla (*P. emblica*) fruits were washed and cut into small pieces to remove the seeds. The fruit pieces were dried with freeze drying method using lyophilizer to maintain its nutritional quality [16, 17] and grinded to fine powder form, which was stored in air tight container. Four iso-proteinous experimental feeds (crude protein 24.70-25.37%) were prepared using locally available ingredients (rice bran, mustard meal and vitamin-mineral mixture), which were supplemented with AFP @ 0% (T_0), 1% (T_1), 2% (T_2) and 3% (T_3), where feed T_0 (without AFP) served as control (Table-1).

Table 1: Details of feed formulation in different treatments in terms of feed ingredients and their inclusion levels.

Ingredients	Treatments			
	T_0	T_1	T_2	T_3
[1]Basal feed (%)	100	99	98	97
[2]Amla fruit powder (AFP) (%)	-	1	2	3

[1]Basal feed-49% Rice bran + 49% Mustard meal + 1.5% Vitamin-mineral mixture (Agrimin-forte) + 0.5% Common salt (T_0)

[2]AFP incorporated in T_1, T_2 and T_3 treatments @10g, 20g and 30g/Kg, respectively.

All the ingredients were mixed thoroughly and sinking pellets were made by using a mechanical pelletizer. The pellets were dried in hot air oven at 40^0C for overnight and were stored in an air tight container at a cool dry place till further use. The proximate analysis (crude protein, ether extract, ash, crude fiber and nitrogen free extract) of feed ingredients and formulated experimental feeds (Table-2) was estimated as per standard methods [18].

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

Ingredients/ Feed	Crude protein	Ether extract	Crude fiber	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
Amla fruit powder	3.15	0.65	10.40	2.40	83.40
Experimental feed T_0	25.37	1.32	15.82	9.40	48.09
Experimental feed T_1	25.08	1.21	15.81	9.10	48.80
Experimental feed T_2	24.85	1.15	15.74	9.03	49.23
Experimental feed T_3	24.70	1.10	15.63	8.91	49.66

*Solvent extracted

All the experimental tanks were cleaned, layered with 10 cm thick soil and limed initially with lime stone ($CaCO_3$) @ 250kg/ha. Bore well water was used for initial and subsequent filling of all the experimental tanks to maintain water depth of 100±5.0 cm during the study. Each experimental tank was manured with farm yard manure @ 5 t/ha as a basal dose, and subsequently @ 0.5 t/ha at monthly interval. After manuring, experimental tanks were left for 10 days for adequate plankton development. Healthy acclimatized fingerlings of *L. rohita* of average (av.) length 9.66±0.08 cm and av. weight 9.84±0.26 g were stocked in each treatment @10,000 fingerlings/ha (80 fingerlings/tank). Fish was fed daily with experimental feeds @ 2% body weight (BW) and feeding ration was adjusted after every monthly sampling.

The physico-chemical parameters of water, including water temperature, pH, dissolved oxygen, total alkalinity, total hardness, ammonical-nitrogen, nitrate nitrogen and orthophosphate content, were estimated at monthly intervals following standard methods [19].

Growth of fish in terms of total body length (TBL) and body weight (BW) was assessed at monthly intervals, by collecting random sample of 10 fish from each treatment. After 180 days of culture period, survival and growth of harvested fish (final TBL and BW) was recorded for each treatment. Growth performance of fish, in terms of net weight gain (NWG), specific growth rate (SGR), protein efficiency ratio (PER), food conversion ratio (FCR) and condition factor (K-value), was calculated as per following formulae [20, 21]

Total Length Gain (TLG) = Av. final TBL (cm) – Av. initial TBL (cm)

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

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

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

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

$$\text{Fulton's Condition Factor (K-value)} = K = \frac{W}{L^3} \text{ X } 100$$

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

Flesh quality of harvested fish, in terms of total protein, total lipids, carbohydrates, moisture and ash contents, was estimated as per the standard methodology [18]

Data was analyzed using one way ANOVA and Duncan's multiple range test using SPSS 16.0 software ($P \leq 0.05$) to

study significant differences among different treatments with respect to water quality, survival, growth and proximate composition of fish.

3. Results and Discussions

In all the treatments, water quality parameters (Table-3), with respect to culture period mean water temperature, pH, dissolved oxygen, total alkalinity, total hardness, ammonical nitrogen, nitrate nitrogen and orthophosphate, were well within the optimum range for carp culture [22-24] and the differences among treatments were insignificant, except pH and ammonical nitrogen. The results indicate that incorporation of AFP in feed of rohu fingerlings had no undesirable effect on the water quality.

Table 3: Culture period mean physico-chemical parameters and nutrient status of water in different treatments

Parameter	Treatments			
	T_0	T_1	T_2	T_3
Water temperature (^{0}C)	28.58^a±1.47	28.65^a±1.44	28.53^a±1.48	28.65^a±1.46
pH	8.30^a±0.086	8.23^a±0.067	8.30^a±0.064	7.86^b±0.115
Dissolved oxygen (mg/L)	9.20^b±0.23	10.33^a±0.39	10.19^{ab}±0.28	9.45^b±0.47
Total alkalinity (mg/L)	177.76^a±13.17	179.57^a±18.61	204.99^a±14.03	180.62^a±16.13
Total hardness (mg/L)	194.52^a±15.36	179.43^a±16.75	180.57^a±11.82	172.76^a±17.03
Ammonical nitrogen (mg/L)	0.055^{ab}±0.003	0.062^a±0.004	0.053^{ab}±0.005	0.044^b±0.003
Nitrate nitrogen (mg/L)	0.53^a±0.05	0.53^a±0.02	0.50^a±0.04	0.52^a±0.05
Orthophosphate (mg/L)	0.052^a±0.006	0.057^a±0.007	0.049^a±0.007	0.039^a±0.006

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

Results illustrated that incorporation of AFP in fish feed significantly improved the growth performance and survival of *L. rohita* fingerlings (Table-4). Among all treatments, 25.61, 46.56 and 58.84% high NWG and 10.00, 17.50 and 20.00% higher SGR was recorded in fish fed with 1, 2 and 3% AFP feeds, respectively with higher fish survival (95.00-96.88%), as compared to control (92.50%). Condition factor of fish also improved significantly (1.18-1.21) in fish fed with 2-3% AFP supplemented feeds (T_2-T_3), indicating better condition and health of fish as compared to control T_0 (1.12). Besides growth promoting efficacy of AFP supplementation in feed of *L. rohita* fingerlings, significantly ($P \leq 0.05$) better FCR was recorded at all AFP inclusion levels from 1-3% (1.65-1.75) as compared to control (1.80), which reveals dual potential of AFP supplementation in respect to fish growth enhancement (more productivity) and FCR improvement (less feed requirement).

Table 4: Growth performance and production of rohu, *L. rohita* fingerlings in different treatments

Parameter	Treatments			
	T_0	T_1	T_2	T_3
Initial total body length (cm)	9.78^a±0.19	9.70^a±0.16	9.67^a±0.15	9.48^a±0.18
Final total body length (cm)	19.80^c±0.20	20.99^b±0.16	21.79^a±0.19	22.16^a±0.22
Initial body weight (g)	9.92^a±0.67	10.03^a±0.46	9.61^a±0.45	9.81^a±0.46
Final body weight (g)	86.79^d±2.15	106.60^c±2.08	122.27^b±3.08	131.89^a±2.94
TLG (cm)	10.02^d±0.27	11.29^c±0.10 (+12.67%)	12.12^b±0.10 (+20.96%)	12.68^a±0.10 (+26.55%)
NWG (g)	76.87^d±1.46	96.56^c±0.84 (+25.61%)	112.66^b±1.80 (+46.56%)	122.08^a±1.49 (+58.84%)
SGR (%)	1.20^c±0.01	1.32^b±0.03 (+10.00%)	1.41^a±0.00 (+17.50%)	1.44^a±0.01 (+20.00%)
Condition Factor (K-value)	1.12^c±0.02	1.15^{bc}±0.01	1.18^{ab}±0.00	1.21^a±0.00
FCR	1.80^a±0.01	1.75^b±0.01	1.70^c±0.01	1.65^d±0.03
PER	2.19^d±0.01	2.28^c±0.01	2.37^b±0.01	2.46^a±0.04
Survival (%)	92.50	95.00	95.63	96.88
Initial total biomass (g/tank)	793.92	802.67	769.07	784.69
Final total biomass (g/ tank)	6422.46	8101.35 (26.14)	9353.91 (45.64)	10221.73 (59.16)
Net fish biomass production (g/tank)	5628.54	7298.68 (29.67%)	8584.84 (52.52%)	9437.04 (67.66)

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

** Values in parenthesis indicate percentage (%) change over control.

Growth enhancement in AFP fed *L. rohita* fingerlings can be attributed to its biological properties promoting health through improvement in digestion, absorption and assimilation of food besides boosting liver health and function [25, 5, 8]. Dietary incorporation of vitamin C has been found to improve survival and growth in *L. rohita* [25, 26]. However, not much work has been done to evaluate the efficacy of AFP as feed additive in *L. rohita*. Significantly higher SGR and better FCR was recorded in tilapia (*Tilapia mossambicus*) fed with diet containing amla extract @ 1-2% inclusion levels, owing to improved palatability, digestion and absorption of nutrients [12] along with improved haematological parameters (total erythrocytes count, total leucocytes counts, haemoglobin and serum total protein level), which improved growth performance of fish due to improved immunity and health. Higher survival and enhanced growth of rohu larvae was also reported, when fed with Immuplus (an amla containing polyherb) at inclusion rate of 0.5g/kg (0.05%) feed [27]. Similarly enhanced growth was also reported in another carp species *Cyprinus carpio* (common carp) fed with ImmuPlus incorporated diets @ 1.5% [28]. Further, improved health and antibacterial activity, against *Aeromonas hydrophila* infection, was reported in gold fish (*Carassius auratus*) fed with feed containing amla leaf extract @ 25mg/g of BW for 12-15 days [10]. In another study, enhanced immunity and health was recorded in tilapia (*Oreochromis mossambicus*) fed with amla fruit extract incorporated feeds [11]. Enhanced growth (NWG and SGR) has also been reported in gold fish

(*C. auratus*) fed with feed containing dried powder of a related *Phyllanthus* species (*P. niruri*) @ 1-1.5% inclusion levels [29]. A recent study [13] has reported significantly higher growth performance (NWG, SGR and FCR) in *L. rohita* fingerlings fed with amla powder supplemented feed @ 10%. Significantly higher (P≤0.05) PER (2.28-2.46) was recorded in all the AFP fed treatments (T_1-T_3), as compared to control (2.19), which further corroborates growth promoting and feed utilization efficacy of AFP inclusion in feed of fingerlings of *L. rohita*. Enhanced PER values in AFP fed fish (T_1-T_3) indicated better utilization of protein towards muscle formation, which is important in view of high cost of protein source ingredient used in fish feed formulation. The present results indicate that, dietary supplementation of AFP in the feed enhanced growth (NWG and SGR) of *L. rohita* fingerlings with additional improvement in FCR and PER.

The results on proximate composition (flesh quality) of *L. rohita* fingerlings in terms of total protein, total lipid, total carbohydrates, ash and moisture content (Table-5) reveal that supplementation of AFP in fish feed improved flesh quality significantly (P≤0.05). Total protein, carbohydrate and ash content was recorded maximum (13.79, 3.57 and 1.51g/100g, respectively) and moisture content was minimum (78.34%) in fish fed with feed containing 3% AFP (T_3), while total lipid content was recorded maximum (2.69 g/100g, respectively) with 2% AFP feed (T_2). Although, no reports are available with respect to efficacy of AFP supplemented feed on proximate composition of *L. rohita* fingerlings, but improved flesh quality has been reported in common carp (*C. carpio*) fed with feed containing plant powder of related *Phyllanthus* species (*P. niruri*) @ 1-2% inclusion levels [30]. As amla strengthens digestion, absorption, liver function, assimilation of food and enhances protein synthesis [8], it resulted into positive growth benefits leading to improved flesh quality thereof.

Table 5: Proximate composition of flesh (on wet weight basis) of *L. rohita* fingerlings in different treatments

Parameters	Treatments			
	T_0	**T_1**	**T_2**	**T_3**
Total Protein (g/100g)	11.98^{d}±0.05	12.59^{c}±0.17	13.25^{b}±0.05	13.79^{a}±0.05
Total Lipid (g/100g)	2.12^{c}±0.04	2.17^{c}±0.04	2.69^{a}±0.04	2.46^{b}±0.05
Total Carbohydrate (g/100g)	2.42^{d}±0.03	2.91^{c}±0.04	3.38^{b}±0.04	3.57^{a}±0.04
Ash (g/100g)	1.08^{d}±0.02	1.22^{c}±0.02	1.35^{b}±0.02	1.51^{a}±0.02
Moisture (%)	81.68^{a}±0.17	80.35^{b}±0.23	78.98^{c}±0.23	78.34^{d}±0.10

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

Owing to higher survival and enhanced growth performance of fish in AFP fed treatments, 29.67, 52.52 and 67.66% higher net fish biomass was harvested from T_1, T_2 and T_3 treatments (Table-4). Feed cost increased from ₹ 17.10 in control (T_0) to ₹ 18.93, 20.76 and 22.59 per kg in 1% (T_1), 2% (T_2) and 3% (T_3) AFP supplemented feeds, respectively (Table-6).

Although, due to increased feed cost of AFP supplemented feeds, total feeding cost increased in all the AFP fed treatments (T_1-T_3), but higher biomass production (owing to enhanced fish growth), not only compensated for the additional feed cost but also resulted in 25.27, 42.46 and 51.94% addition income (with respect to feed cost) in 1% (T_1), 2% (T_2) and 3% (T_3) AFP treatments, respectively (Table-6).

Table 6: 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*)	5.63	7.30	8.58	9.44
Feed cost /kg feed (₹)	17.10	18.93	20.76	22.59
Feed cost /kg fish production (₹)	30.78	33.13	35.29	37.27
Total feed cost/tank (₹)	173.29	241.83	302.81	351.86
Total income (Fish value @ ₹ 100/kg)	563.00	730.00	858.00	944.00
Net income with respect to feed cost (₹)	389.71	488.17	555.19	592.14
Additional net income over control (%)	-	25.27	42.46	51.94

* $80m^2$

4. Conclusion

The outcome of this study reveals positive effect of AFP supplemented feeds on the survival, growth and flesh quality of *L. rohita* fingerlings. Therefore, it is concluded that amla fruit powder can be used as a growth promoting additive @ 3% inclusion level for higher growth, enhanced survival and improved flesh quality of *L. rohita* in grow out ponds.

5. Acknowledgement

The authors are thankful to Dean, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana for providing the necessary facilities for conducting the research work.

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