Dietary effect of fenugreek (*Trigonella foenum graecum*) and liquorice (*Glycyrrhiza glabra*) on growth performance, antioxidant activities and stress resistance in Oreochromis niloticus

Sarah A. Basha[1], Eman A. Abd El-Gawad[2], Ashraf A. Abd EL latif[2] and Amany A. Abbass[2*]

[1]*Directorate of Veterinary Medicine Gharbiya Governorate, Egypt.*

[2]*Department of Aquatic Animal Diseases and Management, Faculty of Veterinary Medicine, Benha University, Egypt.*

**Correspondence to: Amany A. Abbass. Email: amanyabbass3@fvtm.bu.edu.eg*

ABSTRACT

This work aimed to investigate the effect of dietary fenugreek seeds and liquorice roots on growth performance, immune status, antioxidant and stress resistance in Nile tilapia (*Oreochromis niloticus*). For this, five groups of fish each in duplicate were used, the 1st 2 groups were fed daily on experimental diets 1% and 2% (wt/wt) fenugreek; the 2nd 2 groups were received 1% and 2 % (wt/wt) liquorice and the remaining group was fed on control diet for 90 days. Growth performance (ABW, WG, WGR, SGR, L and LGR) and intestino-somatic index of fish fed 2% fenugreek supplemented diet were significantly increased ($P < 0.05$) compared to control. Hematological parameters (RBCs, WBCs, PCV, hemoglobin concentration and DLC) revealed significant increase in groups fed 2 % fenugreek and 2% liquorice. Serum total protein and globulin were significantly increased in groups received 1 and 2 % liquorice supplemented diets. Liver enzyme (ALT) in fish fed 1 and 2 % liquorice diets was lower than control. Blood cortisol and glucose showed significant decrease with 1 % fenugreek. Liquorice treated groups exhibited significant decrease in lactate dehydrogenase (LDH). Hepatic antioxidant enzymes [(SOD), (CAT), and (GSH)] showed significant increase with 2 % dietary fenugreek and 2% liquorice, while Malondialdehyde (MDA) was significantly decreased compared to control. Post feeding, sub-groups of fish were exposed to 6 days starvation and hypoxia stress and the results showed that, cortisol and glucose levels were significantly decreased in groups fed 2 % fenugreek and liquorice treated diets and the antioxidant enzymes SOD, CAT and GSH response were characterized by significant improvement with lower oxidative stress (MDA) level compared to control. Results suggested that dietary supplementation of fenugreek seeds and liquorice roots enhance growth performance and improve resistance against starvation and hypoxia in *Oreochromis niloticus*.

Keywords: Growth, Fenugreek, Liquorice, Immunity, tilapia fry, stress.

(http://www.bvmj.bu.edu.eg) **(BVMJ-34(3): 355-370, 2018)**

1. Introduction

Aquaculture is an important source for seafood than fisheries. Fish consumption is reported to increase further in countries such as Africa, Asia, America, and European regions during 2010–2030 (Kobayashi *et al*., 2015). There are many management practices such as overcrowding, sudden changes in temperature, poor water quality, low oxygen level, bad handling and starvation which lead to physiological disturbance, immune suppression and increase susceptibility to infectious diseases (Cabello, 2006; Quesada *et al*., 2013). The main method to control diseases is antibiotics but it has disadvantages such as accumulation of residues in fish tissue or in the environment and drug resistant pathogen (Santos and Ramos, 2016). Although, vaccination is a good method of disease prevention, but it is too expensive and unpractical for wide use in fish farms (Harikrishnan *et al*., 2011). Therefore, there is a great attention for use the whole herbal plants or its extracts as an alternative method due to its cheap price, provide better growth for fish and protection against disease (Bahi *et al*., 2017) and also as appetite stimulation, antimicrobial, immune stimulation and stress resistant in fish (Chakraborty and Hancz., 2011).

Fenugreek seeds have antioxidant, immuno modulatory effect, anti-carcinogenic, anti-ulcer due to their bioactive contents of flavonoids such as: apigenin, kaempferol and quercetin and saponins including diosgenin and yamogenin. (Olaiya and Soetan, 2014). Enhancement of growth performance and fish immune system after dietary administration of fenugreek seeds was recorded by Bahi *et al*., (2017) and Guardiola *et al*., (2018).

Liquorice roots possess a considerable number of properties including those that are anti-inflammatory, anti-viral, and antimicrobial (Asl and Hosseinzadeh, 2008). Glabridin is a polyphenolic flavonoid bioactive compound from liquorice has immunostimulants properties (Zore *et al*., 2008). This study aimed to evaluate the effects of dietary fenugreek seeds and liquorice roots on the growth performance, Immuno-haematological parameters, antioxidant activities and resistance against starvation and hypoxia stress in *Oreochromis niloticus* fry.

2. Materials and methods

2.1. Fish

Nile tilapia, *O. niloticus* fry with an average body weight 2±0.4 g was obtained from fish farm at Kafr El Sheik province, Egypt. Fish were stocked in well prepared fiberglass tanks (750L) for 2 weeks acclimation period and fed twice daily on basal diet (42 % crude protein). The health conditions of fish were examined for any diseases as described by Austin and Austin (1989). The water temperature was adjusted to 25±2°C and dissolved oxygen was maintained at 5mg/L

2.2 Herbal plants

Herbal plants fenugreek *(Trigonella foenum graecum)* seed sand liquorice (*Glycyrrhiza glabra)* roots were purchased from local hypermarket in El-Gharbiya province, Egypt. The dried seeds of fenugreek and liquorice roots were grinded to fine powder by electrical blinder and preserved at 4^{o}C in refrigerator till use.

2.3. Preparation of experimental diets

The experimental diets were prepared from the following ingredients according to NRC, (2011) as follow; rice bran 125 g , wheat bran 23 g, fish meal 300 g, yellow corn 150 g, soybean 350 g, glutean meal 25 g, vegetable oil 25 g and vitamin premix 3g . Five diets were prepared; first diet (basal diet 0%) was kept as control, second and third diets were incorporated with 1 and 2 %

(wt/wt) fenugreek. The last two diets were incorporated with 1 and 2 % (wt/wt) liquorice respectively. A Suitable amount of water containing the adequate amount of either fenugreek seed powder or liquorice root power was mixed with all ingredients forming moist dough and pelleted. The obtained pellets were allowed to dry at room temperature for 48 days then packed in clean dry tightly closed plastic containers and kept at 4ºC until use.

2.4. Experimental design

The experiment was designed to include two trials:

2.4.1 Feeding experiment

Fish was divided into 5 groups in fiberglass tanks (750 L capacity) in duplicate. The control group was fed a basal diet (0%) and other four groups received basal diets incorporated with fenugreek at (1 and 2%) and liquorice at (1 and 2%). All experimental groups were fed twice daily at 15 % of their body weight. Each two weeks, fish weighted to adjust the feeding rate and the rate decreased to 10% in the 2nd month and to 7% in the 3rd month of experiment. The water temperature was adjusted to 25±2 °C and the oxygen level was maintained at 5mg/L. All tanks were siphoned to remove excreta and two third of water was exchanged daily. Dead fish was also recorded.

2.4.2. Stress experiment

At the end of feeding experiment, two sub-groups from all treated fish and control were distributed into well prepared glass aquaria (30×40×90 cm) in duplicate (n=20 fish). The first sub-group was exposed to starvation by holding the experimental diets for six days.

The second sub-group was subjected to hypoxia stress by stopping the aeration with measuring dissolved oxygen conc. (DO) every hour using an oxygen meter to 3.5 mg/l and fish showed signs of hypoxia as surfacing with rapid movement of operculum.

2.5. Blood and tissue sampling

Blood and tissue samples were taken on day 90 post feeding, and after exposure to starvation stress for 6 days and to hypoxia stress (stress experiment).

Blood samples were withdrawn from the caudal blood vessels of anesthetized fish using syringe moisten with EDTA anticoagulant for hematological parameters assay, and another amount without anticoagulant to separate serum and stored at –80°C until used

Liver tissues were carefully taken from dissected fish and kept in cold phosphate buffer saline (pH 7.2) and then stored at –80°C until used.

2.6. Determination of growth performance and biosomatic indices

After 90 days feeding, ten fish per replicate (20 fish per group) were weighted and length of each fish was measured for evaluating growth parameters including final body weight , weight gain (WG), weight gain rate (WGR), length gain rate (LGR), feed conversion ratio (FCR), and feed efficiency ratio (FER). Moreover, specific growth rate (SGR) was determined according to the formula, SGR= [(Ln (Wf) _ Ln (Wi) /number of days] ×100. Where, Wf: final weight ; Wi: Initial weight

Hepato-somatic (HSI) and Spleno-somatic (SSI) according to Yun *et al*., (2012), and Intestino-somatic index (ISI) according to Zhang *et al., (*2014) were estimated.

2.5. Immuno-haematological parameters assay

Total erythrocyte (RBCs) and leucocyte number (WBCs) were counted as Kanaev, (1985). Hemoglobin concentration and differential leukocytes count (DLC) were

detected by Stoskopf, (1993); Packed cell volume (PCV %) and blood indices (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were estimated as described by Dacie and Lewis, (1991).

Serums total protein was assayed according to Gornall, (1949) and albumin was determined according to Doumas *et al*., (1971). The globulin level was calculated by subtracting albumin level from total protein. Albumin /globulin ratio was calculated by dividing albumin values by globulin values.

2.6. Liver function enzymes assay

Serum Liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated according to Kiln, (1970) .

2.7. Biochemical parameters assay

Serum levels of cortisol (Braham and Trinder, 1972), glucose (Rittler and Geisler, 1983), Insulin (immuno diametric assay based on coated-tube separation using Insulin-IRMA–CT by Temple *et al*., 1992) and Lactate dehydrogenase (LDH) (Bais and Philcox ., 1994) were estimated.

2.8. Determination of antioxidant enzymes activity:

Liver antioxidant enzymes activities were evaluated using Commercial kits (Bio-dignostic Company, Egypt). Liver homogenates were centrifuged at 4000 ×g for 15 min using cooling centrifuge at 4°C. The supernatants were taken for assaying Superoxide Dismutase (SOD) and Catalase (CAT) enzymes according to Fossati *et al., (*1980), and Glutathione Reductase (GSH-Rx) and Malondialdehyde (MDA) were estimated by Satoh, (1978).

2.9. Statistical Analysis

The data was analyzed by one- way analysis of Variance (ANOVA) and Duncan's multiple range tests to determine significant differences between groups using the statistical package for the social sciences (SPSS) software (Version 17.0). A value of $P < 0.05$ was considered significant.

3. Results

3.1. Effect on growth performance and bio-somatic indices

Fish fed on fenugreek 2% showed significant increase ($P < 0.05$) of all parameters (ABW, WG, WGR, SGR, L and LGR), while those received liquorice at both doses revealed no significance difference compared to control (Table 1). Hepato and Spleno somatic indices exhibited no significance ($P > 0.05$) change in all groups except liquorice1% showed significant decrease in HIS. While, Intestinal somatic index revealed significant increase with fenugreek 2% compared to control ($p< 0.05$) (Table, 2).

3.2. Effect on immune-hematological parameters

Fish fed with fenugreek 2% and liquorice 2% enriched diets showed significant increase in PCV, HB, RBCs and MCV. Total leucocytic count was significantly increased in fenugreek 2%, liquorice 1 % and liquorice 2% treated groups and MCH and MCHC increased significantly with liquorice 2% (Table 3).

Both groups fed on liquorice 1% and 2% exhibited significant increase in serum total protein and globulin levels. A/G ratio recorded significant decrease with 1% liquorice diet and no significant difference in other treated groups (Table, 4).

3.3. Effect on liver function enzymes

Fish received 1% and 2% liquorice supplemented diets recorded significant decrease in ALT than control. While, AST showed no significance in all groups compared to control (Table, 5).

3.4. Effect on biochemical parameters

Before stress, glucose level was significantly decreased with fenugreek 1% and 2% treated groups. Cortisol level showed significant decrease at fenugreek 1% and LDH revealed significant decrease at both groups fed liquorice1 % and 2% enriched diet (Table, 6).

Post exposure to starvation stress, glucose and cortisol level exhibited significant decrease at fenugreek 1%, 2% and liquorice 2% treated diets, and LDH reported significant decrease at liquorice 2% (Table, 7).

After hypoxia stress, glucose and cortisol level significantly decreased at fenugreek 2% and liquorice 2% incorporated diets. Similarly, LDH significantly decreased at fenugreek 1% and liquorice 2% enriched diets (Table, 8).

3.5. Effect on antioxidant enzymes activities

Before stress, MDA revealed significant decrease at all treated groups compared to control, while SOD and GSH significaly increased at fenugreek 1, 2% and liquorice 2% (Table, 9).

Post starvation stress, MDA showed significant decrease at fenugreek 1%, fenugreek 2% and liquorice 2%, but SOD, CAT and GSH showed significant increase at fenugreek2%, and liquorice 2% showed significant increase at CAT and GSH (Table, 10).

Post hypoxia stress, MDA recorded significant decrease at fenugreek 2% and liquorice 2% incorporated diets. While, SOD and GSH showed significant increase at fenugreek 2% and liquorice 2% (Table,11).

Table (1): Effect of dietary supplementation of fenugreek and liquorice on growth performance of *O. niloticus*.

Group	Wo(gm)	FBW(gm)	WG(gm)	WGR%	SGR%	FCR	FER	L0(cm)	L(cm)	LGR
Control	2.10 ±0.06	8.32^{b}±0.59	6.22^{b}±0.57	294.47^{b}±25.94	1.48^{b}±0.07	7.72±0.69	0.15±0.01	1.89 ± 0.05	8.00^{b} ± 0.20	333.38^{b} ± 19.70
Fenugreek1%	2.18 ±0.02	10.00^{ab}±0.71	7.83^{ab}±0.70	357.43^{ab}±30.41	1.65^{ab}±0.06	6.37±0.41	0.17±0.01	1.90 ± 0.03	8.38^{b} ± 0.19	341.80^{b} ± 9.18
Fenugreek2%	2.36 ±0.10	13.60^{a}±1.91	11.24^{a}±1.85	454.42^{a}±63.09	1.79^{a}±0.11	6.24±0.61	0.21±0.03	1.65 ± 0.07	9.59^{a}± 0.40	503.36^{a} ± 34.92
Liquorice1%	2.02 ±0.06	10.68^{ab}±2.02	8.66^{ab}±1.97	400.01^{ab}±79.17	1.62^{ab}±0.13	7.06±0.77	0.21±0.04	1.66 ± 0.07	8.03^{b} ± 0.44	391.93^{b}± 23.42
Liquorice2%	2.00 ±0.08	8.13^{b}±0.88	6.13^{b}±0.81	292.75^{b}±27.54	1.48^{b}±0.07	7.37±0.55	0.15±0.01	1.72 ± 0.05	7.79^{b}± 0.43	353.09^{b}± 18.83

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=10)/replicate.

Table (2): Effect of dietary supplementation of fenugreek and liquorice on Bio-somatic indices of *O. niloticus*.

Group	HSI	SSI	ISI
Control	3.73^{ab}±0.13	0.36±0.04	4.93^{b}±0.23
Fenugreek 1%	4.05^{a}±0.23	0.61±0.06	5.20^{b}±0.34
Fenugreek 2%	3.29^{bc}±0.18	0.53±0.05	7.71^{a}±0.37
Liquorice 1%	2.78^{c}±0.23	0.84±0.39	3.51^{c}±0.14
Liquorice 2%	3.19^{bc}±0.13	0.54±0.05	4.66^{b} ±0.27

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=10)/replicate.

Table (3):Effect of dietary supplementation of fenugreek and liquorice on hematological parameters of *O.niloticus*

Group	PCV%	HB g/dl	RBC $X10^{12}$/ L	MCV(fl)	MCH(pg)	MCHC%	TLC $\times10^{9}$/L	Monocyte%	Lymphocyte%	Neutrophils_%	Eosinophili%
Control	0.95 ^{b} ± 0.08	4.30^{b} ± 0.35	13.07 ^{b} ± 1.16	114.93^{b} ±11.16	40.90 ^{b} ± 2.26	33.03 ^{b} ± 0.48	80.00 ^{c} ± 0.52	6.28 ^{b} ± 0.62	69.40 ^{b} ± 3.64	6.26 ^{b} ± 1.11	0.84 ± 0.09
Fenugreek1%	0.91 ^{b} ± 0.04	4.00 ^{b} ± 0.21	11.60^{b} ± 0.76	120.40 ^{ab}± 4.08	43.75 ^{b} ± 0.41	31.23 ^{b} ± 1.64	85.70 ^{bc} ± 2.79	9.47 ^{a} ± 1.12	70.66 ^{b} ± 0.39	5.67 ^{b} ± 1.07	0.88 ± 0.45
Fenugreek 2%	1.53 ^{a} ± 0.21	6.30^{a} ± 0.61	17.53 ^{a} ± 1.50	137.57 ^{a} ± 1.60	45.47 ^{ab} ± 0.90	35.87 ^{ab}± 1.68	88.40 ^{b} ± 3.99	8.90^{a} ± 0.46	74.37 ^{ab}± 1.90	9.35 ^{a} ± 0.72	1.15 ± 0.23
Liquorice1%	0.94 ^{b} ± 0.03	4.50^{b} ± 0.15	11.43 ^{b} ± 0.24	119.66 ^{ab} ± 4.54	42.17 ^{b} ± 1.13	35.27 ^{ab}± 0.56	96.73 ^{a} ± 0.69	9.07 ^{a} ± 0.33	77.59 ^{a} ± 0.29	8.10 ^{ab}± 0.36	1.42 ± 0.27
Liquorice 2%	1.60 ^{a} ± 0.06	6.80^{a} ± 0.35	19.30^{a} ± 1.30	140.63^{a} ± 5.85	49.75 ^{a} ±1.70	39.53 ^{a} ±1.89	97.13 ^{a} ± 0.84	9.00^{a} ± 0.31	77.67 ^{a} ± 0.13	9.37 ^{a} ± 0.72	0.94 ± 0.03

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=3)/replicate

Table (4): Effect of dietary supplementation of fenugreek and liquorice on total protein and albumin of *O.niloticus.*

Group	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio
Control	3.41^{b} ±0.01	1.83^{ab} ± 0.17	1.58^{b} ± 0.18	1.21^{a} ±0.25
Fenugreek 1%	2.90^{b} ± 0.19	1.43^{c} ± 0.13	1.47^{b} ± 0.07	0.97^{ab} ±0.04
Fenugreek2%	3.53^{b} ± 0.28	1.80^{ab} ± 0.05	1.73^{b} ± 0.33	1.14^{a} ±0.26
Liquorice1%	4.42^{a} ± 0.24	1.58^{bc} ± 0.08	2.84^{a} ± 0.16	0.56^{b} ±0.00
Liquorice2%	5.16^{a} ± 0.40	2.06^{a} ± 0.01	3.10^{a} ± 0.40	0.69^{ab} ± 0.09

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=3)/replicate

Table (5): Effect of dietary supplementation of fenugreek and liquorice on liver enzyme of *O.niloticus.*

Group	AST (U/l)	ALT (U/l)
Control	314.13 ± 7.14	286.30^{a} ± 24.19
Fenugreek 1%	295.95 ± 9.56	164.60^{bc} ± 3.03
Fenugreek 2%	318.48 ± 0.59	327.80^{a}± 10.33
Liquorice1%	305.13 ± 29.49	209.50^{b} ± 28.23
Liquorice2%	ND	116.12^{c} ± 1.51

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=3)/replicate. ND: not detected due to insufficient serum samples.

Table (6): Effect of dietary supplementation of fenugreek and liquorice on stress parameters of *O.niloticus* before stress.

Group	Glucose (mg/dl)	Cortisol (mg/l)	Insulin (pmol/l)	LDH (U/l)
Control	202.57 ^{a} ± 9.77	236.10^{a} ± 6.52	10.85 ^{ab} ± 0.09	2084.27^{a} ± 481.65
Fenugreek 1%	95.76 ^{c} ± 2.01	189.60 ^{b} ± 6.29	10.50^{b} ± 1.04	1687.50^{a} ± 324.76
Fenugreek 2%	171.62^{b} ± 5.24	219.04^{ab} ± 17.89	10.00 ^{b} ± 0.12	1902.50^{a} ± 283.77
Liquorice1%	221.84 ^{ab}± 21.09	228.20 ^{ab} ± 16.51	11.35 ^{ab} ± 0.61	700.75 ^{b} ± 38.83
Liquorice2%	186.71 ^{ab} ± 8.02	254.48^{a} ± 9.48	13.35 ^{a}± 1.36	740.00 ^{b} ± 42.72

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=3)/replicate

Table (7): Effect of dietary supplementation of fenugreek and liquorice on stress parameters of *O.niloticus* after starvation stress.

Group	Glucose (mg/dl)	Cortisol (mg/l)	Insulin (pmol/l)	LDH (U/l)
Control	72.10^{a} ± 2.12	251.12^{a} ± 7.41	9.87^{b} ± 0.23	1387.00^{a} ± 95.77
Fenugreek1%	35.43^{c} ± 5.26	214.00^{b} ± 8.08	10.40^{b} ± 0.35	1290.75^{a} ± 34.21
Fenugreek2%	52.58^{b} ± 3.40	191.10^{c} ± 8.72	12.13^{a} ± 0.25	1232.88^{a} ± 44.38
Liquorice1%	63.40^{ab} ± 4.65	244.34^{a} ± 5.73	10.02^{b} ± 0.58	1388.00^{a} ± 39.26
Liquorice2%	56.53^{b} ± 2.26	182.80^{c} ± 2.37	11.28^{ab} ± 0.70	1022.00^{b} ± 51.96

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=3)/replicate

Table (8): Effect of dietary supplementation of fenugreek and liquorice on stress parameters of *O.niloticus* after hypoxia stress.

Group	Glucose (mg/dl)	Cortisol (mg/l)	Insulin (pmol/l)	LDH (U/l)
Control	164.15^{a} ± 7.25	269.20^{a} ± 16.05	10.80 ± 0.58	715.00 ^{a} ± 34.06
Fenugreek1%	144.01 ^{a} ± 6.17	190.06^{b} ± 12.85	11.15 ± 0.45	496.00^{b} ± 94.25
Fenugreek2%	95.84^{b} ± 2.80	169.50^{b} ± 15.47	11.10 ± 0.91	561.17^{ab} ± 66.35
Liquorice1%	164.54^{a} ± 7.01	288.32^{a} ± 6.84	9.73 ± 0.88	746.67^{a} ± 46.40
Liquorice2%	114.81^{b} ± 7.80	200.80^{b} ± 7.36	10.42 ± 0.29	402.00^{b} ± 1.73

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=3)/replicate

Table (9):Effect of dietary supplementation of fenugreek and liquorice on antioxidant enzyme of *O.niloticus* before stress .

Group	MDA (nmol/ g)	SOD (U/g)	CAT (U/g)	GSH (mg/g)
Control	870.17^{a} ±20.70	132.07 ^{c} ± 0.48	493.41 ^{c} ± 5.70	45.46 ^{c} ± 3.46
Fenugreek1%	474.33^{bc}± 23.09	334.21^{b} ± 28.84	609.44 ^{b} ± 10.61	90.46 ^{b} ± 4.97
Fenugreek2%	280.46^{d}± 1.77	403.32^{a} ± 26.51	756.96 ^{a} ± 39.31	133.38^{a} ± 7.27
Liquorice1%	544.16^{b} ±38.80	109.95^{c} ± 2.10	528.18^{bc} ± 36.50	59.72 ^{c} ± 3.08
Liquorice2%	346.67^{cd}± 99.67	334.83^{b} ± 19.56	599.11^{bc} ± 50.24	104.65^{b} ± 2.69

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=3)/replicate

Table (10): Effect of dietary supplementation of fenugreek and liquorice on antioxidant enzyme of *O.niloticus* after starvation stress.

Group	MDA (nmol/ g)	SOD (U/g)	CAT (U/g)	GSH (mg/g)
Control	743.53^{a}± 85.90	417.67^{b} ± 45.60	438.87^{b} ± 70.78	46.66^{b} ± 4.62
Fenugreek1%	340.71^{b}± 38.32	339.66^{b} ± 26.84	308.02^{b} ± 0.74	53.06^{ab} ± 3.08
Fenugreek2%	278.41^{b}±62.16	804.71^{a} ± 24.46	761.35^{a} ± 65.30	61.72^{a} ± 1.77
Liquorice1%	688.10^{a}± 57.62	732.70^{a} ± 80.25	503.85^{b} ± 4.75	55.46^{a} ± 0.16
Liquorice2%	350.40^{b}± 38.24	399.39^{b} ± 27.50	700.96^{a} ± 91.93	59.46^{a} ± 1.08

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=3)/replicate

Table (11): Effect of dietary supplementation of fenugreek and liquorice on antioxidant enzyme of *O.niloticus* after hypoxia stress .

Group	MDA (nmol/ g)	SOD (U/g)	CAT (U/g)	GSH (mg/g)
Control	544.22^{a}±38.52	136.11^{c} ± 8.70	380.31^{b} ± 62.76	46.53^{c} ± 3.46
Fenugreek1%	532.31^{a}± 52.83	135.58^{c} ± 0.28	372.07^{b} ± 32.65	73.19^{b}± 5.93
Fenugreek 2%	250.03^{b}± 66.77	440.12^{a} ± 18.31	478.04^{b} ± 42.66	126.92^{a} ± 1.23
Liquorice1%	578.20^{a}± 10.91	130.13^{c} ± 3.43	455.42^{b} ± 25.06	55.99^{c}± 6.31
Liquorice2%	256.54^{b}± 63.37	335.90^{b} ±34.32	901.35^{a} ± 44.70	77.28^{b} ± 0.95

Values (means± SEM) with different letters in the same column are significantly different (P<0.05, n=3)/replicate

4. Discussion

There is positive relation between a proper diet and health to prevent outbreaks of disease (Guardiola *et al*., 2016). Using herbal plant as alternative to chemical treatment and growth promoter has been greatly increased in aquaculture. In this study, fish fed on fenugreek 2% showed significant increase in growth performance. A similar finding were recorded by Awad *et al*., (2015), Bahi *et al*., (2017) and Zaki *et al*., (2012) in other fishes fed with fenugreek supplemented diets. This result may be due to ability of fenugreek seeds to improve protein digestion and fat absorption capacity (Mansour and El-Adawy, 1994) and their contents of flavonoids which essential for health and growth (Jiang *et al*., 2007).

Liquorice showed that both doses revealed no significant changes at growth parameters. Similarly, Elabd *et al*., (2016) recorded no significant difference in growth performance in Yellow perch received diets with 1% and 2 liquorice.

The bio-somatic indices are considered as an environmental stress indicators of fish (Morgan *et al*., 2008). In this study HSI and SSI exhibited no significant change in all treated groups, while ISI

revealed significant increase at fenugreek 2% treated group. In the same respect, there was significant increase of ISI in *O.niloticus* fed 2% ginger (Islam, 2015), thyme (Doaa et al., 2017) and pollens (ElAsely *et al*., 2014). The increase of ISI may be due to increase in thickness of intestinal tract villi (Wang *et al*., 2007).

Blood is a patho-physiological indicator of the entire body and the counts of hematological parameters indicate the health statue of fish (Tewary and Patra, 2011). This work revealed that fenugreek 2% and Liquorice 2% enriched diet showed significant increase at PCV, HB, RBCs count, MCV and WBCs in *O. niloticus*. Nearly similar observations were detected by many authors (Antache *et al.,* 2014*;* Gültepe *et al.,* 2014; Awad, *et al.,* 2015 and Roohi *et al*., 2017).

Serum globulins are the source of immune-globulins, so their level is an index for the concentration of antibodies and the immune health of fish (Goda, 2008). In this study, fish fed on liquorice 1% and 2% exhibited significant increase in total protein and globulins. The same findings were observed in previous studies (VasudevaRaoa *et al*., 2006; Abdel-Zaher *et al*., 2009; Kaleeswaran *et al*., 2012 and Kumar *et al*., 2017). A/G ratio in this study significantly decreased with 1% liquorice enriched diet. This result corroborate with El-Asely *et al*., (2014) and Abd El-Gawad and Abdel Hamid (2014). Also Guardiola *et al.*, (2018) observed that Gilthead seabream fed on 1%, 5% and 10% fenugreek enriched diets showed a significant decrease in A/G ratio, due to the increase in the total globulin level.

Liver enzymes are important for deamination and synthesis of amino acid also important for assessing the health state of liver (Verma *et al*., 1981). In this study, fish received 1% and 2% liquorice supplemented diets recorded significant decrease of ALT activity compared to control. While AST level showed no significance difference in all treated groups. These results are supported by those of Zaki *et al*., (2012) who recorded significant decrease at ALT and AST level of Nile tilapia fed on fenugreek meal, and Elabd *et al*., (2016) who observed decreasing in ALT and AST activities of yellow perch fed liquorice.

Plasma cortisol and glucose levels are used as environmental stress indicators in fish (Barton and Iwama, 1991). In this work, pre exposure to stress the glucose level were significaly decreased at fenugreek 1% and 2% treated groups ,cortisol level showed significant decrease at fenugreek 1%, LDH revealed significant decrease at both groups liquorice1and 2% enriched diet. In the same respect (Elabd *et al*., 2016) revealed that yellow perch received 2 % liquorice four weeks of feeding gave significant decrease in glucose and cortisol levels.

Exposure to starvation resulted in significant decrease of glucose and cortisol levels with fenugreek 1%, 2% and liquorice2% treated diets and significant decrease in LDH level at liquorice 2% incorporated diets. While hypoxia stress induced significantly decrease of glucose and cortisol level at fenugreek 2% and liquorice 2% and for LDH at fenugreek 1% and liquorice 2%. A similar finding by Elabd *et al.,* (2016) recorded that incorporating the diets with 1% liquorice exhibited the most significant decrease in glucose and cortisol levels after 1-week starvation stress.

Antioxidant enzymes are important for improvement the imbalance in biological systems (Madeira *et al*., 2013). Pre exposure to stress MDA level revealed significant decrease in all treated groups compared to control while SOD and GSH significaly increased at fenugreek 1, 2% and liquorice 2%. In the same manner Emeish and Saad EL-

Deen, (2016) observed that sharp tooth catfish fed on fenugreek 1% for 30 days revealed significant increase in CAT activity. Ravikumar and Anuradha, (1999) observed CAT activity of gilthead sea bream was significantly higher in group fed on fenugreek.

Post exposure to starvation results of MDA showed significant decrease at fenugreek 1% and 2%, and liquorice 2%. SOD, CAT and GSH recorded significant increase in fenugreek 2% and significant increase of CAT and GSH with liquorice 2%. After hypoxia stress, MDA reported significant decrease at fenugreek 2% and liquorice 2% while, SOD, CAT and GSH showed significant increase at fenugreek2% and liquorice 2%. Similarly, Elabd *et al*., (2016) observed that yellow perch fed with 1% Liquorice at post-starvation exposure showed significant increase in SOD level.

In conclusion, dietary supplementation of fenugreek and liquorice enhance growth performance, immuno-hematological parameters and beneficial for controlling starvation and hypoxia stress in *O.niloticus.*

5. References:

Abd El-Gawad, E A. and Abdel Hamid OM. (2014): Effect of vitamin C dietary supplementation in reducing the alterations induced by fenitrothion in *Oreochromis niloticus*. Fish Physiol Biochem 40:787-796

Abdel-Zaher A., Mostafa M., Ahmad M.H., Mousallamy A. and Samir A. (2009):Effect of using dried fenugreek seeds as natural feed additives on growth performance, feed utilization, whole-body composition and entro pathogenic *Aeromonas hydrophila* challange of monsex Nile tilapia fingerlings. Aust. J. Basic Appl. Sci. 3: 1234-1245.

Antache A., Cristea V., Grecu I., Dediu L., Cretu M., Bocico E. and Petrea S.M. (2014): Effects of Dietary Supplementation at Nile tilapia with *Thymus vulgaris, Trigonella foenum graecum* and *Azadirachta indica* on Welfare Status. Bulletin UASVM Animal Science and Biotechnologies 71(2):115-122.

Asl M.N. and Hosseinzadeh H. (2008) : Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytother. Res. 22, 709–724.

Austin B. and Austin D.A. (1989): Method for microbiological examination of fish and shellfish. Chichester: Ellis Horwood; New York: Halsted Press, pp 317-327.

Awad E., Cerezuela R., Esteban M.A. (2015): Effect of fenugreek (*Trigonella foenum graecum)* on gilthead seabream (*Sparus aurata L*.) immune status and growth performance. Fish and shell fish immunology 45:454-464.

Bahi A. , Guardiola F., Messina C., Mahdhi A. , Cerezuela R . , Santulli A. Bakhrouf A. and Esteban M.A. (2017): Effects of dietary administration of fenugreek seeds, alone or in combination with probiotics, on growth performance parameters, humoral immune response and gene expression of gilthead seabream (*Sparus aurata L.)*, Fish and Shellfish Immunol. 60 -50-58.

Barton B.A. and Iwama G.K. (1991): Physiological changes in fish from stress with emphasis on the response

and effects of corticosteroids, Ann. Rev. Fish. Dis. 1 , 3-26.

Braham D. and Trinder P.(1972): Enzymatic Calorimetric Test Method (GOD_PAP method) without Deproteinization. Analyst , 97:142:145.

Bais, R., and Philcox, M. s., (1994) : Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzyme .Part 8.IFCC method for LDH, Eur J Clinical Chem Biochem. (32) No8.p 639.

Cabello F.C. (2006): Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment, Environ. Microbiol. 8, 1137-1144.

Chakraborty S.B. and Hancz C. (2011): Application of phytochemicals as immunostimulant,anti pathogenic and antistress agents in finfish culture. Rev. Aquac. 3, 103–119.

Dacie J.V., Lewis S.M.(1991): Practical Haematology, 7th Edition, Churchill Livingstone, Edinburgh, pp.54-79.

Doaa A. Y, Abd El Gawad E A. and Abbass A A. 2017 Effect of herbal plants "*Thymus vulgaries* and *Rosmarinus officinale*" on growth performance and protection of *Oreochromis niloticus* against *Aeromonas hydrophila* infection. The 2nd international Sino Egyptian Congress on Agriculture, Veterinary Sciences and Engineering (ISEC-AVE), Benha University, Egypt 7-10 October 2017.

Doumas B, Watson W .and Biggs M . (1971): Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chim Acta 31: 87-96.

El abd H., Wang H. P., Shaheen A., Yao H. and Abbass.A (2016): Feeding *Glycyrrhiza glabra* (liquorice) and *Astragalus membranaceus* (AM) alters innate immune and physiological responses in yellow perch (*Perca flavescens*). Fish & Shellfish Immunology 54 :374-38.

El-Asely A. M., Abbass AA.and Austin B. (2014) : Honey bee pollen improves growth, immunity and protection of Nile tilapia (*Oreochromis niloticus*) against infection with *Aeromonas hydrophila*. Fish & Shellfish Immunology 40 : 500-506.

Emeish W.F .and Saad EL-Deen A. (2016): Imuno modulatory effects of thyme and fenugreek in sharp tooth catfish , *Claris Garipinus,* Assiut Vet. Med. J. Vol. 62 No. 150 July, 1-7.

Fossati P., Prencipe L. , and Berti G.(1980): Use of 3,5- Dichloro-2hydroxy benzene sulfonic Acid/4-Aminophenazone Chromogenic System in Direct Enzymic Assay of Uric Acid in Serum and Urine .Jornal of Clinical Chemistry ,26(2):227-231.

Goda A.M.A.S. (2008): Effect of dietary ginseng herb (Ginsana _ G115) supplementation on growth, feed utilization, and hematological indices of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. Journal of world Aquaculture society, 39(2): 205-214.

Gornall A.J. (1949): Calorimetric determination of protein total in serum and Plasma using the Biuret Reaction .Biochemistry, 177:751.

Guardiola F.A., Porcino C., Cerezuela R., Cuesta A., Faggio C., Esteban M.A. (2016): Impact of date palm fruits extracts and probiotic enriched diet on antioxidant status, innate immune response and immune-related gene

expression of European sea bass (*Dicentrarchus labrax).* Fish and Shellfish Immunol. 52 :298-308.

Guardiola F.A., Bahi A. and Esteban M.A (2018): Effects of dietary administration of fenugreek seeds on metabolic parameters and immune status of gilthead seabream (*Sparus aurata L*.), Fish and Shellfish Immunology 74:372–379

Gültepe N., Bilen S., Yılmaz S. ,Güroy D. , and Aydın S. (2014): Effects of herbs and spice on health status of tilapia (*Oreochromis mossambicus*) challenged with *Streptococcus iniae*. Acta Vet. Brno 83:125–131.

Harikrishnan R., Balasundaram C. and Heo M. S. (2011): Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish, Aquaculture 317: 1-15.

Islam M.N. (2015): Effect of some immunostimulants on growth performance and immune status of Nile tilapia *(Oreochromis niloticus)*. M.V.SC. Thesis (Fish Diseases and Management), Faculty of Veterinary Medicine, Benha University.

Jiang J., Zhu L., Zhang W., Sun R., (2007) :Characterization of galactomannan gum from fenugreek (*Trigonella foenum-graecum*) seeds and its rheological properties Int J Polym Mater 56 , 1145- 1154.

Kaleeswaran B., Ilavenil S. and Ravikumar S. (2012): Changes in biochemical, histological and specific immune parameters in Catla catla (Ham.) by *Cynodon dactylon (*L.). Journal of King Saud University- Science 24, 139–152.

Kanaev, A.E. (1985): Veterinary Hygiene in Fish Farming. Moscow., 140-194.

Klin Z. (1970): Kinetic determination of Glutamate pyruvate transaminase and Glutamate oxaloacetate transaminase –IFCC version without pyridoxal phosphate activation. Biochemistry, 8:658-658.

Kobayashi M., Sang M., Batka S.M., Vannuccini S., Dey M.M. and Anderson J.L. (2015): Fish to2030: the role and opportunity for aquaculture, Aquac. Econ. Manag. 19 (3) 282–300.

Kumar A., Vasmatkar P., Baral P., Agarawal S. and Mishra A. (2017): Immuno modulatory and Growth Promoting Effect of Dietary Fenugreek Seeds in Fingerlings of Common Carp (*Cyprinus carpio Lin*.) .Fishery Technology 54: 170 – 175.

Madeira D., Narciso L., Cabral H.N., Vinagre C. and Diniz M.S. (2013): Influence of temperature in thermal and oxidative stress responses in estuarine fish, Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 166: 237-243.

Mansour E., El-Adawy T. (1994): Nutritional potential and functional properties of heat-treated and germinated fenugreek seeds, LWT-Food Sci Technol 27,568-572.

Morgan A.L., Thompson K.D., Auchinachine N .A. and Migaud H. (2008): the effect of seasonality on normal hematological and innate immune parameters of rainbow trout *Oncorhynchus mykiss*. Fish and shellfish immunology, 25:791-799.

Olaiya C.O., Soetan K.O., (2014). A Review of the health benefits of fenugreek (*Trigonella Foenum- Graecum L*.):

nutritional, biochemical and pharmaceutical perspectives, Am. J. Soc. Issues Humanit ,3-12

Quesada S.P., Paschoal J.A.R., and Reyes F.G.R. (2013): Considerations on the aquaculture development and on the use of veterinary drugs: special issue for fluoroquinolones . J. food Sci. 78, 1321-33.

Ravikumar P., and Anuradha C.V (1999): Effect of fenugreek seeds on blood lipid peroxidation and antioxidants in diabetic rats. PhytotherRes13 (3): 197–201.

Rittler M. and Geisler D. (1983): Radioimmunoassay of urinary cortisol: comparison of the procedures with and without previous cortisol extraction. Meth. Find. Exptl. Clin. Pharmacol. 5(6), 403-406.

Roohi Z., Reza Imanpoor M., Jafari V. and Taghizadeh V.,(2017): The use of fenugreek seed meal in fish diets: growth performance, hematological and biochemical parameters, survival and stress resistance of common carp (*Cyprinus carpio L.)* Aquaculture Research, 48(3), 1209-1215.

Santos F. and Fernando Ramos (2016): Analytical strategies for the detection and quantification of antibiotic residues in aquaculture fishes: a review, Trends in Food Science and Technology, 52: 16-30.

Satoh K., (1978): Serum lipid peroxide in cerebrovascular disorders determined by anew colorimetric method .Journal of Clinical Chemical Acta,90:37-43.

Stoskopf M.K. (1993): Fish medicine. Sounders Company, U.S.A Pp 882.

Temple R.C ., Clark P.M . and Hales C.N. (1992): Measurement of insulin secretion in type 2 diabetes : problems and pitfalls. Diabetic medicine, 9:503-512.

Tewary A. and Patra B.C., (2011): Oral administration of baker's yeast (*Saccharomyces cerevisiae*) acts as a growth promoter and immuno modulator in *Labeo rohita* (*Ham*.). Journal of Aquaculture Research and Development 2 (1), 1–7.

Vasudeva Raoa Y., Das B.K., Jyotyrmayee P., Chakrabarti R. (2006): Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish & Shellfish Immunology 20: 263-273.

Verma S.R., Sarita R and Dalela R.C.(1981): Isolated and combined effect of pesticides serum transaminase in *Mystus Vittatus* (African catfish). Toxicological Letters, 8:67-71.

Wang J., Li S., Wang Q., Xin B. and Wang H. (2007): Trophic effect of bee pollen on small intestine in broiler chickens. Journal of Medicinal Food, 10: 276-80.

Yun B., Ai Q., Mai K., Xu W., Qi G. and Luo Y. (2012): Synergistic effects of dietary cholesterol and taurine on growth performance and cholesterol metabolism in Juvenile turbot (*Seophthalmus Maximus*) fed high plant protein diets. Aquaculture, vol 324-325, pp. 85-91.

Zaki M.A., Labib E.M, Nour A.M, Tonsy H. D. , and Mahmoud S. H.(2012): Effect Some Medicinal Plants Diets on Mono Sex Nile Tilapia (*Oreochromis niloticus*), Growth Performance, Feed Utilization and Physiological Parameters, APCBEE Procedia 4: 220-227.

Zhang Q., Wu J., Chen J.S. and Feng Y. (2014): Influences of organic pollutants in water on antioxidant enzyme in zebra fish. Journal of chemical and pharmaceutical Research, 6 (4), 1014-1021.

Zore GB., Winston UB ., Surwase BS., Meshram NS., Sangle V.D., Kulkarni S S. , Mohan Karuppayil S (2008): Chemo profile and bioactivities of *Taverniera cuneifolia* (Roth) Arn.: a wild relative and possible substitute of *Glycyrrhiza glabra L*. Phytomedicine 15:292–300.

Dietary effect of fenugreek (*Trigonella foenum graecum*) and liquorice (*Glycyrrhiza glabra*) on growth performance, antioxidant activities and stress resistance in Oreochromis niloticus