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Effect of dietary inclusion of *Leucaena leucocephala* on the growth performances of Nile Tilapia

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Abstract

In this research five feed ingredients were selected for the experiment entitled effect of dietary inclusion of *Leucaena leucocephala* on the growth performances of Nile Tilapia, which were Soybean meal, Rice bran, Mustard oil cake, Wheat flour and Leucaena leaf meal. Five treatments were T1 which was control in which no Leucaena leaf meal was used, T2 in which 10% replacement of Soybean meal was done by LLM, T3 in which 20% replacement was done and 30% and 40% replacement was done in T4 and T5 respectively and in each aqauarium 10 fingerlings were stocked. During the research, water quality parameters like temperature, pH and DO was measured in daily basis and Ammonia was measured in weekly basis. After three month of research, significantly the maximum weight gain was obtained in treatment 2 (52.48 g), followed by treatment 3 (143.60 g) and minimum weight gain was obtained in treatment 5 (129.14 g) followed by treatment 4 (130.17 g). Treatment 2 shows the highest average daily weight gain (0.116 g), followed by treatment 3 (0.110 g). Lowest average daily weight gain was observed in treatment 4 and 5 (0.09 g). Treatment 2 shows the highest Specific growth rate (SGR) value (1.30), followed by treatment 3 (1.22). Similarly, in treatment 4 and 5 the lowest SGR value (1.11) and (1.10) was obtained respectively. Lowest Feed Conversion Ratio (FCR) was obtained in treatment 2 (2.64) followed by treatment 3 (2.71). Highest FCR value obtained in treatment 4 (2.96) followed by treatment 5 (2.94). Comparatively treatment 2 shows the better growth performances than other treatments.

Keywords: aquarium, anti-nutrient, fingerlings, analysis, *Leucaena leucocephala*

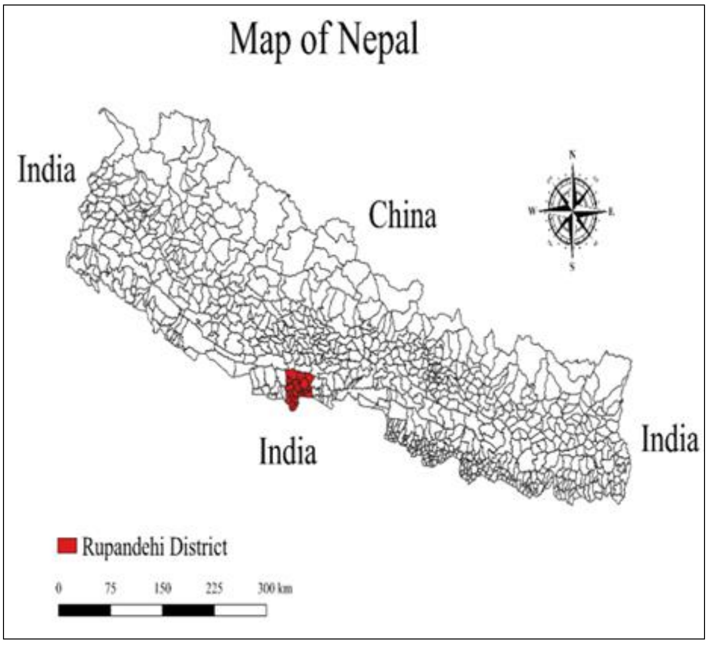
Introduction

Tilapia is an African fish used in warm water aquaculture throughout the world and also considered as the major source of protein in many developing countries. Although it is endemic to Africa its distribution has been widened worldwide due to its aquaculture potential since 1950s to include much of the tropics and subtropic (Pullin and Maclean., 1992) [6]. *Oreochromis niloticus* and *O. mossambicus* are two species of tilapia which are introduced in Nepal in 1985 for the first time in Thailand (Shrestha, 1994) [12]. Tilapias are tropical fishes that are sturdy, fertile, and fastgrowing, and also they are showing short food chain, generally feed on plankton, aquatic macrophytes, and algae (Saikia and Das., 2015) [8]. During the 1970's, this fish inspired the International Development Agencies to name it "Aquatic Chicken" and twenty years later as "fish of the 1990's". Presently it is considered as "Food fish of the 21st Century" (Ramnarine and Singh., 2005) [7]. Initially, all the tilapias are assembled under the genus *Tilapia* which are commercially important, but in the mid-1970s the mouthbrooding species were separated from the species that incubated their eggs externally and were put in the genus *Sarotherodon.* Nile tilapia was reported as *Oreochromis niloticus* which was then called *Sarotherodon niloticus* in the literature of the late 1970's, and before that, it was identified as *Tilapia nilotica* (Popma and Lovshin., 1996) [5]. The major cultivable species of tilapia are *Oreochromis niloticus* (Nile tilapia), *Oreochromis mossambicus*, (Mozambique tilapia), *Oreochromis aureus* (Blue tilapia), *Oreochromis hornorum (*Zanzibar tilapia), and *Oreochromis* hybrids (Red tilapia). *The original Oreochromis* hybrids (Red tilapias) are genetic mutants and has become most popular for culture because of their high market value. Tilapia farming is more popular in developing countries like China, Bangladesh, Indonesia, Laos, Malaysia, Taiwan, Philippines and Vietnam. Nile tilapia are well known to feed upon phytoplankton, periphyton, water plants, invertebrates, benthic organism, detritus, bacterial films, and even the body of other fish and fish eggs (Rakocy, 2005) [4]. (Schroeder, 1978) [10] reported that 30 to 50 % of natural food organisms are required for the tilapia growth in intensive culture ponds with heavy feeding and its contribution is much greater in pond culture of tilapia than for culture of channel catfish or marine shrimp in ponds. Generally, common carp disturb the ground level of the pond but tilapias do not disturb when they are feeding. Male fish initiate breeding with the creation of a spawning nest at the ground level of the pond (generally in water shawoller than 3 feet), which is fiercely guarded and mate with several females. The number of eggs a female will produce is dependent on body size. The production of eggs ranges from 100 to 1500. During brooding, females can't spawn but on favorable environmental conditions males impregnate the eggs of multiple females and also found that the survival of *O. niloticus* between 11 and 42°C was found more effective (Rakocy, 2005) [4]. Due to the distribution of salinity and temperature in torrential river systems Nile tilapia will not thrive except that it can survive in any watered habitat (Shipton *et al.*, 2008) [11]. Nile tilapia can tolerate at low levels concentration (3-4 mg/l) of dissolved oxygen (Boyd, 2004) [2]. Temperature is also a major factor that affects the metabolic functions of fish. Optimal growing temperatures are typically between 22°C (72°F) and 29°C (84°F); spawning at 22°C (72° F) temperatures found more. Survival of tilapia species are found difficult at below 10°C (50°F), growth and development are stunted at below 20°C (68°F) temperature. Tilapia can tolerate higher concentrations of fiber and carbohydrate than cultured fish. Successful tilapia culture requires well-balanced food for growth and development of the fish to obtain higher yield but nutrients requirements are primarily affected due to the size of the fish. The stunted in age and size of the tilapia was found due to higher concentrations of crude protein (30-56%) required for fry, for juvenile (30-40%), and larger tilapia lower protein levels (28-30%) required (Twibell and Brown., 1998). (Jauncey, 2000) reported the concentration of fat and maximizing the utilization of protein between 8 and 12% for tilapia up to 25g, and 6 to 8% for larger fish. As with most fish, tilapia appears to require n-6 (linoleic) fatty acids, and to a lesser extent, a requirement for n-3 (linolenic) fatty acids. Dietary lipids should supply at least 1% of n-6 fatty acids (Teshima *et al*., 1982). Carbohydrates provide a relatively inexpensive source of energy compared to protein and their inclusion can improve the quality of pelleted feeds. Tilapia can effectively utilize carbohydrate levels up to 30 to 40% in the diet, which is considerably more than other fish (Anderson *et al*., 1984). *Leucaena leucocephala* belongs to the family Fabaceae and it is a small in size, fast growing tree that has several common names identified worldwide by which it is known as White Lead tree, White Popinac, Jumbay, and Tamarind. *Leucaena leucocephala* is a quick-growing multipurpose legume plant that has been introduced in many humid and subtropical regions of nations having the protein value ranging from 15.2-34.3% dry matter (Sheet, 1997). The incorporation of *Leucaena leucocephala* leaf as a meal at 12.5% in the diet of *O. niloticus* did not effects the development but at 25% it's general effects on the growth of tilapia (Santiago *et al.*, 1988). The Leucaena leaf used as a meal for the fish diet in *Clarias gariepinus* for 56 days using hapa system found that 20% Leucaena leaf meal shows the best performances in gaining weight, specific growth rate of the fish, and Feed Conversion Ratio (P<0.05). The broodstock of Nile tilapia fed on four isonitrogenous diets (20% crude protein) containing varying amounts of leucaena leaf meal (0%, 20%, 40%, and 80%). Gaining the weight of female fish is decreased due use of leucaena leaf as a meal and also losing their weight by 80%.

Materials and methods

Site Location of the experiment

The experiment was conducted at the Aquaculture Laboratory of Institute of Agriculture and Animal Science (IAAS), Paklihawa campus, Rupandehi, Bhairahawa for 90 days. The site is located 4 km southwest of Bhairahawa. The altitude of this site is about 256 masl with (27.50 N, 83.450 E). The research was conducted from January 2021 to April 2021.



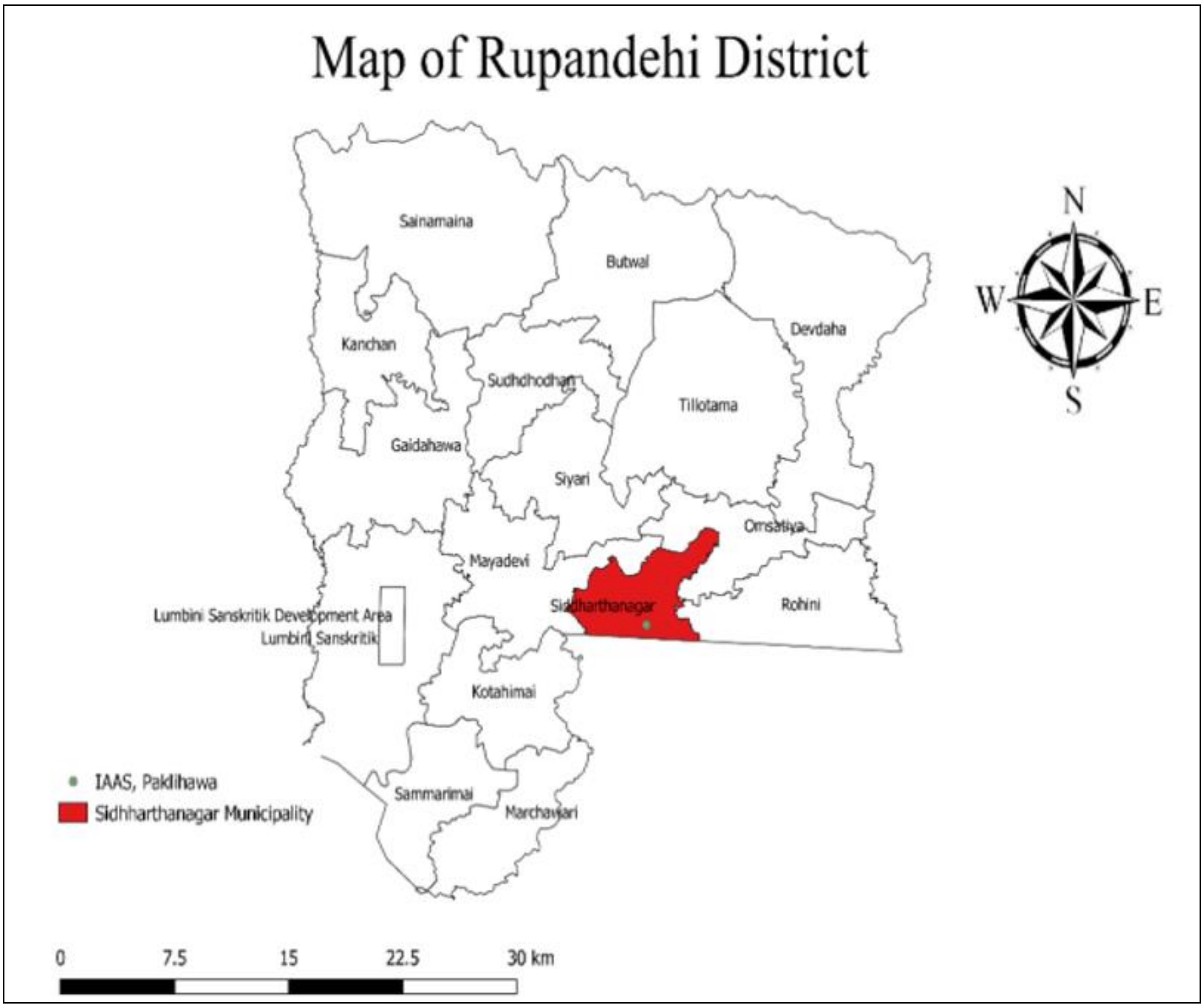


Fig 1: Administrative map showing research site at IAAS, Rupandehi district.

Experiment Details

The research was conducted in 15 aquariums with a volume of (1 x 2 x 1 cubic feet). The research is based on replacing a much more expensive Soybean meal with locally available alternative plant protein sources i. e, *Leucaena leucocephala.* For the experiment, a Completely Randomized Design was used having the five treatments and three replications.

Five experimental diets/treatments include,

- **Diet 1(Treatment 1):** containing Soybean meal, rice bran, mustard oil cake, and wheat flour. (Control)
- **Diet 2(Treatment 2):** 10% replacement of Soybean meal with Leucaena leaf meal powder.
- **Diet 3(Treatment 3):** 20% replacement of Soybean meal with Leucaena leaf meal powder.
- **Diet 4(Treatment 4):** 30% replacement of Soybean meal with Leucaena leaf meal powder.
- **Diet 5(Treatment 5):** 40% replacement of Soybean meal with Leucaena leaf meal powder.

All the treatments were randomized by lottery method.

Collection of feed ingredients

Five ingredients Soybean meal, Rice bran, Mustard oil cake, Wheat flour, and Leucaena leaf meal were selected for experimental diet preparation. Soybean meal was selected as a protein source and Rice bran was selected as a carbohydrate and energy source. Also, Wheat flour was used which acts as a binding agent and helps to bind all the feed ingredients together. All four ingredients Soybean meal, Rice bran, Mustard oil cake, and Wheat flour were purchased from the local market of Bhairahawa. Before mixing all the ingredients were grinded with the help of a grinder into fine powder form. Before grinding heat treatment of Soybean was done to remove the anti-nutritional factors present in it and also it makes it easier to grind after heat treatment. In the case of the Mustard oil cake, it was pre-soaked overnight before grinding. Leucaena leaves were collected from the paklihawa campus periphery and kept soaked for 2 days followed by sun-drying for further 3 days. This technique helps to reduce the anti-nutritional factors present in it. After sun drying, leaves were also grinded into powder form and all the ingredients were passed through a 0.5mm mesh size. Then further all the ingredients were sent to National Animal Feed and Livestock Quality Management Laboratory, Hariharbhawan, Lalitpur for proximate analysis. Proximate composition of feed ingredients were presented in Table 1.

Table 1: Proximate composition of feed ingredients

S.N	Parameter	Soyabean meal (%)	Mustard Oil cake (%)	Rice bran (%)	Leucaena leaf meal (%)	Wheat flour (%)
1	Moisture	7.1	7.9	9.1	7.4	7.5
2	Crudeprotein (CP)	47.1	38.9	12.2	30.1	14
3	Crude fat	13.4		3.9		4.6
4	Ash	7.7				
5	Aflatoxin					B2
6	Aflatoxin					B2
7	Others	5.1		9.1		

Source: (National Animal Feed and Livestock Quality Management Laboratory Hariharbhawan, Lalitpur).

Feed formulation and preparation

Feed formulation was done by Pearson's Square method with the help of the value obtained after proximate analysis. Five diets with the same level of dietary protein 30% were prepared. Control diet containing no Leucaena and 10%, 20%, 30%, and 40% replacement of Soybean meal with Leucaena leaf meal was done. During the feed formulation, the quantity of Soybean meal and mustard oil cake to be used in the control (T1) diet was obtained 28g/100g, and rice bran and wheat flour was obtained 22g/100g of feed to make 30% CP feed. Then the amount of Soybean meal used in the control diet was replaced by 10% in treatment 2 (T2) and similarly, a replacement was done by 20%, 30%, and 40% in T3, T4, and T5 respectively.

Also, Vitamin premix was applied in each treatment @1kg/100kg of feed. All the ingredients were mixed and then put into a manual-operated pellet machine (Engineering industries Pvt. Ltd) and a pellet diet of 3 mm size was made. The prepared feed pellet was sun-dried for up to 1 week. Ingredients and compositions (%) of the experimental diets were presented in Table 2.

Table 2: Ingredients and compositions (%) of the experimental diets

Ingredients	Control T1(g)	T2 (g)	T3 (g)	T4 (g)	T5 (g)
Soybean meal	28	25.2	22.4	19.6	16.8
Mustard oil cake	28	28	28	28	28
Leucaena leaf meal	0	2.8	5.6	8.4	11.2
Rice bran	22	22	22	22	22
Wheat flour	21	21	21	21	21
Vitamin and Mineral Premix* (Agrim Fort)	1	1	1	1	1
Total	100	100	100	100	100

*Vitamin mineral premix /Kg contains the following: Vitamin A 7,00,000 I.U, Vitamin D3 70,000 I.U, Vitamin E 250mg, Cobalt 250mg, copper 1200mg, Iodine 325mg, Iron 1500mg, Magnesium 6000mg, Potassium 100mg, Sodium 5.9mg, Manganese 1500mg, Sulphur 0.72%, Zinc 9600mg, DL-Methionine 1000mg, Calcium 25.5%, Phosphorus 12.75%.

Transportation of Fingerlings and Acclimatization

Viable fingerlings of Tilapia size (3-4g) were collected from the Shanti Matshya Hatchery, Bhagalapur, Rupandehi. Fingerlings were transported with proper handle and care. No mortality of fingerlings was observed during the transportation. All the fingerlings were kept in a separate tank for acclimatization and allowed to acclimatize up to 14 days before the start of the experiment in research aquaria. During the acclimatization period feeding of extruded commercial feed having crude protein, 28% was done before using experimental diets. Then the fingerling was transferred to the individual aquarium with densities of 10 fish /aquarium and was fed with feed with respective diet prepared as per treatment twice a day.

Research setup

Before the start of the research, all the equipment that should be required in the research was well sanitized with $KMnO_4$ and Malachite green solution. All the equipment was dipped into the solution for 30 min and then sun-dried. Also, the aquariums were cleaned with salt solution (3%) before stocking the fingerlings. Aerators were fixed in positions to maintain an adequate level of oxygen in each aquarium.

Stocking

During stocking, healthy fingerlings were selected for research purposes. Before stocking all the fingerlings were well sanitized with $KMnO_4$ solution (2ppm) for 5 min and then transferred to an individual aquarium with the density of 10 fingerlings in each aquarium. During transferring in each aquarium all the fingerlings were weighed and their weight and total weight were measured. The weight difference between individual fish was kept at only 1-2g. The total weight difference in each aquarium was ranges from 46 – 48g. Proper handling and stocking of fingerlings were done and no mortality of fingerlings was observed due to stress.

Feeding

Fingerlings were hand-fed with prepared pellet feed following the total body weight of the fishes of each aquarium. Feeding was done two times a day, at 8 AM and 4 PM. Before feeding, the prepared pellet feed was grinded in a mortar pistle to make small size particle that makes it easier to swallow for fish. Fishes were fed 10% of their body weight for the first month and were adjusted to 7.5% & 5% in the following months. During the initial days, fingerlings did not show the feeding response properly and feed loss was seen during those days. The reason behind that might be low temperature.

Cleaning and Exchanging Aquarium Water

The water of the aquarium was changed daily. Water exchange was done using the suction pump for draining water from the aquarium. About 2/3 part by volume water will be exchanged using underground pump water. During water exchange, the temperature between the aquarium water and pump water was maintained at the difference of 1 degree only. Entire feed wastage was sucked using a suction pump.

Water quality analysis

Dissolved Oxygen (DO), pH, and temperature were measured every day. A portable pH meter (Hana instrument, accuracy ± 0.1) was used to monitor pH. Likewise, the thermometer was used to measure water temperature. Similarly, a dissolved oxygen meter (Lutron PDO-519) was used to measure dissolved oxygen. The temperature in degree centigrade (ºC), dissolved oxygen in parts per million (mg/l) was noted for each value monitored.
Also, other water quality parameters Ammonia was measured weekly using Chemical Test Kit.

Procedure for Ammonia Test

5ml sample in the vial with the help of 5ml syringe was taken and 1 drop Ammonia Reagent I in the Vial sample was taken and

- shaked.
- Then 3 drops of Ammonia Reagent II will be added and finally, the observed color will be immediately compared with the Ammonia color chart.

Data collection protocol

Monthly from each aquarium entire fish was collected by seining and its growth performance was assessed. Data was collected on a weight (g) basis using an electronic compact scale (kerro series P3 BL5002 Max-500g, D=0.01g).

Growth Parameters

- Mean Weight Gain (MWG) (g): Final Mean Weight(g) – Initial Mean Weight(g)
- Specific Growth Rate (%): [[log (final weight)(g)] – [log (initial weight)(g)]/ time interval in days] X 100
- Feed Conversion Ratio (FCR): Dry weight of feed gain (g) / Wet weight gain (g)
- Daily weight gain(g): Average final weight(g) – Average stocked weight(g) / culture period
- Survival Rate %: (total number of fish harvested / total number of fish stocked) x 100

Harvesting

Final harvesting of Tilapia fish was done after 90 days by draining each aquarium completely on termination of research. Harvested fish weight was measured using an electronic Compact scale (kerro series P3 BL5002 Max-500g, D=0.01g). Fish was counted and their batch weight (g) was recorded. Batch weight was recorded by measuring the entire 10 fishes using an electronic compact scale (kerro series P3 BL5002 Max-500g, D=0.01g).

Statistical Analysis

The data was collected over time and based on individual fish observations, the population means for each growth parameter was computed. The analysis of variance was used to compare different growth parameters using R-Stat. The mean and standard errors were calculated for each treatment. The data entry was done through MS Excel 2016. The accepted level of significance was $p<0.05$.

Results

The effect of dietary inclusion of *Leucaena leucocephala* leaf meal on growth parameter, and its survivability, is presented under the following headings.

Proximate composition of experimental diets

Proximate analyses of different experimental diets are presented in Table 3. The dietary crude protein level of all five experimental diets was almost the same. Diet 1 contained 30.96% crude protein, 5.96% crude fat 8.75% Ash, and 1.64% Crude fiber. Diet 2 contained 30.31% crude protein, 5.11% crude fat, 8.49% ash, and 2.11% crude fiber. Diet 3 contained 30.63% crude protein, 5.97% crude fat, 9.20% ash, and 1.85% crude fiber. Similarly, Diet 4 contained 30.46% crude protein, 5.78% crude fat, 9.27% ash, and 2.08% crude fiber, and Diet 5 contained 30.52% crude protein, 5.92% crude fat, 9.05% ash, and 2.14% crude fiber.

Table 3: Proximate composition of different experimental diets used in experiment

S.N.	Parameter	Diet 1 (T1) (%)	Diet 2 (T2) (%)	Diet 3 (T3) (%)	Diet 4 (T4) (%)	Diet 5 (T5) (%)
1	Mositure	11.84	12.29	11.93	11.93	11.67
2	Crude protein	30.96	30.31	30.63	30.46	30.52
3	Crude fat	5.96	5.11	5.97	5.78	5.92
4	Ash	8.75	8.49	9.20	9.27	9.05
5	Crude fibre	1.64	2.11	1.85	2.08	2.14

Source: National Animal Feed and Livestovk Quality Management Laboratory Hariharbhawan Lalitpur.

Growth parameters

The total stock weight, average stock weight total harvest weight, average harvest weight, average daily weight gain, its survivability, specific growth rate (SGR) and Feed conversion ratio (FCR) are presented in table 4. There was no significant difference between all 5 treatments in terms of stocking weight (p>0.05). In treatment 1 stocking weight was (47.51±0.10) g which is the weight of 10 fish. Similarly, in treatment 2 stocking weight was (47.12±0.33) g, in treatment 3 (47.75±0.61) g which is the highest stocking weight as compared to other treatments, in treatment 4 (47.51±0.69) g, and in treatment 5 (47.72±0.68)g. But statistically, there was a similar stocking weight of all 5 treatments during the stocking day of fish. Also, there was no significant difference between the average stocking weight of fish (p>0.05). This shows that there was not much difference in the weight of individual fish in each treatment during stocking. Also, we can say that statistically there was the same weight of individual fish in each treatment. If we observed the data of harvest there is a significant difference between the treatments T1, T2, and T3 (p<0.05). Maximum weight gain was observed in Treatment 2 i, e (152.48±3.96) g followed by treatment 3 i, e (143.6 ± 0.50) g and then in treatment 1 i, e (139.79±2.73) g. The lowest weight gain was observed in treatment 5 i, e (129.14±3.86) g followed by (130.17±4.68) g in treatment 4. There was no significant difference between treatment 4 and treatment 5 (p>0.05). It means statistically they both have the same weight gain.

Table 4: Mean value of growth parameter of Tilapia during the experimental period of 90 days

Treatment	Growth Parameters							
	SW	ASW	Harvest	AHW	ADWG	Survivability	SGR	FCR
T1	47.51±0.10	4.75±0.00	139.79±2.73^{bc}	13.97±0.27^{bc}	0.10±0.00^{b}	100±0.00	1.19±0.02^{bc}	2.80±0.07^{b}
T2	47.12±0.33	4.71±0.03	152.48±3.96^{a}	15.24±0.39^{a}	0.11±0.00^{a}	100±0.00	1.30±0.02^{a}	2.64±0.06^{c}
T3	47.75±0.61	4.77±0.06	143.6±0.50^{ab}	14.36±0.05^{ab}	0.11±0.00^{ab}	100±0.00	1.22±0.01^{ab}	2.71±0.04^{b}
T4	47.51±0.69	4.75±0.06	130.17±4.68^{c}	13.01±0.46^{c}	0.09±0.00^{c}	100±0.00	1.11±0.05^{bc}	2.96±0.05^{a}
T5	47.72±0.68	4.77±0.06	129.14±3.86^{c}	12.91±0.38^{c}	0.09±0.00^{c}	100±0.00	1.10±0.04^{c}	2.94±0.10^{a}
LSD (=0.05)	6.87	0.17	10.95	1.09	0.01	-	0.10	0.12
CV (%)	7.04	1.95	4.33	4.33	7.16	-	5.18	4.46
Significance	Ns	Ns	**	**	**	Ns	*	*

Means followed by a same letter within a column are not significantly different by DMRT (α=0.05), CV: Coefficient of variation; LSD: Least significant difference; **- significant at p=0.01, ns- non significant.

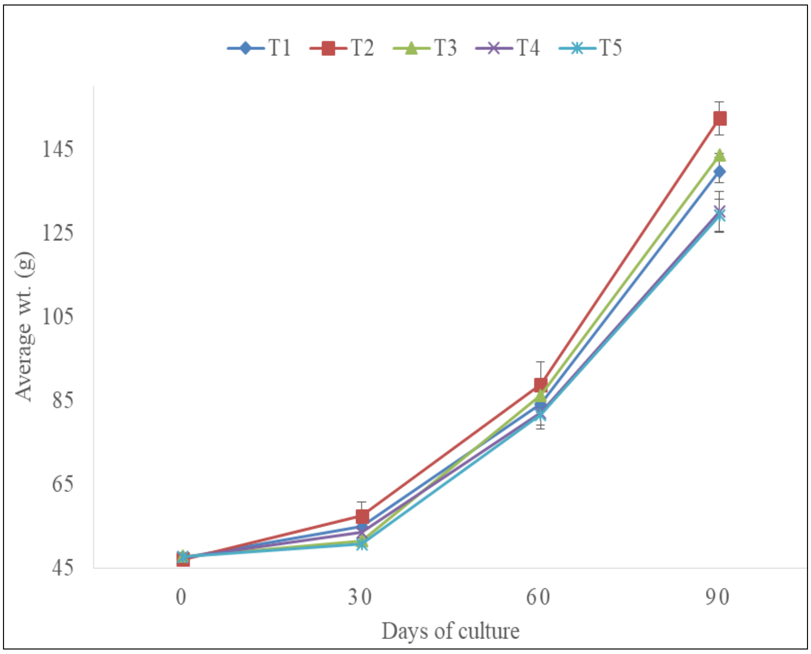


Fig 2: Growth trends of tilapia fry in different treatments during experimental period of 90 days.

According to figure no 2, the effect of Leucaena leaf meal on the weight gain of fish after 1 month, 2 months, and 3 months of stocking.

After one month of stocking, the weight gain of fish in all treatments was almost statistically the same. A similar case was observed after 2 months of stocking. Statistically, the same weight gain was observed.

Also, Table 4 shows the average daily weight gain of fish during the 90 days of the research period. There was a significant difference in average daily weight gain of fish between treatments 1, treatments 2, and treatments 3 ($p<0.05$). But the treatments 4 and 5 show a non-significant difference from each other ($p>0.05$). Those treatment 4 and 5 shows statistically the same average daily weight gain i, e 0.09g. The highest average daily weight gain was observed in treatment 2 i, e 0.116g followed by 0.110 in treatment 3 and 0.10 in treatment 1. The highest and lowest value is shown in terms of numeric value.

Above table 4 shows the specific growth rate of fish in which there was a significant difference obtained in all five treatments ($p<0.05$). The lowest specific growth rate was found in treatment 5 i, e (1.10±0.04) followed by treatment 4 in which SGR obtained was (1.11±0.05). The highest SGR was observed in treatment 2 i, e (1.30±0.02) which means 10% replacement of Soybean meal with Leucaena leaf meal result in higher biomass production in a certain period, and in treatment 3 and treatment 1 SGR was obtained (1.22±0.01) and (1.19±0.02) respectively.

Also, FCR shows a significant difference in each treatments ($p<0.05$). The lowest value of FCR was obtained in treatment 2 i, e (2.64±0.06) which means the efficiency that the fish body converted the feed into desired output was found higher in treatment 2 in which 10% replacement of soyabean meal with Leucaena leaf meal was done. Further, treatment 3 shows the FCR value (2.71±0.04) and treatment 4 and treatment 5 shows the FCR value (2.96±0.05) and (2.94±0.10) respectively. Table 4 shows that there was no significant difference in survivability in each treatment ($p>0.05$). No mortality of fish occurred during the entire period of research. Hence we can say that the survivability rate of fish was 100% in all treatments.

Water quality parameters

Water quality parameters temperature, pH, Dissolved Oxygen and Ammonia was measured during the experiment period. Ammonia was measured in weekly basis and temperature, pH and DO was measured in daily basis.

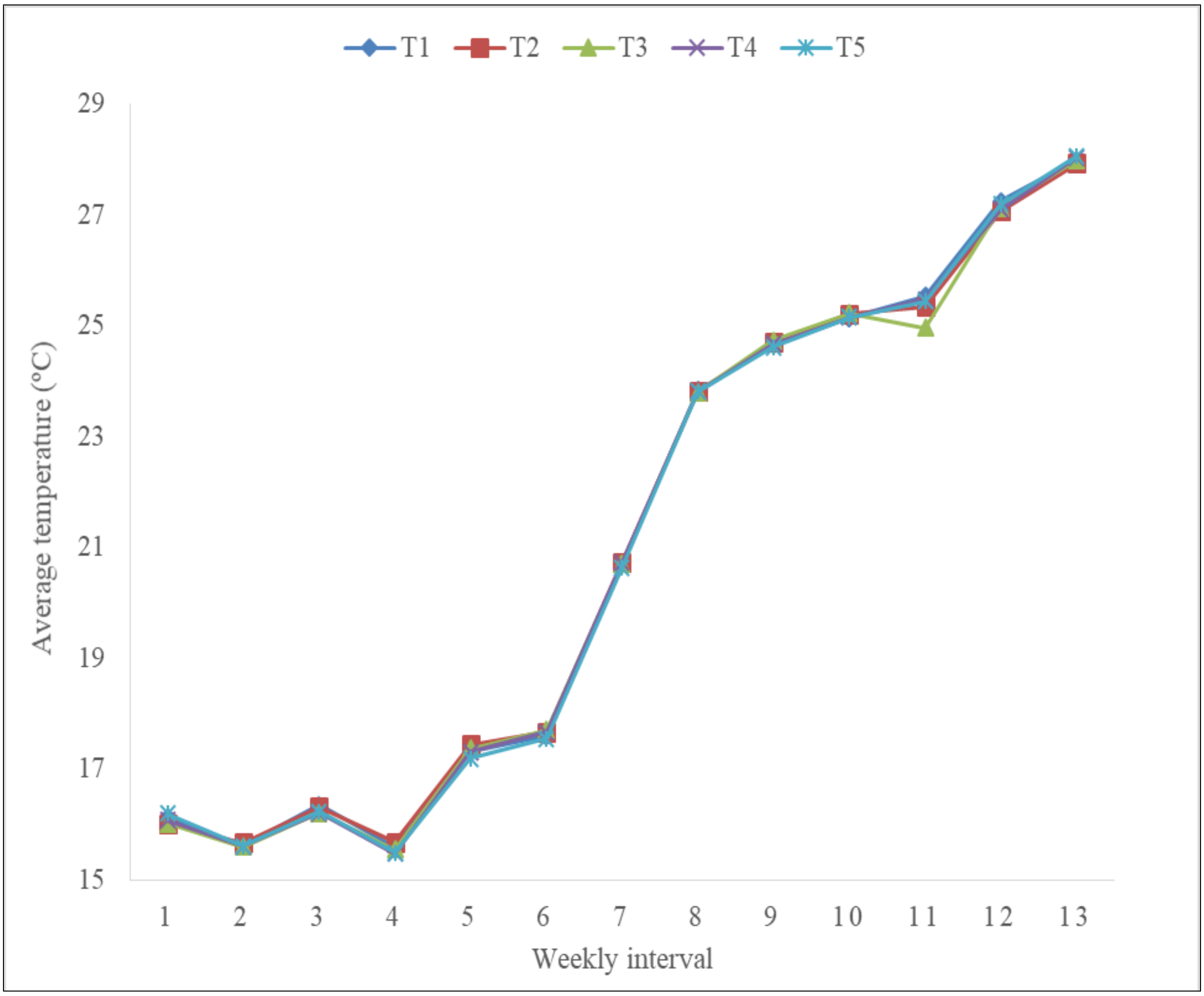


Fig 3: Weekly trend of average temperature in different treatments during experimental period.

In figure no. 3, the average temperature in weekly intervals. This figure clearly shows that during the first 4 weeks the average temperature ranges between 15-170C in all treatments. The reason behind the lower temperature was cold and foggy weather. Also due to the lower temperature, the growth of fish was not properly gained. Different literature shows that at temperatures 16 -17 0C the feeding rate of fish starts decreasing. So this might be the reason that affects the growth of fish.

During the 5th to 8th week, the average temperature was found higher than previous weeks and the temperature value ranges between 17 to 24 0C. During this temperature level, the fish showed the feeding response properly and a better growth rate of fish in the second month than the first month was achieved.

After the second month, the graph shows the average temperature ranges between 25 to 29 0C which is optimum for better growth development. Some literature says that the optimum level of temperature is between 27-30 0C. So this level of temperature affects the growth rate of fish and better growth was observed after the harvesting. In the above graph, the highest average temperature recorded was 28 0C and the minimum average temperature recorded was approximately 15 0C during the entire research period.

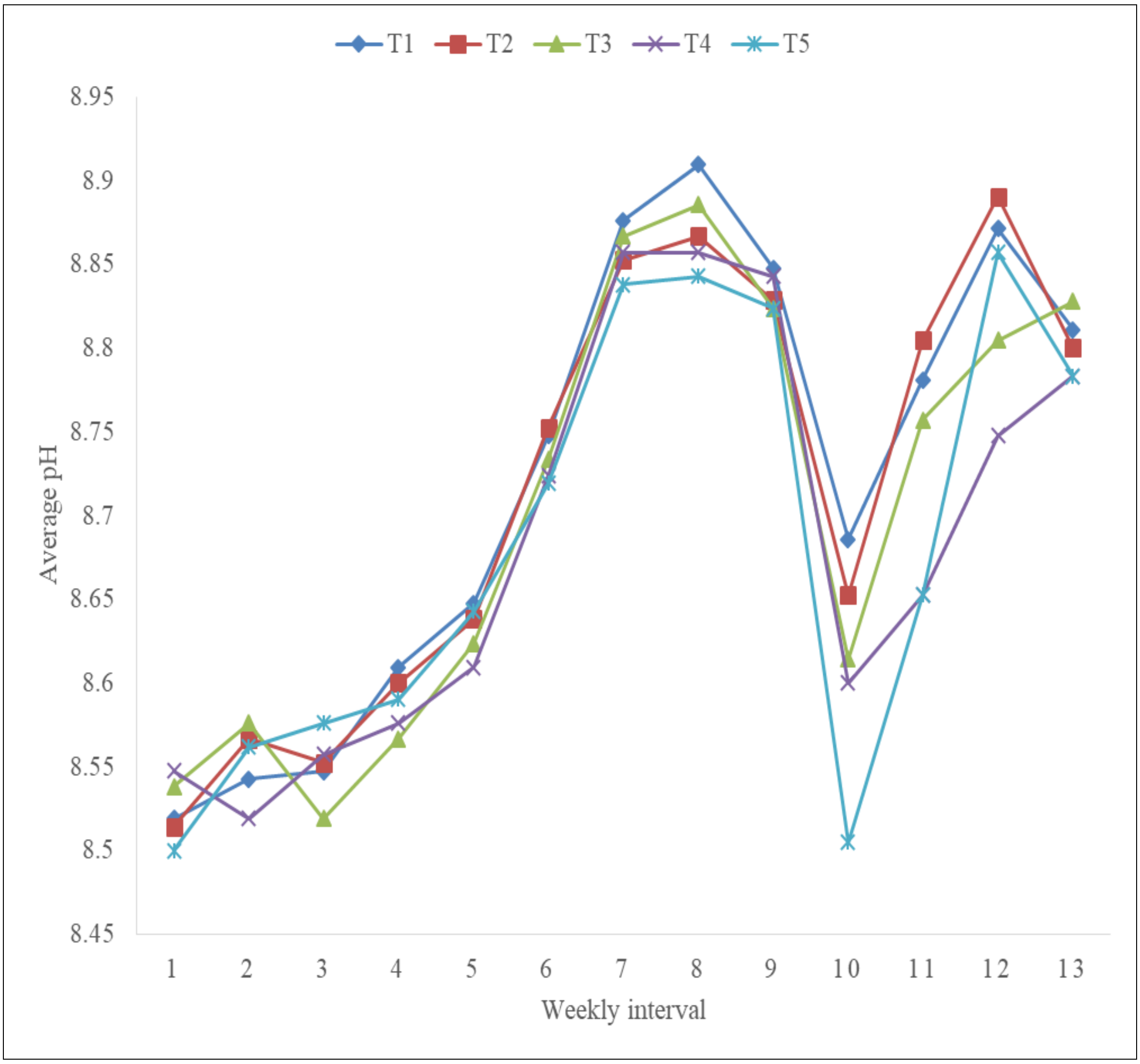


Fig 4: Weekly trend of the average pH during the experiemental period of 90 days.

In figure no.4, the average pH in the weekly interval. During the first 4 weeks, the average pH value ranges from 8.5 to 8.6 which was found optimum for the growth of tilapia according to different kinds of literature. During the first 4 weeks, the lower pH was observed in treatment 5 of the first week i, e 8.5, and higher pH was found in treatment 1 of 4th week i, e 8.6. Although Tilapias can tolerate the lower pH up to 3 and higher pH up to 11, the best growth can be obtained in pH values ranging from 7 to 9.

During the 5th to 8th week, the average pH recorded was ranging from 8.6 to 8.9. The lower value of average pH was observed in treatment 4 of 5th week i, e 8.6, and the higher value of average pH was recorded in treatment 1 of 8th week i, e 8.9. Better growth performances were observed in this period.

Similarly, during the 9th to 13th week, the average pH recorded was ranging from 8.5 to 8.85. During this period the lowest value of average pH was recorded in treatment 5 i, e 8.5, and the highest value of average pH was recorded in treatment 2 i, e. 8.85.

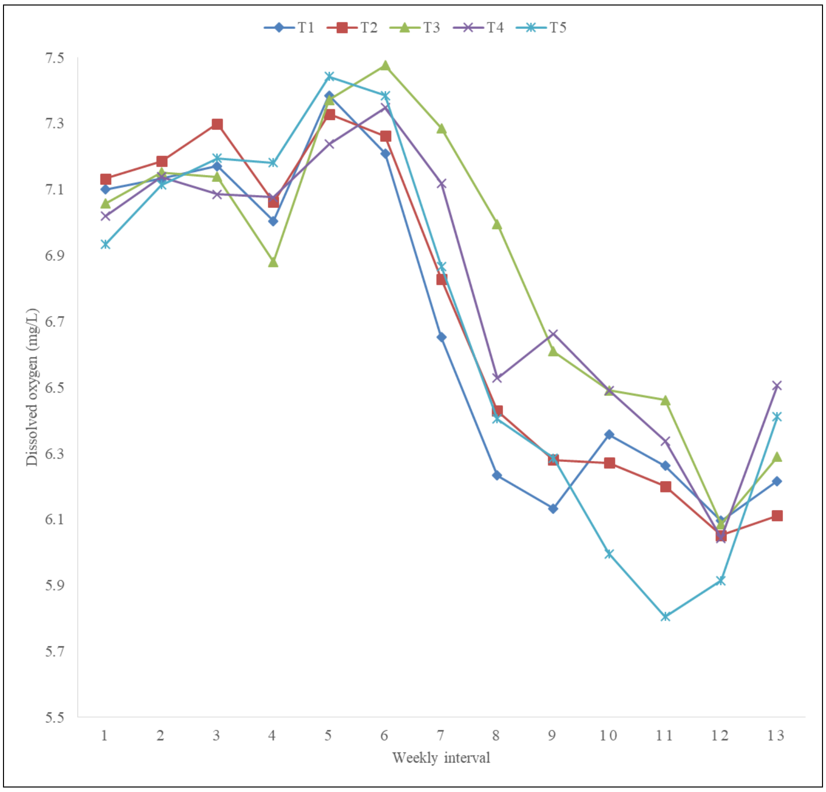


Fig 5: Weekly trend of average Dissolved Oxygen during the experimental period of 90 days.

According to Figure no. 5 The dissolved oxygen (mg/L) in the weekly interval. Dissolved oxygen is one of the most important water quality parameters that affect growth. Due to the high tolerant capacity of tilapias they can tolerate the DO level up to 0.1 mg/L. During the first 4 weeks, the Dissolved oxygen ranges from 6.8 to 7.3 mg/L. During this period the lowest value of DO was observed in treatment 3 of 4th week i, e 6.8 mg/L, and the highest value of DO was observed in treatment 2 of 3rd week i, e 7.3 mg/L. During the 5th to 8th week, the Dissolved oxygen ranges from 6.2 to 7.4 mg/L. During this period the highest value of Dissolved oxygen was recorded in treatment 3 of the 6th week and the lowest value of DO was observed in treatment 1 of 8th week i, e 6.2 mg/L. Similarly, during the 9th to 13th month, the Dissolved oxygen value ranges from 5.8 to 6.6 mg/L. During this period the highest value of Dissolved oxygen was recorded in treatment 4 of 9th week i, e 6.6 mg/L, and the lowest value of Dissolved oxygen was recorded in treatment 5 of 11th week i, e 5.8 mg/L.

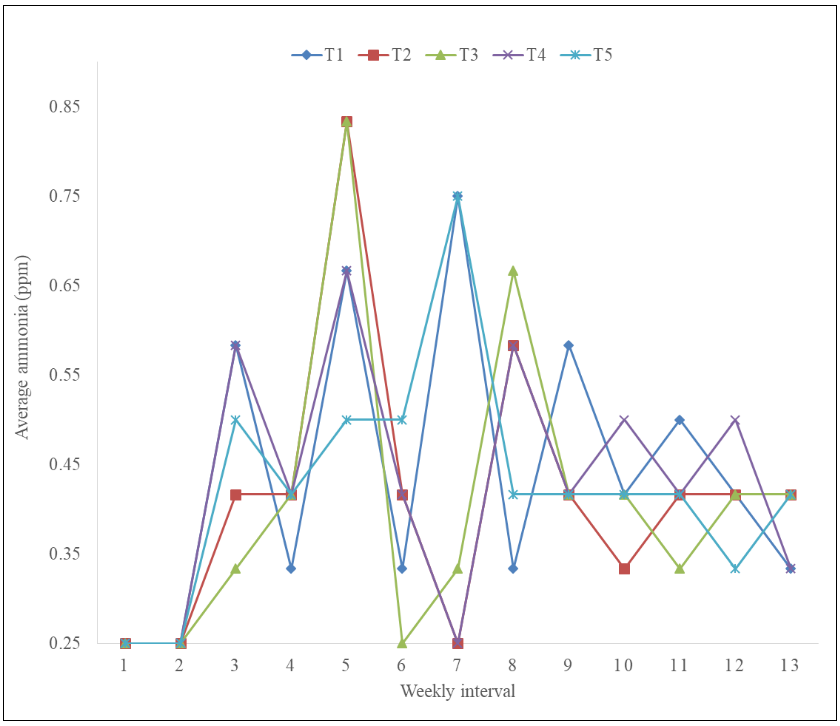


Fig 6: Weekly trend of average ammonia during the experimental period of 90 days.

In figure no.6, the average ammonia concentration during the entire research period in the weekly interval. During the first 4 weeks, the average ammonia concentration ranges between 0.25 to 0.60 ppm in which the lowest concentration was found during Ist and 2nd week in all treatments i,e 0.25 ppm and the highest concentration was found in treatment 1 and treatment 4 of 3rd week i, e 0.60 ppm.
During the 5th and 8th months, the average ammonia concentration ranges from 0.25 to 0.80 ppm in which the highest average concentration was observed in treatment 2 and treatment 3 of 5th week i,e 0.80 ppm and the lowest concentration was also observed in treatment 2 and treatment 3 of 7th and 6th week i, e 0.25 ppm.
During the 9th and 13th months, the average ammonia concentration ranges between 0.35 to 0.60 ppm in which the highest concentration was recorded in treatment 1 of 9th week i,e 0.60 ppm and the lowest concentration was recorded in all treatments during 10th, 11th,12th, and 13th-week i,e 0.35 ppm.

Discussion
Feed is the most important factor in aquaculture production which comprises about 70% of the total cost of production. Locally available plant-based protein feed can be a great substitute for expensive commercial feeds in aquaculture. Optimum utilization of those plant-based proteins as a fish feed can help reduce the cost. The present study aimed to evaluate the effect of plant-based protein feed i, e *Leucaena leucocephala* leaf meal by replacing the most expensive soyabean meal in the fish diet. In this experiment, 10%, 20%, 30%, and 40% soyabean meal was replaced by Leucaena leaf meal. Leucaena leaf can be locally available which is the cheapest source of protein feed. The result showed that the total harvest weight was ranged from 129.14g to 152.48g among treatments after a rearing period of 90 days in the aquarium. The harvest weight of T5 (129.14±3.86 g/tank) was found to be lower than other treatments (T1, T2, T3, and T4). But T5 was found non-significantly difference ($p>0.05$) with T4 (130.17±4.68 g/tank) and all three treatments T1, T2, and T3 were found significantly different ($p<0.05$) from each other. The maximum weight gain was found in treatment 2 (152.48±3.96 g/tank).
Our result also showed the average daily weight gain of fish in which T4 and T5 showed nonsignificant differences with each other ($p>0.05$). Both treatments 4 and 5 showed the average daily weight gain of (0.09±0.00)g. The highest average daily weight gain was observed in treatment 2 (0.11±0.00). Also if we observed the SGR, the maximum SGR was obtained in treatment 2 i, e (1.30±0.02), and the lowest was found in treatment 5 which was (1.10±0.04). Also, FCR was found lower in treatment 2 which was (2.64±0.06) and higher FCR was found in treatment 4 i, e (2.96±0.05). It means the efficiency of fish to convert feed into output is found higher in treatment 2. So overall treatment 2 shows better growth performances as compared to other treatments. Measurement of temperature, pH, Dissolved Oxygen was taken daily in the morning before exchanging water from the aquarium. After 1 month of stocking, there was not so much weight gain of fish in all treatments. Due to the cold and foggy weather, the temperature ranges between 15-17 degrees Celcius in the first four weeks of stocking. Generally, at this temperature ranges tilapia fish stops feeding. Feeding usually stops at temperatures less than 16 or 17 ^{O}C, and temperatures below 10 to 11 ^{O}C is lethal (Suresh and Lin, 1992). In the following month's temperature gradually rises and reached the optimum. During this period a satisfactory growth rate was obtained. If we observe the ammonia concentration then we can see a value below 1ppm during the entire research period. Regular exchange of water daily and removal of wastes and feed residue might be the reason for low ammonia concentration during the entire period.

Highlight points
1. The research was based on replacing expensive soyabean meals with locally available alternative plant protein sources *Leucaena leucocephala* for the growth of Nile Tilapia.
2. Five ingredients: Rice bran, Mustard oil cake, Soyabean meal, Wheat flour, and leucaena leaf meal were selected and research is carried out in CRD design having 5 treatments and 3 replications.
3. Overall better growth performances of Nile tilapia were observed in treatment 1 in which 10% replacement of soyabean meal was done with leucaena leaf meal.

Conclusion
Experiment entitled, Effect of dietary inclusion of *Leucaena leucocephala* on the growth of Nile Tilapia was successfully conducted at the Aquaculture lab of Institute of Agriculture and Animal Science, Paklihawa Campus, Rupandehi. This experiment clearly shows the importance of plant-based protein fish feed that can be used as an alternative source of other expensive commercial fish feed. Due to the perennial nature, abundance, and enough protein content of *Leucaena leucocephala,* one can easily use it as a feed for fish. As aquaculture is used as a means of poverty reduction among poor communities, the number of farmers who can afford commercial feeds is low. So it is better to use locally available plant-based feeds which can easily replace the commercial feed.
From the experiment, we can conclude that *Leucaena leucocephala* can be used as a source of protein for fish but to a certain extent only. Excessive use of LLM can slow the growth rate of fish. In treatments 4 and 5 the growth rate was comparatively lower than other treatments in which replacement of soyabean was done by 30% and 40% respectively. Better growth performances were seen in treatment 2 and then in treatment 3. The reason behind the slower growth rate may be due to the excessive presence of mimosine and tannin in treatments 4 and 5, which is responsible for retardation of the growth. Feeds that can give satisfactory growth and are readily

available at a reasonable price and don't rely on fish and their products are the essence to sustain the aquaculture industry.

Conflict of Interest

As my research was carried out totally on the research institute Paklihawa campus there is no conflict of interest. The research institute selected me as a researcher in his institute based on the objective and uniqueness of the research. Thus, financially I would like to declare no conflict of interest. Also, I am not linked with a research institute based on family relations or any other professionalism. The research also does not consist of any interest from peer review or other editorial staff and supervisors. The research has been completed fully under the academic guidance of my supervisors.

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Author Contributions

Conceptualization, writing a research paper, calculation, and analysis made by Nabin Chalaune; removal of plagiarism, format preparation, grammer checking, and editing made by Aman Kumar Gupta; Supervision under Shailesh Gurung and Suraj Kumar Singh; helps in data collection and analysis made by Ashish Chaudhary.

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