

## Original Research Article

# **Aloe barbadensis EFFECT ON GROWTH PERFORMANCE AND GASTROINTESTINAL TRACT OF *Labeo rohita***

### **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  $14.30 \pm 3.51$ g were bought from Government Fish Hatchery, Faisalabad. *Aloe barbadensis* leaf's powder were prepared and 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 90 days. This feeding trial remains continued till 180 days. Iso nitrogenous diets having protein level  $33 \pm 1.45$  but different concentration of *Aloe barbadensis* were prepared and fed to fingerlings. Ingredients quantity was

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**Comment [a2]:** Unit?

calculated by using Pearson square method. Conventional feed ingredients i.e. fish meal, rice polish, corn and wheat brane were used in feed form. 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<sub>3</sub>) 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 (Bene et al., 2007). More than 50% of dietary protein and minerals requirements are fulfilled in poor countries (Richardson et al., 2011). Fish is a rich source of different nutrients and help to reduce the inadequacy of calcium, vitamin A, iron and cure many diseases (Shah et al., 2018; Harris, 2004). 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 (Suplicy et al., 2017).

### 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 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 protein various protein percentage as 35 to 65%, also including imported fish meals. Fishmeal is normally used in order to reduce feed cost (Alam et al, 2017).

Soybean meal protein could be replaced by fishmeal protein upon 60% without amino acid supplementation and at upon 70% with amino acid such as methionine and lysine. It has been reported that digestibility of main feed ingredients in fish fingerlings (Tran et al. 2010).

**Comment [a3]:** Explain exactly

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**Comment [a5]:** 2023?

**Comment [a6]:** Which species?

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*Labeo rohita* belongs to the carp family Cyprinidae order Cypriniformes of Actinopterygii. The body of *Labeo 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 and well-known for its food and economic importance (Datta and Sarivastava., 1988).

#### 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 (Chitrarasu, 2002).

Several medicinal plants such as *Phyllanthus niruri*, *Azadirachta indica*, *Acalypha indica*, *Piper bettle*, *Mentha piperita*, *Allium sativum* and *Astragalus membranaceus* found bioactive compounds which increase growth and act as disease resistance against pathogenic bacteria in fish (Jegade, 2012; Zahran, 2014).

#### *Aloe barbadensis* as a powerful medicinal herb

*Aloe barbadensis* (Aloe Vere) is a member of a *Liliaceae* 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, prevailed 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 (Plaskett., 1996).

*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 (Surjusheet al., 2008; Adesuyi et

**Comment [a9]:** At what age and weight do males and females become adults? How to catch? The amount of production-catch-breeding in Pakistan and the world? Cost price and sale price?

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al., 2012). Medicinal properties of *Aloe barbadensis* like antibacterial , anti-septic , anti- inflammatory , immune modulatory effects , ant- oxidant (Poorfarid et al., 2012), growth and gastrointestinal promoting effects have been examined (Heidarieh et al., 2013). Pharmaceutical effects like Skin lesions, antiviral, antibacterial and wound healing effects of *Aloe barbadensis* have been reported (Alishahi et al. , 2010: Zodape, 2010)

#### **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 natural 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. (Christaki and Florou-Paneri, 2010).

*Aloe barbadensis* used as resources of functional food which are yogurt and preparation of health drinks including tea (Gage, 1996; Surjushe et al, 2008; 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* (Eshun and He 2004). 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 I 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, 1999; Vienna et al 2007).

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## **2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY**

### **STUDY DESIGN**

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

### **3.2 SETTINGS**

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

### **3.3 STUDY DURATION**

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

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### 3.4 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  $12.44 \pm 2.67$ g,  $14.37 \pm 2.80$ g,  $14.35 \pm 2.47$ g and  $12.30 \pm 2.88$ g these groups were termed as T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively.

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Fifteen fingerlings were kept in each aquarium 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  $33.15 \pm 1.45$  was prepared and fed to fingerlings twice a day i.e 8:am and 4 p.m. @ 4% body weight. Aquarium water was changed weekly to keep the environment safe and clean for fishes.

**Comment [a18]:** Dimensions and type of aquarium?

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**Comment [a20]:** Were waste materials siphoned?  
Number of fish in each treatment? Aeration? Light regime?

### 3.5: SAMPLING TECHNIQUES

Random sampling technique was used for sample selection.

### 3.6: SAMPLE SELECTION

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

### 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, 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.

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### TREATMENT DIETS PREPARATION

The fish feed was prepared by using pearson square method (Wagner & Stanton, 2012). And feed was prepared by followings (Haghighi et al, 2014). Pellet alternate 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

**Comment [a26]:** Which company

fourth diets was control having 0% *Aloe barbadensis* powder. These diets were termed as D<sub>0</sub>, D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub>. 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. From all groups required parameter evaluation was checked after fifteen days.

## GASTROINTESTINAL ANALYSIS

### 3.13.1 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 visraand 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.

### 3.13.2 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 histopathologically study following (Aldoghachi et al., 2015). 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 °C 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 sections were covered with cover slips and were viewed under microscope at 10×, 40× and 100×.

## STATISTICAL ANALYSIS

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Comment [a28]: Proximate composition and percentage of ingredients of the diet?

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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. (Nazari and Roozbehani, 2015).

### 3. RESULTS AND DISCUSSION

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 *F. vulgare* extract mingles diet on the growth and reproductive performance of *Poeciliareticulata*. According to results of the present study, FCR decreased in T<sub>2</sub> as compared to other treatments. *Foeniculumvulgare* seed extract contains the Anethole and Estragole perform the function as a digestive stimulant may be a reason for the decrease in FCR (Frankic et al., 2009).

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Comment [a34]: *Aloe barbadensis*???

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More than this growth of *Poeciliareticulata* significantly ( $p < 0.05$ ) positive increase in T<sub>2</sub>. The growth parameter, the survival rate was uniformly the same in all treatments which are about 100% in all treatments.

Comment [a36]: Re-wright

This justified that *Foeniculumvulgare* 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. (2012). His research investigated that dietary thyme improved the growth performance of *Dicentrarchuslabrax*.

Comment [a37]:

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°C to 30°C.

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, (2012). They evaluated that a dietary cumin level of 1% provides the best survival rate for tilapia, *Oreochromismossambicus*, with no inauspicious effect on growth performance simultaneously.

*TribulusTerrestris* 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*, *Rosmarinusofficinalis*, and *Trigonellafoenumgraecum* had a positive response towards the growth performance of *Dicentrarchuslabrax* (Yilmaz et al., 2010).

After the chemical analysis of *Foeniculumvulgare* seed extract resulted that *Foeniculumvulgare* 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 *Poeciliareticulata* 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, (2015). Their finding showed that the fertility rate of *Poeciliareticulata* enhanced when used *Foeniculumvulgare* extract in the diet.

Among dietary treatments used in this research, T<sub>2</sub> show positive increase in GSI ( $8.69 \pm 0.2$ ), Fecundity ( $9.4 \pm 0.19$ ), 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 *Foeniculumvulgare*.

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 *Pterophyllumscalare* 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, (2006) treated *Poeciliareticulata* 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* (Yilmaz et al., 2006). Current research finding indicated that *Poeciliareticulata* treated with *Foeniculumvulgare* seed extract, increased number of young's ones produced by guppy fish in

T<sub>2</sub> 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 0.3 and 0.5 ml/kg and 3000 IU/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. *Natrummuriaticum* showed a positive effect on the spawning performance of *Poeciliareticulata* (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 conchoniis* treated with similar herbal medicine *Natrummuriaticum* on spawning response.

**Table 1: Concentration of different ingredients used in different diets.**

Ingredients	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Fish meal %	35.50	34	32.50	30
Wheat Brane %	35.50	34	32.50	30
Rice Polish %	14	13	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
<i>Aloe barbadensis</i> %	00	05	10	15
Total	100	100	100	100

Comment [a38]: Why are the values different?

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<sub>1</sub>, 10% *Aloe barbadensis* in T<sub>2</sub>, 15% *Aloe barbadensis* in T<sub>3</sub> were labeled and diet without *Aloe barbadensis* served as control T<sub>0</sub>. Conventional feed ingredients such as fish meal, wheat brane, rice polish and corn flour were weighed and mixed in a drum mixer.

Proximate of Diet

**Table 2: Proximate analysis of prepared treatment diets**

Parameters	Control (T <sub>0</sub> )	5% <i>Aloe barbadensis</i> (T <sub>1</sub> )	10% <i>Aloe barbadensis</i> (T <sub>2</sub> )	15% <i>Aloe barbadensis</i> (T <sub>3</sub> )

Moisture	5.91±1.02 <sup>c</sup>	8.56±1.5 <sup>a</sup>	6.94±1.22 <sup>b</sup>	6.02±1.34 <sup>bc</sup>
Crude				
VALUES				
MISSED	31.49±1.06 <sup>c</sup>	32.05±1.56 <sup>b</sup>	31.40±1.26 <sup>c</sup>	33.30±1.38 <sup>a</sup>
protein				
Ether				
Extract	06.02±1.04 <sup>b</sup>	06.02±1.58 <sup>b</sup>	06.44±1.28 <sup>c</sup>	05.36±1.34 <sup>a</sup>
Ash	10.97±1.08 <sup>c</sup>	12.24±1.54 <sup>a</sup>	11.97±1.24 <sup>a</sup>	11.44±1.32 <sup>b</sup>
Dry matter	94.09±1.06 <sup>a</sup>	91.44±1.52 <sup>c</sup>	93.06±1.24 <sup>b</sup>	93.98±36 <sup>b</sup>

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

All treatments diets were almost iso-nitrogenous having same protein level Table 2. Protein contents among T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> 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 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.

#### Water Quality Parameters

**Table 3: Water quality parameters recorded during project.**

S.no	Parameters	Treatments			
		Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
1	Temperature <sup>0</sup> C	28.8 ± 0.75 <sup>a</sup>	28.4 ± 0.80 <sup>a</sup>	28.2 ± 0.40 <sup>a</sup>	28.6 ± 0.48 <sup>a</sup>
2	DO(mg/l)	5.30 ± 0.11 <sup>a</sup>	5.88± 0.09 <sup>b</sup>	5.96 ± 0.08 <sup>b</sup>	5.76 ± 0.12 <sup>ab</sup>
3	pH	7.2 ± 0.31 <sup>a</sup>	7.4 ± 0.25 <sup>a</sup>	7.5 ± 0.13 <sup>a</sup>	7.3 ± 0.28 <sup>a</sup>
4	TDS (Total dissolved	531.6	536.6 ±	534.4. ±	521.6 ±

	solvents)(mg/l)	$\pm 6.22^c$	6.25 <sup>a</sup>	6.37 <sup>b</sup>	6.28 <sup>d</sup>
5	Conductivity(us/cm)	$726.8 \pm 11.9$ a	722.4 $\pm 11.42^b$	$719.2 \pm 11.87$ c	$717.6 \pm 11.22^d$
6	TSS(Total suspended solid)(mg/l)	$82.06 \pm 2.36$ a	$79.02 \pm 2.79^c$	$81.34 \pm 2.84^{ab}$	$82.66 \pm 2.85^d$
7	Hardness(mg/l)	$109.4 \pm 5.46$ b	$112.6 \pm 5.57$ ab	$113.2 \pm 5.63^a$	$110.8 \pm 5.97^a$

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

The recorded values indicate that, *Aloe barbadensis* leaves mingled diets has no effect on water quality. Highest TSS were recorded as  $82.66 \pm 2.85$  and lowest value was  $79.02 \pm 2.79$  and hardness values were  $113.2 \pm 5$  in T<sub>2</sub> to  $109.4 \pm 5.46$  in T<sub>0</sub>, 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  $536.6 \pm 6.25$  and lowest TDS were  $521.6 \pm 6.28$ . 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.

### 3.1 GROWTH PERFORMANCE PARAMETERS

**Table 4: Growth performance of *Labeo rohita*, fed on different diets treatments**

Comment [a39]: During 90 day culture period

Treatments	Total weight gain	Total length gain	FCR	Survival rate
T <sub>0</sub>	$8.88 \pm 1.46^c$	$4.51 \pm 0.89^b$	$4.18 \pm 0.61^a$	100±0.00
T <sub>1</sub>	$13.04 \pm 2.26^b$	$4.72 \pm 0.48^b$	$3.04 \pm 0.85^b$	100±0.00
T <sub>2</sub>	$14.60 \pm 1.95^a$	$5.15 \pm 0.32^a$	$3.61 \pm 0.51^{bc}$	100±0.00
T <sub>3</sub>	$15.17 \pm 2.70^a$	$5.40 \pm 0.40^a$	$2.63 \pm 0.91^b$	100±0.00

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

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  $8.88 \pm 1.46$ ,  $13.04 \pm 2.26$ ,  $14.60 \pm 1.95$  and  $15.17 \pm 2.70$  in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. Weight gain increased as level of *Aloe*

Comment [a40]: Not bold

*barbadensis* increase. Statistical analysis showed that T<sub>0</sub> and T<sub>1</sub> show significant difference (P≤0.05) between weight gain, while weight gain among treatments, T<sub>2</sub> and T<sub>3</sub> 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<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. Statistical analysis showed that lengths gain among T<sub>2</sub> and T<sub>3</sub> were none significantly different while T<sub>0</sub> and T<sub>1</sub> length gain were significantly differed from T<sub>2</sub> and T<sub>3</sub> (P<0.05). The recorded values show that as the concentration of *Aloe barbadensis* increases the weight and length also increases. 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).

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#### Feed Conversion 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<sub>0</sub> There was a significant decrease in FCR value of T<sub>4</sub>. FCR value was highest in T<sub>0</sub> while it was lowest in T<sub>3</sub>. The results indicate that T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> were none significantly differing from each and while other T<sub>3</sub> was significantly differed from T<sub>0</sub>, T<sub>1</sub> The results about FCR showed that *Aloe barbadensis* also affect the Feed conversion ratio (fig 2).

Comment [a42]: o

#### 4.1.2 Survival rate

*Aloe 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*.

Comment [a43]: *Aloe barbadensis* for the first time and then *A. barbadensis*

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

Growth rate increased was observed fed with various *Aloe barbadensis* concentration (T<sub>0</sub>, fed with no *Aloe barbadensis*) (T<sub>1</sub>, fed with 5% *Aloe barbadensis*) (T<sub>2</sub>, fed with 10% *Aloe barbadensis*) (T<sub>3</sub>, fed with 15% *Aloe barbadensis*). According to the calculations, the best results were shown by feeding with 15% *Aloe barbadensis* (T<sub>4</sub>). 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* experimental treatments

## GASTEROINTESTINAL PARAMETERS

Like other carps, normal distal intestinal cross section 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 No 5 as the level of Aloe Vera increased gaps between villus increased and widest villus gap was observed in T<sub>3</sub>(17.18±1.48) whereas narrowest villus gap was observed in T<sub>0</sub> (17.18±1.48). Statistical analysis shows T<sub>3</sub> is significantly differed from T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> while T<sub>3</sub> and T<sub>3</sub> 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<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. The observed value shows that VW increased as Aloe vera level increase. Statistical analysis showed that maximum VW was observed in T<sub>3</sub> while minimum VW was recorded in T<sub>0</sub>, respectively. Length of villi was measured as (212±0.14), (223±0.27), (237±0.34) and (245±0.48) in treatments T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. Statistical analysis showed, the maximum Villi length was measured with high level of Aloe vera groups T<sub>3</sub> whereas minimum villi length was observed in low Aloe vera groups T<sub>0</sub>. Results indicates with increasing level of *Aloebarbadensis*, length, Width, gap of villi increased which showed that *Aloe barbadensis* powder with 15% concentration in diet has significant results (P<0.05).

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

Treatments	Gap between villi ( $\mu\text{m}$ )	Width of villi ( $\mu\text{m}$ )	length of villi ( $\mu\text{m}$ )
T <sub>0</sub>	08.12±1.12 <sup>c</sup>	41.38±0.25 <sup>c</sup>	212±0.14 <sup>c</sup>
T <sub>1</sub>	11.15±1.28 <sup>b</sup>	45.25±0.34 <sup>b</sup>	223±0.27 <sup>c</sup>
T <sub>2</sub>	14.16±1.34 <sup>ab</sup>	47.36±0.36 <sup>b</sup>	237±0.34 <sup>b</sup>
T <sub>3</sub>	17.18±1.48 <sup>a</sup>	51.41±0.54 <sup>a</sup>	245±0.48 <sup>a</sup>

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

## 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 (Frankic, et al 2009). 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 ( Ali et al, 2009). 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 (Haghighi et al, 2014). In current study, Proximate analysis in which Protein contents among T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> were recorded as 31.49, 32, 31.40 and 33.30, respectively. Moisture, 05.91, 8.56, 6.94 and 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<sub>1</sub> (10.97), T<sub>2</sub> (12.24), T<sub>3</sub> (11.97) and T<sub>4</sub> (11.44), respectively.

water temperature (°C), DO (mg /l), 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. ( Abid and Ahmed 2009). 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 (Surjusheet *al.*, 2008). 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<sub>4</sub> as compared to other groups. More than this growth of *Labeo rohita* is significantly increase in T<sub>4</sub>. The growth

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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, (Divyagnaneswari *et al.*, 2007), in tilapia, and (Misra *et al.*, 2006), in Indian major carp (*Labeo rohita*) stated, an enhance in serum bactericidal activity.

Comment [a45]: Et al in italic or not?

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<sub>4</sub> (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. (Ferraris *et al.*, 1990; 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 (Bakke *et al.*, 2011). Maximum numbers of villi which needed to do the maximum absorption (Bakke *et al.*, 2011). Similarly, villi lengths oin anterior intestine were significantly taller. To provide surface area for absorption of nutrient-rich feed particles more efficiently (Nordrum *et al.*, 2000; Bakke *et al.*, 2010). In agreement, conclusion that absorption of nutrients like protein, carbohydrate and lipid occurred at a faster rate in proximal or anterior regions of intestine (Collie, 1985; Buddington and Diamond 1987; Dabrowski, 1990; Bakke-McKellep *et al.*, 2000; 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 (Yilmaz *et al.*, 2007; Ngamkala *et al.*, 2010; Heidari *et al.*, 2012). 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 (Samanya and Yamauchi, 2002; Miles *et al.*, 2006). In general, these villi provided large surface area for absorption of available nutrients.

Comment [a46]: Some where in the text comes in italic?

## 5.2 CONCLUSION

The present study, demonstrated that *Aloe barbadensis* has positive effect on *Labeo rohita* growth performance and gastrointestinal tract. *Aloe barbadensis* has a signigance 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<sub>3</sub> (15%/kg feed *Aloe barbadensis*).

Comment [a47]: For the first time mention it as *Labeo rohita* and then *L. rohita*

Comment [a48]:

## REFERENCES

- Alam, M. J., Ang, K. J., Begum, M., Balaji, K., Sahu, N. P., Tripathi, S. D., ... & Tacon, A. G. J. (2017). Aquaculture feed and fertilizer resources information system.
- Alishahi, M. Ranjbar, M. M Ghorbanpour, M. Mesbah and M. RaziJ,M, (2010 )Int J of veterinary res, , 4, 189-195
- Aldoghachi, M. A., Azirun, M. S., Yusoff, I., & Ashraf, M. A. (2015). Ultrastructural effects on gill tissues induced in red tilapia *Oreochromis sp.* by a waterborne lead exposure. *Saudi journal of biological sciences*, 23(5), 634-641.
- Bene C., Macfadyen, G., & Allison, E. H. (2007). *Increasing the contribution of small-scale fisheries to poverty alleviation and food security* (No. 481). Food & Agriculture Org..
- Chitrarasu, P., Ali, A. J., & Babuthangadurai, T. (2013). Study on the bioaccumulation of heavy metals in commercially valuable and edible marine species of Ennore creek, South India. *International Journal of Pharma and Bio Sciences*, 4(2), 1063-1069..
- Eshun, K., & He, Q. (2004). Aloe vera: a valuable ingredient for the food, pharmaceutical and cosmetic industries—a review. *Critical reviews in food science and nutrition*, 44(2), 91-96.
- Jegede, T. (2012). Effect of garlic (*Allium sativum*) on growth, nutrient utilization, resistance and survival of *Tilapia zillii* (Gervais 1852) fingerlings. *Journal of Agricultural Science*, 4(2), 269.
- Nazari, A., & Roozbehani, S. (2015). Influence of fennel *Foeniculum vulgare* extract on fertility, growth rate and histology of gonads on guppy *Poeciliareticulata* *Turkish Journal of Fisheries and Aquatic Sciences*, 15(3), 457-463.
- Plaskett, L. G. (1996). *The health and medical use of Aloe barbadensis*. Life Sciences Press.
- Poorfarid, M., KarimiJashni, H., & Houshmand, F. (2012). The effects of Aloe barbadensis sap on progesterone, estrogen and gonadotropin in female rats. *Journal of Jahrom University of Medical Sciences*, 10(4), 7-12.
- Tran T.T.H., Thai T.T.T., Nguyen H.D.T. & Tran L.C.T. 2010. *Study on the dietary lysine and methionine requirement in feed for striped catfish Pangasianodon hypophthalmus*. Can Tho University. Project Final Report, 89 pp. (in Vietnamese).
- Richardson K., Steffen, W., & Liverman, D. (Eds.). (2011). *Climate change: Global risks, challenges and decisions*. Cambridge University Press. - [books.google.com](https://books.google.com)

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Comment [a51]: All references must be edited according to journal guidelines

- Shah, S. B. H., Mu, Y., Abbas, G., Pavase, T. R., Mohsin, M., Malik, A., ... & Soomro, M. A. (2018). An economic analysis of the fisheries sector of Pakistan (1950-2017): Challenges, opportunities and development strategies. *International Journal of Fisheries and Aquatic Studies*, 6(2), 515-524.
- Suplicy, F. M., Vianna, L. F. D. N., Rupp, G. S., Novaes, A. L., Garbossa, L. H., de Souza, R. V., ... & dos Santos, A. A. (2017). Planning and management for sustainable coastal aquaculture development in Santa Catarina State, south Brazil. *Reviews in Aquaculture*, 9(2), 107-124.
- Yilmaz, E., Genc, M. A. and Genc, E. (2007). Effects of dietary mannan oligosaccharides on growth, body composition, and intestine and liver histology of rainbow trout, *Oncorhynchus mykiss*. *Isr. J. Aquacult.*, 59: 182–188.
- Zahran, E., Risha, E., AbdelHamid, F., Mahgoub, H. A., & Ibrahim, T. (2014). Effects of dietary Astragalus polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, 38(1), 149-157.

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