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Effects of dietary fenugreek (*Trigonella foenum-graecum*) on growth performance, body composition and hematological and immune parameters of Nile tilapia (*Oreochromis niloticus*) fry

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Abstract
A 60 days feeding trial was performed to assess the effects of fenugreek (*Trigonella foenum-graecum*) on the growth performance, haematological and immunological response in Nile tilapia (*Oreochromis niloticus*) fry. Five diets incorporating fenugreek diet at 0, 0.5, 1, 1.5 and 2% were prepared. The results showed that fenugreek diet in feed led to significantly ($p<0.05$) improved survival, weight gain and feed efficiency for treated groups over the control. Dietary fenugreek significantly ($p<0.05$) increased erythrocytes, leucocytes, haematocrit, haemoglobin in treated fish. Significantly higher ($p<0.05$) serum protein, albumin and globulin levels were observed in treated groups over the control. In conclusion, the impact of fenugreek has shown effective therapeutic results to consider incorporating this plant in fish feed. Also it was possible to meet the dietary fenugreek requirement of tilapia that was estimated to be 0.98 g/kg and 0.99 on the basis of maximum weight gain and protein efficiency ratio respectively.

Keywords: fenugreek, hematological, *O. niloticus*, serum

Introduction
Herb or spices have reported to promote various functions like growth, appetite stimulation, anti-stress, immune functions [1], skin coloration [2], egg-hatching rates haematological and biochemical status [2] and also increase disease resistance [3] in fish culture due to different active components. Fenugreek (*Trigonella foenum graecum*) is an annual herb of the leguminoseae family. Its seeds are used a spice and its leaves are used as a vegetable which is rich in vitamins and minerals. The seeds are protein rich; it is also an important source of diosgenin [4]. Fenugreek is rich in flavonoids (such as apigenin, kaempferol and quercetin) and saponins (such as diosgenin and yamogenin). Their characteristic functions are to protect the oxidative damage and immunostimulatory properties [5].
Therefore, this study was conducted to evaluate the effects of fenugreek seed as a feed additive in fish diets and its impact on the growth performance, blood haematological and immune parameters and survival of Nile tilapia (*Oreochromis niloticus*) fry.

Materials and methods
Experimental fish
Juvenile *Oreochromis niloticus* of total length 4.76 ± 0.04 cm (mean ± SE) and weight 1.68 ± 0.11 g were selected in the experiment. The fish, procured from the Fish Hatchery, Pune and held in plastic pools (1000 L) with continuous aeration for 15 days and then stocked into the plastic aquarium tanks one week before beginning of the experiment. The experimental set-up comprised of 20 FRP tanks (100 L capacity), with four tanks of fish for each of five treatments. Lengths and weights of fish were measured and 10 fish were then stocked into each tank (200 fish in total). The experimental tanks were cleaned manually and siphoned every day to remove fecal matter and an equal volume of clean water was filled in tanks. Length and weight of individual fish were measured at 0, 15, 30, 45, and 60 days of the experiment. 3 fish per tank (12 fish per treatment) were sampled at the end of the experiment to analyze carcass

composition and to evaluate condition factor.

Preparation of herbal diet and feeding

Ingredient constitution of the basal diet includes fish meal, groundnut cake, wheat bran, wheat flour, rice flour, fish oil, and vitamins and minerals (pre mixture) and proximate composition of feed by the standard methods [6]. Fenugreek was purchased from the local market in Mangalore. Fenugreek seeds were a shade dried and crushed into powder form using a household electric

Table 1: Proximate composition of ingredients used for preparation of treatment diets

Ingredients	Moisture	Dry matter	Ash	Protein	Ether extract	Nitrogen free extract
Fish meal[1]	8.47	91.53	24.88	46.11	9.31	19.7
Groundnut oil cake	7.42	92.58	14.79	42.34	9.38	33.49
Wheat flour[2]	5.34	94.66	2.43	11.09	3.02	83.46
Wheat bran[2]	6.59	93.41	3.69	14.58	3.14	78.59
Rice flour[2]	5.64	94.36	2.65	11.87	1.88	83.6

1 Star fish meal plant, Veraval, Gujarat, India

2 Local market, Veraval, Gujarat, India

Table 2: The formulation of control and treatment diets

Ingredients	Diets				
	T1 (Control)	T2	T3	T4	T5
Groundnut oil cake (42.34 CP)	52	52	52	52	52
Fish meal (46.11 CP)	40	40	40	40	40
Wheat bran (14.58 CP)	1	1	1	1	0.5
Rice Flour (11.87 CP)	1	1	1	1	1
Wheat Flour (11.09 CP)	2	1.5	1	0.5	0.5
Tapioca	1	1	1	1	1
Fish Oil[2]	2	2	2	2	2
Vitamin Mixture[3]	1	1	1	1	1
Total	0	0.5	1	1.5	2
Proximate analysis (determined on dry matter basis)					
Crude protein (CP) (%)	40.46	40.47	40.44	40.42	40.4
Ether extract (%)	10.07	9.96	9.93	9.88	9.86
Ash (%)	21.94	22.62	22.94	23.28	23.37
Moisture (%)	7.59	7.64	7.71	7.74	7.78
Nitrogen free extract (%)	19.94	19.31	18.98	18.68	18.59

1Gemini sunflower oil

2 seven sea cods

3 Vitamin and mineral mixture/Kg premix: Vitamin A-7,00,000IU, VitaminD3-70,000IU, Vitamin E-250 mg, Nicotinamide-1000 mg, Cobalt-150mg, Copper-1200mg, Iodine-25g, Iron-1500 mg, Magnesium-6000 mg, Manganese-1500 mg, Potassium-100mg, Sodium- 5.9mg, Sulphur-0.72%, Zinc-9600 mg, Calcium-25.5%, Phosphorus-12.75%.

grinder, and mixed directly with fish feed contents to achieve four modified diets at 5, 10, 15 and 20 g/kg of feed and control was without fenugreek (Table 1 and 2). Test diets were prepared and stored in airtight containers. The feed was provided at the rate of 10% of body weight initially, and after 10 days fishes were fed *ad libitum* three times daily (09.00, 13.00 and 18.00 hours) till the end of the experiment.

Blood collection for haematological and immunological parameters

Blood and serum samples for haematological, biochemical and immunological parameters which were assayed in randomly-picked fish (n = 6) that were collected from fish in each group and examined according to methods previously described by [7].

Calculations

The following calculations were made

Length gain (%) = (Final length – Initial length / Initial length) x 100

Weight gain (%) = [(Final weight – Initial weight)/Initial weight] x 100

Feeding Rate, FR (% Body weight/day) = [Dry feed intake/{60 days (FBW + IBW)/2}] x 100

Feed efficiency, (FE) = [Wet weight gain (g)/ total amount of the feed consumed (g)] x 100

Protein efficiency ratio, (PER) = Increment in body weight (g)/Protein intake (g)

Protein Productive Value [PPV (%)] = [Body wet protein gain (g)/Protein intake (g)] x 100

Protein growth rate [PGR (%day^{-1}] = [(Log$_e$ final protein content – Log$_e$ initial protein content)/days of feeding] x 100

Condition factor (CF) = Weight of fish (g)/[Length of fish (cm)]^3 x 100

Survival (%) = (No. of fish survived after rearing/No. of fish stocked) x 100

MCH (pg) = [Haemoglobin (g dl^{-1}/)/RBC x (10^{-6} C mm^{-1})] x 10

MCV (fl) = [Haematocrit (%)/RBC x (10^{-6} C mm^{-1})] x 10

MCHC (g/dl) = [Haemoglobin (g dl^{-1})/Haematocrit (%)] x 100

Statistical analysis

One way Analysis of Variance (ANOVA) and least significantly difference (LSD) [8] was applied to test the level of significance amongst the treatments.

Results

Table 3 shows the effects of different protein sources on fish performance. The growth curve is plotted to determine the average growth rate (SGR and ADG) of tilapia fry during the experimental period (Fig 1 and 2). Weight gain of fish fed

with diet T3 was found to be significantly ($p<0.05$) higher as compared to other treatment diets. Similarly, PER, PPV and PGR were found significantly ($p<0.05$) higher in the fish fed with diet T3 and. FE was significantly ($p<0.05$) lower in the fish fed with diet T3. The rate of fish survival ranged 100% ($p>0.05$) in all

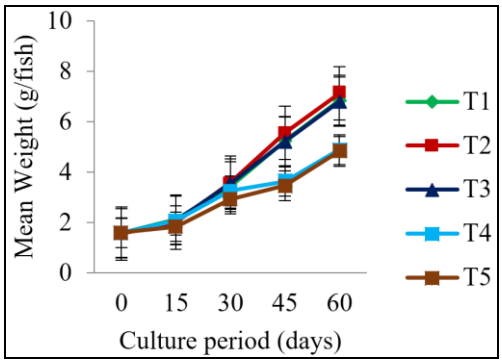

Fig 1: Average growth (g/fish) *O. niloticus* fed. different experimental diets for 60 days.

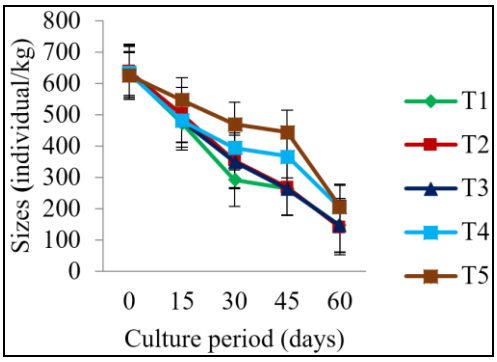

Fig 2: Average growth (individual/kg) of *O niloticus fed* different experimental diets for 60 days.

the treatment groups. A quadratic regression analysis indicated that weight gain and PER reached the maximum value at 0.98 g/kg and 0.99 respectively (Fig 3 and 4). Significantly ($p<0.05$) higher RBC, hemoglobin, WBC, HCT, MCV, MCH and MCHC values were obtained in group T3 (Table 4). Immunological parameters such as total plasma protein, albumin, globulin and total immunoglobulin were found significantly ($p<0.05$) higher in the diet T3 (Table 5). There was no significant ($p<0.005$) changes observed for proximate body composition of experimental fish (Table 6).

Table 3: Growth, food conversion ratio, protein efficiency ratio and survival of *O. niloticus* fed the test diets for 60 days

Parameters	Treatment				
	T_1 (Control)	**T_2**	**T_3**	**T_4**	**T_5**
L_1 (cm)	4.77±0.02^a	4.76±0.05^a	4.75±0.02^a	4.76±0.01^a	4.75±0.01^a
L_2 (cm)	7.46±0.21^c	8.44±0.02^b	8.81±0.11^a	8.33±0.01^b	7.49±0.01^c
Length gain (%)	65.39±4.65^c	77.6±2.00^b	85.56±1.53^a	74.95±0.19^b	57.72±0.35^c
W_1 (g)	1.68±0.00^b	1.68±0.00^{bc}	1.67±0.0^c	1.68±0.00^{bc}	1.69±0.00^a
W_2 (g)	4.99±0.03^c	6.95±0.05^b	7.23±0.07^a	6.90±0.03^b	4.92±0.08^c
Weight gain (%)	196.28±1.21^c	314.77±2.19^b	333.56±3.18^a	311.02±1.31^b	190.39±4.22^c
FR (% bw day^{-1})	2.73±0.01^d	3.34±0.04^b	4.95±0.03^a	3.09±0.03^c	2.16±0.01^e
FE (%)	48.75±1.16^d	67.51±0.83^b	74.22±0.41^a	59.08±0.53^c	41.19±0.34^e
PER	1.22±0.03^d	1.69±0.02^b	1.86±0.01^a	1.48±0.01^c	1.03±0.01^e
PPV (%)	50.25±1.19^d	69.16±0.85^b	76.01±0.42^a	60.91±0.54^c	42.19±0.35^e
PGR (% day^{-1})	0.56±0.00^b	0.57±0.00^a	0.57±0.00^a	0.56±0.00^b	0.56±0.01^b
CF	1.15±0.00^b	1.66±0.01^a	1.70±0.14^a	1.20±0.01^b	1.06±0.03^b
Survival (%)	100±0.00^a	100±0.00^a	100±0.00^a	100±0.00^a	100±0.00^a

*****Mean±SE within a row followed by with different superscripts are significantly different ($p<0.05$) from each other.
L1- Initial length, L2- Final length, W1- Initial weight, W2- Final weight, FR- Feeding Rate (% Body weight day-1), FE- Feed Efficiency, PER- Protein Efficiency Ratio, PPV- Protein Productive Value (%), PGR- Protein growth rate (% day-1), CF- Condition Factor

Discussion

In this study, fenugreek-supplemented diet improved the growth performance which agreed with findings of [9] who reported that the fenugreek seed as feed additive improved growth parameters (body weight, weight gain and specific growth rate) in Nile tilapia fingerlings. This observation is also in agreement with the finding of [10]. The beneficial effect of fenugreek seed may be due to its contents of active materials according to [11] who reported that fenugreek seeds have antioxidant activity.

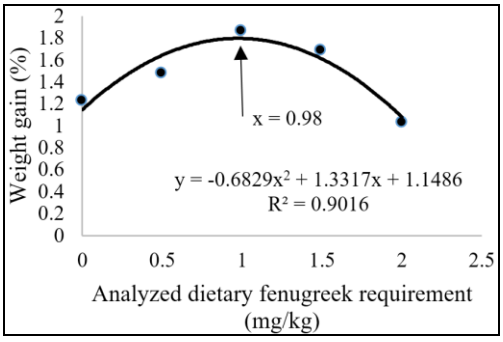

Fig 3: The relationship between weight gain and dietary fenugreek level in tilapia fry for 60 days.

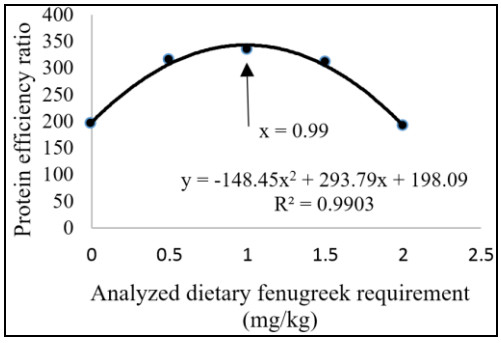

Fig 4: The relationship between protein efficiency ratio and dietary fenugreek level in tilapia fry for 60 days.

Table 4: Haematological parameters of O. niloticus fed the test diets for 60 days (Mean ± SE)

Parameters	Treatment				
	T_1 (Control)	**T_2**	**T_3**	**T_4**	**T_5**
RBC (x 106 mm^{-3})	1.85 ± 0.06^{b}	2.19 ± 0.04^{a}	2.24 ± 0.09^{a}	2.17 ± 0.03^{a}	1.83 ± 0.04^{b}
Hb (g dl^{-1})	7.71 ± 0.09^{b}	9.29 ± 0.07^{a}	9.35 ± 0.11^{a}	9.25 ± 0.06^{a}	7.68 ± 0.07^{b}
HCT (%)	22.36 ± 0.92^{c}	29.76 ± 0.94^{a}	31.38 ± 0.81^{a}	28.43 ± 0.82^{b}	20.89 ± 0.85^{c}
MCV (fl)	124.25 ± 0.84^{c}	135.59 ± 1.72^{b}	142.53 ± 1.20^{a}	133.54 ± 0.11^{b}	117.79 ± 1.05^{d}
MCH (pg)	47.37 ± 0.75^{bc}	56.41 ± 0.79^{a}	58.39 ± 0.82^{a}	48.59 ± 1.03^{b}	44.67 ± 0.87^{c}
MCHC (g dl^{-1})	37.95 ± 0.73^{b}	43.48 ± 0.64^{a}	44.86 ± 0.97^{a}	41.76 ± 0.68^{a}	36.74 ± 0.98^{b}
WBC (x10^3 mm^{-3})	59.62 ± 1.08^{c}	67.87 ± 0.11^{ab}	69.39 ± 0.12^{a}	65.59 ± 1.25^{b}	57.80 ± 1.16^{c}
Eosinophils (% mm^{-3})	0.48 ± 0.04^{a}	0.21 ± 0.04^{c}	0.18 ± 0.04^{c}	0.38 ± 0.03^{b}	0.50 ± 0.03^{a}
Basophils (% mm^{-3})	0.20 ± 0.03^{bc}	0.25 ± 0.02^{a}	0.27 ± 0.03^{a}	0.24 ± 0.03^{ab}	0.16 ± 0.04^{c}
Neutrophils (% mm^{-3})	2.79 ± 0.17^{a}	2.05 ± 0.03^{c}	1.87 ± 0.02^{c}	2.28 ± 0.05^{b}	$2.94\pm.04^{a}$
Lymphocytes (% mm^{-3})	85.1 ± 0.82^{c}	89.8 ± 0.42^{ab}	90.6 ± 0.59^{a}	88.3 ± 0.88^{b}	84.5 ± 0.68^{c}
Monocytes (% mm^{-3})	11.43 ± 0.87^{a}	7.69 ± 0.07^{bc}	7.08 ± 0.04^{c}	8.80 ± 0.41^{b}	11.90 ± 0.71^{a}

*Mean±SE within a row followed by with different superscripts are significantly ($p<0.05$) different from each other
RBC- Red Blood Cells; Hb- Haemoglobin; WBC- White Blood Cells; HCT- Haematocrit; MCV- Mean corpuscular volume; MCH- Mean corpuscular haemoglobin; MCHC- Mean corpuscular haemoglobin concentration

Table 5: Blood serum biochemical parameters of *O. niloticus* fed the test diets for 60 days (Mean± SE)

Parameters	Treatment				
	T_1 (Control)	**T_2**	**T_3**	**T_4**	**T_5**
Total protein (g dl^{-1})	4.68 ± 0.05^{a}	4.71 ± 0.08^{a}	4.84 ± 0.05^{a}	3.91 ± 0.08^{b}	3.88 ± 0.09^{b}
Total immunoglobulin (g dl^{-1})	0.89 ± 0.05^{a}	0.90 ± 0.04^{a}	0.94 ± 0.04^{a}	0.79 ± 0.03^{b}	0.77 ± 0.02^{b}
Albubin (g dl^{-1})	1.45 ± 0.03^{a}	1.49 ± 0.04^{a}	1.52 ± 0.05^{a}	1.16 ± 0.04^{b}	1.12 ± 0.06^{b}
Globulin (g dl^{-1})	2.33 ± 0.05^{a}	2.34 ± 0.12^{a}	2.36 ± 0.14^{a}	2.18 ± 0.09^{b}	2.16 ± 0.08^{b}
A/G	0.62 ± 0.03^{ab}	0.64 ± 0.04^{a}	0.64 ± 0.06^{a}	0.53 ± 0.07^{bc}	0.52 ± 0.05^{c}

*Mean±SE within a row followed by with different superscripts are significantly ($p<0.05$) different from each other

Haematological analysis, including erythrocyte and leucocyte count, have provide valuable knowledge for fishery biologistsin the evaluation of fish health and in monitoring stress responses [12]. The present results are in agreement with that previous research in which feeding gilthead seabream specimens with fenugreek results in an increment in WBC and RBC counts. It has been suggested that those increments are due to the effectiveness role of fenugreek as natural antioxidant [13]. The present results on the effects of fenugreek on antioxidant gene expression in liver from seabream specimens (discussed below) seem also to corroborate this possibility.

The concentration of total protein, albumin, globulin and the total immunoglobulin in blood serum is used as a basic index for the health status of brood fish [14]. As there is a close relationship between the rate of protein synthesis in the liver tissue and total protein concentrations in the serum, the increase in the total protein levels in treated fish probably reflects the increase in the protein synthesis in liver tissue. In our study, total protein of serum was significantly enhanced in T3 group compared to control ($P<0.05$) which agrees with findings of [9]. [15] reported that the increase in serum total protein indicates that fish are immunologically strong.

Table 6: Proximate analysis of fish carcass (Dry weight basis)

Parameters	T1 (Control)	T2	T3	T4	T5
Crude protein	40.84±0.73	40.76±1.01	40.63±0.50	40.44±1.59	40.32±0.62
Ether extract	14.97±0.70	14.85±0.55	14.74±0.24	14.61±0.74	14.55±0.72
Ash	21.98±1.04	22.15±1.51	22.29±0.60	22.43±0.64	22.58±1.26
Moisture	20.56±0.94	20.62±71	20.76±0.99	20.88±0.37	20.95±.03
Nitrogen free extract	1.65±0.04	1.62±0.04	1.58±0.04	1.64±0.01	1.60±0.04

*Mean±SE within a row followed by with different superscripts are significantly ($p<0.05$) different from each other

Conclusion

In conclusion, the results of this study revealed that fenugreek seed is beneficial dietary supplement for improving growth performance and blood parameters in Nile tilapia fry; the best results were obtained at the 1% level. However, the quadratic regression analysis suggested that the optimal fenugreek requirement was 0.98 g/kg and 0.99 based on maximum weight gain and protein efficiency ratio respectively.

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