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Aloe barbadensis Effect on Growth Performance and Gastrointestinal Tract of *Labeo rohita*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Fish is a rich source of nutrients and help to reduce the inadequacy of calcium, vitamin A, iron, protein and cure many diseases. *Labeo rohita (*Rohu) is commercial species of Pakistan and main food item. The base of this study is to enhance the growth of *Labeo rohita* under special dietary condition. The aim of this research project was to check the effect of traditional herb *Aloe barbadensis* on Growth rate, FCR, Survival rate and Gastrointestinal villi histopathology of *Labeo rohita* fed on different *Aloe barbadensis* concentration containing diet.

Methodology: Initial stocks of 120 *Labeo rohita* fingerlings having average weight 14.30 ±3.51g were bought from Government Fish Hatchery, Faisalabad. *Aloe barbadensis* leaf's powder were prepared ad mixed with different concentration of conventional feed ingredients like such as fish meal, rice polish, corn, wheat bran. Four treatments were designed including a control and *Aloe barbadensis* incorporated in the fish feed at 0%, 05% and 10%, 15% which were administrated for a period of 180 days. This feeding trial remains continued till 180 days in aquarium. Iso nitrogenous

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diets having protein level 33±1g.45 but different concentration of *Aloe barbadensis* were prepared and fed to fingerlings. Ingredients quantity was calculated by using Pearson square method. Conventional feed ingredients i.e. fish meal, rice polish, corn and wheat brane were used in feed in pellet forn. At the end of feeding trial three fish from each aquarium were selected randomly for gastrointestinal analysis. Gastrointestinal Villi parameters like length, width of intestine villi were measured at the end of trail.

Results: According to results, different levels of *Aloe barbadensis* leave's powder had significant positive effect upon Growth, Survival rate, and FCR. Results showed that *Aloe barbadensis* at 15% (T_3) resulted in improved growth rate, Survival rate and feed conversion ratio (FCR). The *Aloe barbadensis*-treated groups showed improvement in intestinal villi length, width and gap between villi. The present study suggests that *Aloe barbadensis* especially at 15% feed administration may enhance effectively the growth performance and gastrointestinal (villi) parameters like length, width and gap between villi of *Labeo rohita*.

Keywords: Labeo rohita; histopathology; survival, proximate analysis; growth.

1. INTRODUCTION

Aquaculture products are considered as greatest source of necessary nutrients especially protein. These products provide near to 20% edible protein source in developing countries [1]. More than 50% of dietary protein and minerals requirements are fulfilled in poor countries [2]. Fish is a rich source of different nutrients and help to reduce the inadequacy of calcium, vitamin A, iron and cure many diseases [3,4]. All over the world, aquaculture is playing an essential role to raise the fisheries production. In Pakistan, the department of fisheries is also playing a significant role to reduce poverty and to achieve food security [5].

1.1 Feed Ingredients

Feed ingredients which have potential sources and quality to formulate supplemented fish feeds are very diverse for both locally produced and imported ingredients. Main feed ingredients in *Labeo rohita* fish feed, are rice bran which are in different types, fishmeal, meat bone meal, blood meal, soybean meal, soybean cake, canola meal, cassava, oils, wheat brane, corn flour etc. Many kinds of fishmeal including local fish meals with various protein percentage as 35 to 65%, also including imported fish meals. Fishmeal is normally used in order to reduce feed cost [6].

Soybean meal protein could be replaced by fishmeal protein upon 60% without amino acid supplementation and at upon 70% with protein such as methionine and lysine. It has been reported that digestibility of main feed ingredients in fish fingerlings [7].

L*abeo rohita* belongs to the carp family Cyprinidae order Cypriniformes of Actinopterygii. The body of L*abeo rohita* is bluish with black on the back and radish black on sides and silver-colored on the flanks and belly. The scales are leather, reddish or orange with center and black margins. The whole body is covered with smooth silver-colored scales which are arranged in rows. In the middle of the scales, this reddish color becomes darker and brighter in the breeding season. Fins are black-colored. Body bilaterally symmetrical, is moderately elongated, conical with cycloid scale and the abdomen is round. The dorsal profile is more arched than the abdomen. The mouth is small and downward and lips are thick and torn above and below the mouth which connect inwards. There is only one pair of barbells on the upper lip of the mouth. Eyes are large in shape, red-colored, with no eyelids and dorsolateral in position. The cornea is transparent covering by skin. The lateral line is completed and there are 40-42 fins along the line while around the caudal fin, 20 scales. They grow up to 200cm in length weight 0.001 g well-known for its food and economic importance [8].

1.2 Uses of Herbs as Growth Promoter in Fisheries

Herbaceous plant's extracts have the potential to increase disease resistance by enhancing specific and nonspecific immunity in fish .Herbs are being used against diseases, as a growth promoter, to boost stress resistance and prevent infections. Herbs are also used as immune supplements by honoring the fish's nonspecific defense mechanisms and boosting specific immunity. Herbs are not only secure for consumers but also available on a wide scale (Logambal et al., 2000). Natural plant products have anti-stress, growth, appetite stimulation tonic and immunostimulating aphrodisiac and

various antimicrobial properties with active principles such as alkaloids, flavonoids, oils, phenolics, terpenoids has been reported [9].

Several medicinal plants such as *Phyllanthus niruri, Azadirachta indica, Acalypha indica, Piper bettle, Mentha piperita, Allium sativum* and *Astragulus membraneceus* found bioactive compounds which increase growth and act as disease resistance against pathogenic bacteria in fish [10,11].

1.3 *Aloe barbadensis as* a Powerful Medicinal Herb

Aloe barbadensis (Aloe Vere) is a member of a *Liliesaceae* family. According to the "Folk medicine" of cultures around the world, it has been used as a powerful medicinal plant for centuries. Liquid's product of *Aloe barbadensis* named gel, juice and whole leaf's extract, prevalled liquid when break down the structure of the *Aloe barbadensis* leaf and to further obtain it by separating the solid residue to leave the approximately obvious solution [12].

Aloe barbadensis stem less and succulent herb which is widely distributed in the tropic and native areas. *Aloe barbadensis* genus comprised 360 species containing more than 70 biological active and popular medicinal compounds. *Aloe barbadensis* gel contains a large amount of polysaccharides, essential amino acids that the human body needs and other compounds required for skin therapy. Moreover, it also contains vitamins, minerals, enzymes, Fatty acids, salicylic acids, lignin, saponins and hormones [13]; Adesuyi et al., 2012). Medicinal properties of *Aloe barbadensis* like antibacterial, anti-septic, anti- inflammatory, immune modulatory effects, ant- oxidant [14], growth and gastrointestinal promoting effects have been examined [15]. Pharmaceutical effects like Skin lesions, antiviral, antibacterial and wound healing effects of *Aloe barbadensis* have been reported [16], (Zodape, 2010).

1.4 Role of *Aloe barbadensis* in Animal Feed

Around the world, various countries have many resources of various kinds of medicinal herbs which is used in feed additives in animals. Growth performance, improvement in immunity, improvement in intestinal micro flora and controlling particular diseases achieved by herbs, used as supplement feeds [11].

Aloe barbadensis used as resources of functional food which are yogurt and preparation of health drinks including tea (Gage, 1996; Surjushe et al, [13]; Vienna et al 2007). The botanical products used greatly utilized such as nutritional supplement to promote health and prevent diseases. The quality and safety of fresh products can be prolonging coating by *Aloe barbadensis* gel (Serrano et al, 2006). Microorganisms growth caused foodborne illness in humans and animals, also food spoilage inhibits by *Aloe barbadensis* [17]. It does not affect the food taste and appearance. *Aloe barbadensis* promise a safe, natural and environmentally alternative solution for conventional synthetic preservatives (Serrano et al, 2006). The gel internal use of like a 'dietary supplement' has approved in the United States, FDA. With Annex 1 of Regulation No 1831/2003, in European Commission (EC), the industries of feed like sensory additive functional group 'flavoring compounds', increase smell or palatability of feedings stuff by *Aloe barbadensis* (World Health Organization, [18]; Vienna et al 2007).

2. MATERIALS AND METHODS

2.1 Study Design

Complete Random Design (CRD) was used for fish sampling to evaluate the variables to be studied for growth and gastrointestinal tract.

2.2 Settings

The present study, was conducted, in Zoology Laboratory, Department of Zoology, The University of Lahore Sargodha campus.

2.3 Study Duration

The preset experiment remained continue for 6 months (180 days). During experiment, different parameters were standard to check the performance of supplement. At the end of experiment histopathology was performed to check effect of *Aloe barbadensis* on intestine of fish.

2.4 Sample Size

Initial stocks of 180 fingerlings of *Labeo Rohita* fish having average weight 14.30 $\pm$3.5 g were bought from Government Fish Hatchery, Faisalabad. Fingerlings were live transported to laboratory and were acclimatized, for fifteen

days, before start of experiment. During this period fingerlings, were keenly observed, and diseased and sick fingerlings were separated out for smooth running of experiment. After fifteen days fingerlings were divided into four groups having average weight 12.44±2.67 g, 14.37±2.80 g, 14.35±2.47 g and 12.30±2.88 g these groups were termed as T_0, T_1, T_2 and T_3 respectivelys, Fifteen fingerlings were kept in each aquarium 50 cm high and 100 cm width after their weight and length measurement. Experiment was run in triplicate. Four iso -nitrogenous treatment diets were prepared with conventional feed ingredients. Feed having protein level 33.15±1.45 was prepared and fed to fingerlings twice a day i.e 8:am and 4 p.m. @ 4% body weight. Aquarium full water was changed weekly to keep the environment safe and clean for fishes.

2.5 Sampling Techniques

Random sampling technique was used for sample selection.

2.6 Sample Selection

Sample was select on the basis of weight and length characteristics individual fingers having an average weight of 12.30 ±2.67 and length 6.80 ± 1.98 were selected for trial. During acclimatized period fishes having weight more than average were excluded from the experiment.

2.7 Preparation of *Aloe barbadensis* Powder

Aloe barbadensis leaves were bought from a plant Nursery situated in Bhakkar, Pakistan. *Aloe barbadensis* leaves were washed with sterile distilled water and identified by the Department of Botany, Lahore University, and Sargodha Campus. The fresh leaves of Aloe barbadensis were dried into electric oven at 100 C temperature after it, dried *Aloe barbadensis* leaves were grinded in an electric grinder for making the powder. The powder was stored, in a container until used.

2.8 Treatment Diets Preparation

The fish feed was prepared by using pearson square method [19]. And feed was prepared by followings [20]. Pellet alternate rehan machine and conventional feed ingredients such as fish meal, rice polish, corn, wheat bran used in feed, were grinded in Electric grinder and then mixed with dried *Aloe barbadensis* powder. These ingredients were weighed and mixed in a drum mixer with dry powder. Four treatments diets with different *Aloe barbadensis* leave concentration were prepared i.e 5%, 10%, 15% while fourth diets was control having 0% *Aloe barbadensis* powder. These diets were termed as D_0, D_1, D_2, and D_3. Distilled water along with corn starch was used as a binder to make pallets of grinded ingredients. Pellet alternate machine was used to make pallets. The pellets were dried in the air and stored in air tight container at room temperature until fed. This feed was fed to fishes, twice a day, at the 4% of body weight, for 90 days 5% of their body weight. From all groups required parameter evaluation was checked after fifteen days.

2.9 Gastrointestinal Analysis

2.9.1 Dissection

Three samples were chosen at random from each group and dissected following Batvari et al,. 2015. Dissection was started by stainless steel scalpels from the neck, then used a knife to slit the fish through the middle, avoiding deep cutting the body of the fish because it damages the internal organs. After slitting the fish, the organs were gently removed with a scalpel or tweezers. After the removal of organs, intestine was separated from the visra and opens it straightly on the clean surface. Intestines from each sample were dissected for analysis. Then, they were washed with distilled water, dried in filter paper, weighed, Preserved in formalin solution and kept at 18°C until analyzed.

2.9.2 Histopathology

Three intestinal segments (Proximal, Middle and Distal) samples were taken from intestine and cut into 1.5 cm pieces. Proximal, Middle and Distal were excised out for histopathologicaly study following [21]. Intestinal segments were washed with distilled water fixed in Bouin's solution. After fixation intestinal segments were preserved in 20% formaline solution. After treatment with Formaline solution were intestinal segments treated with 50%, 70%, 90% and 100% alcohol for 24 hours, respectively for alcoholic grading. After alcoholic grading, organs were placed in Xylene for 2-3 hours to remove remaining of alcohol. Intestinal segments were placed in mixture of Xylene and wax @ 50:50 ratio v/v for 3 hours. After three hours this mixture was heated in oven at 80 ^oC and intestinal segments

tissue was excised out and dipped in pure wax. This wax was melted and then again freeze dried to make blocks for cutting with microtome. A wooden block attached with paraffin block and it was placed for hardness 1-2 days, now it was ready to use in microtome. Machine oil was used to improve microtome functioning. Wooden block was adjusted into holders and a sharp blade was adjusted in to shafts. Microtome was adjusted at 6µ and flywheel was rotated anticlockwise with hands. Sections obtained after microtome cutting were stained with Hematoxylin and Eosin solution. At last scale use to sections were covered with cover slips and were viewed under microscope at 10×, 40× and 100×.

2.10 Statistical Analysis

Recorded data was analyzed by SPSS software ver. (19). One way ANOVA (Analysis of variance) was performed to check difference of mean values. Tukey's post- hoc test was applied [22].

3. RESULTS

The success of aquaculture depends on a number of parameters, the most important is a suitable diet that contains the complete balanced nutritional diet for the average growth of fish (Salehi, 2008).

The aim of this research was to investigate the effect of aloe badbadiensis extract mingles diet on the growth and reproductive performance of Labeo rohita*.* According to results of the present study, FCR decreased in T_2 as compared to other treatments. *Foeniculum vulgare* seed extract contains the Anethole and Estragole perform the function as a digestive stimulant may be a reason for the decrease in FCR [23].

More than this growth of *Poecilia reticulata* significantly ($p < 0.05$) + increase in T_2. The growth parameter, the survival rate was uniformly the same in all treatments which are about 100% in all treatments.

This justified that *Foeniculum vulgare* has no ($p > 0.05$) effect on the survival rate. There are similarities between the current study and those investigated by Sotoudeh & Yeganeh (2017). The finding of the research is also co-related with the work of Yilmaz et al. [24]. His research investigated that dietary thyme improved the growth performance of *Dicentrarchus labrax*.

Water parameters recorded during the experiment were temperature, alkalinity, dissolved oxygen, TDS, TSS, and EC. Water temperature recorded during the experiment was range from 24^0C to 30^0C.

Environmental parameter, the temperature was accordingly to the work of Dawes, (1991). The results of this study are congruous with the finding of Yilmaz et al. [24]. They evaluated that a dietary cumin level of 1% provides the best survival rate for tilapia, *Oreochromis mossambicus*, with no inauspicious effect on growth performance simultaneously.

Tribulus Terrestris showed the same results with a significant difference ($P<0.05$) in weight gain (Yeganeh et al., 2017). The present investigation showed similar findings as *Thymus vulgaris, Rosmarinus officinalis,* and *Trigonella foenum graecum* had a positive response towards the growth performance of *Dicentrarchus labrax* [25].

After the chemical analysis of *Foeniculum vulgare* seed extract resulted that *Foeniculum vulgare* extract contains the trans-anethole (64.49%) which is similar in structure to 17-beta-estradiol.

The presence of trans-anethole may cause a significant increase in estrogenic hormones; lead to an increase in reproductive performance and fecundity (Albert-Puleo, 1980). An increase in the reproduction activities of *Poecilia reticulata* fed feed containing *F.vulgare* seed extract, a reason for increasing the level of estrogenic hormones lead to increase the reproductive performance. A similar result was reported by Nazari and Roozbehani, [22]. Their finding showed that the fertility rate of *Poecilia reticulata* enhanced when used *Foeniculum vulgare* extract in the diet.

Among dietary treatments used in this research, T_2 show positive increase in GSI (8.69 ± 0.2), Fecundity (9.4 ± 0.19), and hatching rate. Dada and Adeparusi (2012) intimate that diets with *Sesa-mum indicum* supplement and seed powder of *Croton zambesicus* were improved female *C. gariepinus* GSI.

Dada and Ajilore (2009) distinguished an increase in egg diameter and fecundity of *C. gariepinus* when treated with *Garcinia* seeds extract. Sadeghpour et al. (2015) commented that increase in serum level estrogen in mice female when injected with extracts of *Foeniculum vulgare.*

The findings of another research are also consistent with this current research, fecundity of guppy was directly proportional to the bodyweight of the fish, which means that the fecundity increased with the increase in body weight (Shahjahan et al., 2013). The use of injection of ovaprim shown the highest fecundity of *Pterophyllum scalare* suggested by Chatterjee, Patra, and Talwar, (2013).

Ghosh et al. (2007) reported that incorporation of probiotics in feed influenced the reproductive performance of livebearers in terms of high fecundity, high Gonadosomatic index, high fry survival rate, reduction in fry mortality and deformity, and higher average weight and length gain of fish fry.

Nielsen and Baatrup, [26] treated *Poecilia reticulata* with estrogens that enter into the aquatic system, results of this research showed that no significant difference was seen in the Gonadosomatic index (GSI).

A similar observation was found with *Cyprinus carpio* when treated with *Ferula coskunii* [24]. Current research finding indicated that *Poecilia reticulata* treated with *Foeniculum vulgare* seed extract*,* increased number of young's ones produced by guppy fish in T_2 were 91±2.1 while the control group produced 45±2.5. Dada (2012) investigated that feed supplement with *G. kola* seed powder improved hatchability and fecundity of *C. gariepinus*.

Successful spawning of *C. punctatus* at 0.3and 0.5 ml/kg and 3000IU/kg weight of HCG was noted by Kather and Sridhar (2002). For *H.fossilis* the utilization of ovaprim came about greatest Successful body spawning (Chatterjee et al., 2013). Effective spawning of *C. punctatus* is when treated with HCG. *Natrum muriaticum* showed a positive effect on the spawning performance of *Poecilia reticulata* (Sudha and Gokula, 2018).

The findings of previous research are similar to the results of the current investigation. There are also similarities between the work of (Sudha and Gokula, 2015) when *Puntius conchonius* treated with similar herbal medicine *Natrum muriaticum* on spawning response.

The fish feed was prepared by using person square method. Concentration of different ingredients is given in Table 1. Ingredients concentration was almost same in all test diets whereas *Aloe barbadensis* concentration varied. Three diets having 32% protein were prepared containing 5% *Aloe barbadensis,* in T_1, 10% *Aloe barbadensis* in T_2 ,15% *Aloe barbadensis* in T_3 were labeled and diet without *Aloe barbadensis* served as control T_0. Conventional feed ingredients such as fish meal, wheat brane, rice polish and corn flour were weighed and mixed in a drum mixer.

3.1 Proximate of Diet

All treatments diets were almost iso-nitrogenous having same protein level Table 2. Protein contents among T_0, T_1, T_2, and T_3 were recorded as 31.49±1.06, 32±1.56, 31.40±1.26 and 33.30±1.38, respectively. statistical analysis showed that proximate composition not consistent as values vary from case to case, ($p<0.05$) in all treatments The level of protein in our experimental diet is in accordance with Abdel-Tawwab et al. (2010) who found that growth of fish is influenced by dietary protein level and their initial body weight. Whereas, Feed conversion ratio and feed intake is also affected by levels of protein only. FCR and food intake

Table 1. Concentration of different ingredients used in different diets

Ingredients	**T_0**	**T_1**	**T_2**	**T_3**
Fish meal %	35.50	34	32.50	30
Wheat Brane %	35.50	34	32.50	30
Rice Polish %	**12**	12	12	12
Corn flour %	14	13	12	12
Vitamin premix%	0.5	0.5	0.5	0.5
Mineral premix %	0.5	0.5	0.5	0.5
Aloe barbadensis %	00	05	10	15
Total	100	100	100	100

Table 2. Proximate analysis of prepared treatment diets

Parameters	Control (T_0)	5%*Aloe barbadensis* (T_1)	10%*Aloe barbadensis* (T_2)	15%*Aloe barbadensis* (T_3)
Moisture	5.91±1.02 [c]	8.56±1.5[a]	6.94±1.22[b]	6.02±1.34[bc]
Crude values missed protein	31.49±1.06 [c]	32.05±1.56 [b]	31.40±1.26 [c]	33.30±1.38 [a]
Ether extract	06.02±1.04 [b]	06.02±1.58 [b]	06.44±1.28 [c]	05.36±1.34 [a]
Ash	10.97±1.08 [c]	12.24±1.54 [a]	11.97±1.24 [a]	11.44±1.32 [b]
Dry matter	94.09±1.06 [a]	91.44±1.52 [c]	93.06±1.24 [b]	93.98±36 [b]

The values given are mean with, standard deviation. Means with, the same letter in the same column, are significant

ratio were also related to initial body weight and level of dietary protein. As the age and weight of fish increased, requirement for protein decreased and advanced juveniles and fingerlings of *Labeo rohita* cannot use excessive protein efficiently.

3.2 Water Quality Parameters

The recorded values indicate that, *Aloe barbadensis* leaves mingled diets has no effect on water quality. Highest TSS were recorded as 82.66±2.85 and lowest value was 79.02±2.79 and hardness values were 113.2±5 in T_2 to 109.4±5.46 in T_0, statistical analysis of TSS and hardness ($P<0.05$) shows that amount of TSS and hardness were significant differed ($p<0.05$) in all treatments. Highest TDS were 536.6±6.25 and lowest TDS were 521.6±6.28. TDS were increased and a significant difference was studied between control and experimental treatments. Electric conductivity vary according to area and also vary according to seasonal variations, statistical analysis shows that amount of Conductivity was significantly differed ($p<0.05$) in all treatments.

3.3 Growth Performance Parameters

Growth parameters i.e. weight gain, Length gain, FCR and survival rate were recorded fortnightly. The recorded values of growth parameters are given in Table 4. Weight gain was recorded as 8.88±1.46, 13.04±2.26, 14.60±1.95 and 15.17±2.70 in T_0, T_1, T_2 and T_3 respectively. Weight gain increased as level of *Aloe barbadensis* increase. Statistical analysis showed that T_0 and T_1 show significant difference ($P\leq.0.05$) between weight gain, while weight gain

Table 3. Water quality parameters recorded during project

S.no	Parameters	Treatments			
		Control	T_1	T_2	T_3
1	Temperature^{0}C	28.8 ± 0.75[a]	28.4 ± 0.80 [a]	28.2 ± 0.40 [a]	28.6 ± 0.48 [a]
2	DO(mg/l)	5.30 ± 0.11[a]	5. 88± 0.09[b]	5.96 ± 0.08 [b]	5.76 ± 0.12[ab]
3	pH	7.2 ± 0.31[a]	7.4 ± 0.25[a]	7.5 ± 0.13[a]	7.3 ± 0.28[a]
4	TDS (Total dissolved solvents)(mg/l)	531.6 ±6.22[c]	536.6 ± 6.25 [a]	534.4. ± 6.37[b]	521.6 ± 6.28[d]
5	Conductivity(us/cm)	726.8±11.9 [a]	722.4 ±11.42 [b]	719.2±11.87 [c]	717.6± 11.22[d]
6	TSS(Total suspended solid) (mg/l)	82.06 ±2.36 [a]	79.02±2.79 [c]	81.34±2.84 [ab]	82.66±2.85 [d]
7	Hardness(mg/l)	109.4±5.46 [b]	112.6±5.57 [ab]	113.2±5.63 [a]	110.8±5.97 [a]

Note: Values given in table are mean with standard. Means with, the same letter in the same column, are significant

Table 4. Growth performance of *Labeo rohita,* fed on different diets treatments for 90 days

Treatments	Total weight gain	Total length gain	FCR	Survival rate
T_0	8.88±1.46[c]	4.51±0.89[b]	4.18±0.61[a]	100±0.00
T_1	13.04±2.26[b]	4.72±0.48[b]	3.04±0.85[b]	100±0.00
T_2	14.60±1.95[a]	5.15±0.32[a]	3.61±0.51[bc]	100±0.00
T_3	15.17±2.70[a]	5.40±0.40[a]	2.63±0.91[b]	100±0.00

Note: Values given are mean ± standard error. The numbers among same row having different alphabet are significantly different (P<0.05)

among treatments, T_2 and T_3 showed none significant difference (P≤.0.05). Length gain was recorded as 4.51 ± 0.89, 4.72 ± 0.48, 5.15 ± 0.32 and 5.40 ± 0.40 in treatments T_0, T_1, T_2 and T_3 respectively. Statistical analysis showed that lengths gain among T_2 and T_3 were none significantly different while T_0 and T_1 length gain were significantly differed from T_2 and T_3 (P<0.05). The recorded values show that as the concentration of *Aloe barbadensis* increases the weight and length also increas. Growth performance results showed that with a concentration of 15% of *Aloe barbadensis* containing had a significant effect (P<.0.05) on the growth performance of *Labeo rohita* (Fig. 1).

3.3.1 Feed conversation ratio

According to the results, presented in Table 4, different concentration of *Aloe barbadensis* had positive effect on FCR of *Labeo rohita*. FCR values were recorded as T_0 There was a significant decrease in FCR value of T_4. FCR value was highest in T_0 while it was lowest in T_3. The results indicate that T_0, T_1 and T_2 were none significantly differing from each and while other T_3 was significantly differed from T_0, T_1 The results about FCR showed that *A. barbadensis* also affect the Feed conversion ratio (Fig. 2).

3.3.2 Survival rate

A.barbadensis powder had no negative effect on Survival rate of *Labeo rohita* in every experimental groups. Survival rate in all experimental and control were consistently high (P > 0.05). Survival rate of all groups were recorded as 100 %. It was inferred that *Aloe barbadensis* powder had no adverse consequence on Survival rate of *Labeo rohita*.

3.3.3 *Aloe barbadensis* effect on survival rate in all treated groups 100% survival.

Growth rate increased was observed fed with various Aloe *barbadensis* concentration (T_0, fed with no *Aloe barbadensis*) (T_1, fed with 5% *Aloe barbadensis*) (T_2, fed with 10% *Aloe barbadensis*) (T_3, fed with 15% *Aloe barbadensis*). According to the calculations, the best results were shown by feeding with 15% *Aloe barbadensis* (T_4). According to the Statistical analysis results with different concentration, of *Aloe barbadensis,* had positive effect on Growth performance, FCR and survival rates, on the *Labeo rohita* in experimental treatments.

3.4 Gasterointestinal Parameters

Like other carps, normal distal intestinal cross session of *Labeo Rohita* shows that Intestine villi length (VL), width (VW) and gap between villi were affected by dietary treatment. Villi length and width in fish fed containing 40% protein, in freshwater were found to be higher than those fed with other diets. VW in the fish fed, with a diet containing 30% and 35% protein, in freshwater was found to be similar to each other. In freshwater, the intestine villi length significantly increased by increasing dietary protein levels. In our present research work gaps between villi, VL and VW were observed in 60 days. According to Table 5 as the level of Aloe Vera increased gaps between villus increased and widest villus gap was observed in T_3(17.18±1.48) whereas narrowest villus gap was observed in T_3 (17.18±1.48). Statistical analysis shows T_3 is significantly differed from T_0, T_1 and T_2 while T_3 and T_3 were none significantly differed. VW was observed as (41.38±0.25), (45.25±0.34), (47.36±0.36) and (51.41±0.54) in treatments T_0, T_1, T_2 and T_3 respectively. The observed value shows that VW increased as Aloe vera level increase. Statistical analysis showed that maximum VW was observed in T_3 while minimum VW was recorded in T_0, respectively. Length of villi was measured as (212±0.14), (223±0.27), (237±0.34) and (245±0.48) in treatments T_0, T_1, T_2 and T_3 respectively. Statistical analysis showed, the maximum Villi length was measured with high level of Aloe vera groups T_3 whereas minimum villi length was observed in low Aloe vera groups T_0. Results

Table 5. Gap between villi, Width of villi, Length of villi of *Labeo rohita* fingerlings

Treatments	Gap between villi (µm)	Width of villi (µm)	length of villi (µm)
T_0	$08.12±1.12^{c}$	$41.38±0.25^{c}$	$212±0.14^{c}$
T_1	$11.15±1.28^{b}$	$45.25±0.34^{b}$	$223±0.27^{c}$
T_2	$14.16±1.34^{ab}$	$47.36±0.36^{b}$	$237±0.34^{b}$
T_3	$17.18±1.48^{a}$	$51.41±0.54^{a}$	$245±0.48^{a}$

Note: Values (mean SEM), superscripted by different alphabets, within the same line are significantly different (P < 0.05)

indicates with increasing level of *Aloe barbadensis* , length, Width, gap of villi increased which showed that *Aloe barbadensis* powder with 15% concentration in diet has significant results ($P<0.05$).

4. DISCUSSION

The success of aquaculture depends on a number of parameters, including a balanced diet that contains the complete balanced nutritional diet for healthy fish (Salehi, 2008). The present research investigated that *Aloe barbadensis* containing diet has the ability to improve growth performance, FCR and Survival rate of *Labeo rohita.* It has been already reported in previous studies that herbs stimulator present in plants extract work to enhance the activity of enzyme involved in digestion [23]. Furthermore, in previous work it has been found that, better growth performance of *Cyprinus carpio* within herb supplemented diet. Also reported *Aloe barbadensis* extract like a growth promoter, appetite stimulator, tonic, and immunostimulant in the diet, can reduce stress, reduce food losses and protect fish in order to better growth (Mahdavi et al. 2013).

Fish feed ingredients such as fish meal, meat, bone meal, shrimp meal and three plant proteins ingredients such as soybean meal, mustard oilcake and rice polish were selected in the study for supplemented feed [3]. The conventional feed prepared in the current study by using fish meal, rice polish, wheat brane and corn flour safely in fish feed with different levels of *Aloe barbadensis.*

Normal fish diet was used to prepared formulated fish feed with 50 percentage crude protein, 18 percentage crude lipid, 1.9 percentage fiber, 1.3percentage, total phosphorus, 8.3 percentage ashes, and 14.8 percentage nitrogen. The extract of dried *Aloe barbadensis* was used at a ratio 1 percentage of weight in feed [20]. In current study, Proximate analysis in which Protein contents among T_0, T_1, T_2, and T_3 were recorded as 31.49, 32, 31.40 and 33.30, respectively. Moisture, 05.91, 8.56, 6.94and 6.02, Ether Extract, 06.02, 06.02, 06.44 and 05.36, Dry matter, 94.09, 91.44, 93.06 and 93.98, Ash contents were recorded as T_1 (10.97), T_2 (12.24), T_3 (11.97) and T_4 (11.44), respectively.

Water temperature (°C), DO (mg/1), pH and TDS (Total dissolved solvents), TSS (Total suspended solids), hardness and electivity remained consistent within treatments (Table 3) and were within acceptable limits for optimum growth of *Labeo rohita* fingerlings [1]. Consistency and uniformity of water quality parameters among treatments revealed that the presence of *Aloe barbadensis* did not have any bearing on water quality.

On the base of present research we concluded that *Aloe barbadensis* in dose (15%) has the potential to use as feed additives for fish. We can say that plants used as medicine will be effect tool to attain the goal of safe production and economy. Growth performance improvement by *Aloe barbadensis* compared to better nutrient digestibility and absorption, improved digestive enzymes, and maintaining the functions, and structure of the small intestine, lead to improve digestive capacity of the gut. The main goals of this study is to investigate the effect of *Aloe barbadensis* on the development, growth and histopathology of *Labeo rohita*. In previous work, *Aloe barbadensis* potentially active compounds: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids [13]. It has been found that, dietary *Aloe barbadensis* dietary feed affected on growth haemato-biochemical, and disease resistance, against *Streptococcus iniae* in tilapia. Fish fed with 0.5, 1, and 2% *Aloe barbadensis* supplemented diet had significantly higher ($p<0.05$) weight gain (Gabriel, et al. 2015).

The present study shows with increasing level of *Aloe barbadensis*, weight gain and length gain increased whereas increasing level of *Aloe barbadensis* reduce the FCR. Likewise in present study, FCR decreased in T_4 as compared to other groups. More than this growth of *Labeo rohita* is significantly increase in T_4. The growth parameter, survival rate was uniformly same in all treatments which is about 100% in all groups. This revealed that *Aloe barbadensis* has no significantly negative effect on the survival rate. In previous work, although supplemented diet with 0.5% and 1% ACE were significantly, increased the serum bactericidal activity, against *A. hydrophila* ($p<0.05$), diet supplemented with 0.1% had no stimulating effect on serum bactericidal activity compared to the control group. In the similar way, [27], in tilapia, and (Misra et al., 2006), in Indian major carp (*Labeo rohita*) stated, an enhance in serum bactericidal activity.

Present study concluded that *Aloe barbadensis* leaves have a significant effect on the histopathology of *Labeo rohita.* Under the effect

of dietary treatment of *Aloe barbadensis* leaves has a significant increase in length of villi, width of villi, gap between villi, so the highest potency of absorption was increase in fish fed with T_4 (15%) *Aloe barbadensis.* Villi specialized tissues, to do the absorption in the small intestine, they have a thin wall, about one-cell thick known as enterocyte [28]; (Oxley et al., 2007). They have a huge surface area due to their 'loops-like' shape, more efficient absorption of nutrients with in the blood stream [10]. Maximum numbers of villi which needed to do the maximum absorption [10]. Similarly,villi lengths oin anterior intestine were significantly taller. To provide surface area for absorption of nutrient-rich feed particles more efficiently [29,10]. In agreement , conclusion that absorption of nutrients like protein, carbohydrate and lipid occurred at a faster rate in proximal or anterior regions of intestine [14]; Buddington and Diamond 1987; Dabrowski, [21]; Bakke-McKellep et al., [10]; Jutfelt et al., 2007. Our study. *Aloe barbadensis* increased the villus length and increased villus width in fish intestine at varios levels of administration. Feeding of different immuno stimulants to fish species, increased villus height, fold height, enterocyte height, and reportedly augmenting surface area of the gut mucosa was observed [25,30,31]. A number of anatomical features determine the total absorptive surface area of the gastrointestinal tract. Taller, narrower, and regularly shaped villi and higher number of villi per unit area are indicators that the function of the intestinal villi is activated [32-45]; (Miles et al., 2006). In general, these villi provided large surface area for absorption of available nutrients [46-57].

5. CONCLUSION

The present study, demonstrated that *Aloe barbadensis* has positive effect on *L.rohita* growth performance and gastrointestinal tract. *Aloe barbadensis* has a signifance effect on growth parameters. Increasing the level of *Aloe barbadensis* in fish feed. Growth performance and intestinal villi such as length, width between villi of the fish was increased. Considering that Aloe barbadensis leaves powders withy in the diet significantly effective for fish. The better growth performance was recorded in treatment T_3 (15%/kg feed *Aloe barbadensis*).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ANNEX 1

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