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Effect of Using Dried Fenugreek Seeds as Natural Feed Additives on Growth Performance, Feed Utilization, Whole-body Composition and Entropathogenic *Aeromonas Hydrophila*-challinge of Monsex Nile Tilapia *O. Niloticus* (L) Fingerlings.

[1,3]Ahmed abdel.Zaher M. Mostafa, [2]Mohammad Hassan Ahmad,
[3]Amani Mousallamy and [2]Asmaa Samir.

[1]King Saud University- college of science and arts- Shagra- Kingdom of Saudi Arabia
[2]Fish nutrition Department- Department of aqua culture research- Abassa- Abu hammad Sharkia- Egypt
[3]Zagazig university -Faculty of science- - Chemistry Department- Egypt

Abstract: The present study was conducted to evaluate the use of Fenugreek Seeds meal (FKSM), as natural feed additives, in diets for fingerlings monsex Nile tilapia, Oreochromis niloticus (9.80±23 g). Four diets are isonitrogenous (30% crude protein),and isocaloric (4.40 kcal / g diet) were categorized into four groups contain 0, 0.5, 1 and 1.5 % of FKSM. The effect of FKSM on growth performance, feed utilization and entropathogenic *Aeromonas hydrophila*-challinge of monxes Nile tolapia O. niloticus (L) fingerlings (L.) were evaluated. Diets were fed to triplicate groups of monosex Nile tilapia at a rate of 4 % of live body weight. The feed was offered twice daily; six days a week for 90 days. Results showed that fish fed diets containing 1 % FKSM had significantly higher ($P<0.05$) growth performance (body weight, weight gain, gain % specific growth rate (SGR). The lowest fish growth was obtained at control diet.Survival rate of Nile tilapia fed all the experiments ranged from 93.33 % to 100 % without significantly difference among them. Feed intake (FI), Feed efficiency ratio (FER), protein efficiency ratio (PER), apparent protein utilization (APU), and energy utilization (EU) increased significantly, while feed conversion ratio (FCR) decreased significantly in diet containing 1 % FKSM. While FCR increased significantly for control diet. There were no significant difference were observed in dry matter, protein, lipid, or ash content of Nile tilapia fed diets containing various levels of FKSM. The highest reduction in feed cost compared with control diet showed to produce one kg fish gain of treatment containing 1 % Fenugreek**.** Results in somatic indices measurements for Nile tilapia of K-factor, LS-index and VS-index similar or little lower than those of fish fed the control diet The results of fish challenge against *A. hydrophila* for 10 days, mortality not observed in fish fed diet containing different level of fenugreek.While mortality percentage in fish fed control diet was 89 %. The samples were analyzed for haematology and serum biochemistry. Hemoglobin (Hb), red blood cells (RBCs) count, haematocrite (PCV) ,serum total protein and globulin significantly ($P < 0.05$) increase with increase fenugreek in the dietary levels but, no significant difference in the aspartate aminotransferase (AST) activity and glucose was observed among the groups after feeding in all trails relative to fed control, whereas, fish fed on 0.5% exhibited significantly increase and the highest values of albumin, alanine aminotransferase (ALT), urea and creatinine. Total lipids and A/G ratio of fish fed on diet exhibited significantly decrease lower values than control.

Key words: Fenugreek, All-male Nile tilapia, growth performance, feed utilization, whole body composition, somatic index, Economic and *A. hydrophyla challenge*

INTRODUCTION

Medicinal and aromatic plants have been used for many years in human nutrition as a spices and medical additives for animal to increase dietary energy utilization, improve the performance efficiency and as a new source of protein (El-Katch, 1990; Abdel El-Aal, and Attia, 1993).

Corresponding Author: Ahmed abdel. Zaher M. Mostafa, King Saud University- college of science and arts- Shagra- Kingdom of Saudi Arabia
Tel: 00966530190723
E-mail amostafa@ksu.edu.sa

Herbal medical is a growing area of alternative medicines nowadays. Many active ingredients in manufactured drugs are derived from plant compounds and have a wide range of use. Plants and plant extracts more safe than chemical products whereas natural products is becoming more popular, since drugs of synthetic origin may have a negative impact on the environment and parasite resistance to poisonous chemicals can develop after repeated applications (Magi, 2003).

Fenugreek (Trigonella foenum –graecum L.) is a leguminous plant grown in northern Africa , the Mediterranean , western Asia , northern India , and currently cultivated in Canada . Fenugreek seeds are found in pods at the extremity of the plant , have been used medicinally and so a food ingredient for years . Over the last few years, researchers are interest in extractable seed components, such as storage carbohydrates & sap onions (Sauvaire *et al.,* 1919). Fenugreek 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 (Food Reference, 2004).

Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans); 20-30% proteins high in lysine and tryptophan; 5-10% fixed oils (lipids); pyridine-type alkaloids, mainly trigonelline (0.2-0.36%), choline (0.5%), gentianine and carpaine; the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin; free amino acids, such as 4- hydroxyisoleucine (0.09%); arginine, histidine and lysine; calcium and iron; saponins (0.6-1.7%); glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin); cholesterol and sitosterol; vitamins A, B1,C and nicotinic acid; coumarin compounds and 0.015% volatile oils (nalkanes and sesquiterpenes) (Blumenthal *et al.,* 1988).

Fenugreek benefits the digestive system as a Laxative, intestinal lubricant, carminative, vomiting, colitis swell, febrifuge, digestive and tonic, helps dissolve fat and cholesterol deposits, prevents fat accumulation and water retention, and helps lower blood sugar levels. It's traditionally used to treat wounds, inflammation, abscesses, arthritis, coughs & bronchitis as well as to reduce mucus production and good for asthma and lung disorder (Castleman, 1919; Ody, 1993; Duke, 2002; Sahalian, 2004) Fenugreek contains an amino acid called 4- hydroxide isolucine which appears to increase the bodies' production of insulin when blood sugar level is high. It may reduce the amount of calcium oxalate in the kidneys and conditions affecting the male reproductive trace. Recent studies suggest that fenugreek ant carcinogenic potential. Studies in rodents stimulating, antioxidant & anti-tumor properties, and protects the liver against alcohol toxicity.

Fenugreek seed powder, an ingredient in spiced foods, contains several potential allergens.

The objectives of the present study were to evaluate the effect of fenugreek (trigonella foenum –graecum seeds addition at different levels (0.5, 1, & 1.5 %) into Nile tilapia, *Oreochromis niloticus (L.)* diets on growth performance, feed utilization, whole- body composition, economic efficiency and *A. hydrophyla challenge.*

MATERIAL AND METHODS

Diet Preparation and Feeding Regime:

Four experimental diets were formulated (30% crude protein and 7 % lipid) contained different levels of fenugreek in Table 1 the diets contained control (0), 0.5, 1.0, 1.5 % of diet. Diets formulation and proximate composition of the experimental diets are shown in Table 1. In the present study, fenugreek (natural product) had been obtained from local market. Fenugreek seeds were grinded in a Maig grinder to pass through a 0.8-mm mesh sieve. The dry ingredients of each diet were thoroughly mixed and then after worlds 100 mL of water per kg diet was supplemented and the ingredients were blended using kitchen blender to make a paste of each diet. Pelleting of each diet was carried out by passing the blended mixture through laboratory pellet machine with (1-mm) diameter matrix. The wet pellets were dried 30 °C at room temperature for two day. The diets were stored in plastic bags in a refrigerator (-20°C) until use. The diets were prepared palletized, stored and previously described by Abd Elghany (2003).The proximate chemical composition of the main ingredients in the diets were analyzed and are shown in Table 1. The caloric value as digestible energy (DG) of each ingredient was estimated on the basis of 5 kcal DE / g protein, 9 kcal DE / g lipid, 3.50 kcal DE / g of carbohydrate. Experimental diets were formulated to meet the nutriments of fish (National, 1994).

Fish Culture Technique:

All male-mono-six Nile tilapia, *O. niloticus* L. fingerling (treated with 17 a- methyltestosterone hormone) were obtained from fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, Abou-Hammad, Sharkia, Egypt. Fish were kept under the same environmental conditions and placed in a fiberglass for 2 weeks as an acclimation period to the Laboratory condition and they fed a commercial diet containing 30% crude protein. Fifteen fish were frozen at – 20 °C for proximate analysis at initial. Acclimated fish were distributed

randomly at a rate of 15 fish/100-L aquarium. The initial weight of fish ranged from 9 to 10 g. Each aquarium was supplied with compressed air via air-stones using aquarium air pumps. Settled fish wastes were cleaned daily by siphoned with a half of aquarium's water, which was replaced by aerated water from the storage tank. Each diet was given to fish at a rate of 3% of live body weight twice daily at 9.00 and 13.00 hours. Each diet was fed to triplicate aquaria of Nile tilapia for a period of 90 days. Fish on each aquarium were weighed biweekly and the amounts of given feed were readjusted according to increase in body weight. The dead fish was daily recorded and removed. At the end of feeding trial each fish weight and length were measured. Three fish from each aquarium were dissected and the liver and viscera of each fish were measured. Different parameter of fish growth, k-factor, somatic indexes and feed utilization were calculated.

Chemical Analysis of Diets and Fish:

The tested diets and fish from each treatment were analyzed according to the standard methods of AOAC (A.O., 1990) for moisture, protein, fat and ash. Moisture content was estimated by heating samples in an oven at 105°C until constant weight and calaculating weight loss. Nitrogen content was measured using a microkjeldahl apparatus and crude protein was estimated by multiplying nitrogen content by 6.25. Total lipids content was determined by ether extraction and ash was determined by combusting samples in a muffle furnace at 550°C for 6 hr. Crude fiber was estimated (Goering and Van, 1970) and gross energy was calculated according to NRC (National, 1994).

Analysis of the Water Quality:

Measurement of water quality parameters revealed that temperature, dissolved oxygen, pH, ammonia, total alkalinity and total hardness in aquaria were monitored every 2 weeks.

Growth Parameters, Somatic Indices and Condition Factor:

Growth performance was determined and feed utilization was calculated as following:

Weight gain = W2 – W1;

Specific growth rate (SGR) = 100 (ln W2 – ln W1) / T; where W1 and W2 are the initial and final weights, respectively, and T is the number of days of the feeding period;

Feed conversion ratio (FCR) = feed intake / weight gain;

Feed efficiency ratio (FER) = weight gain/ feed intake;

Protein efficiency ratio (PER) = weight gain / protein intake;

Apparent protein utilization (APU %) = 100 [protein gain in fish (g) / protein intake in diet (g)].

Energy utilization (EU %) = 100 [Energy gain in fish (g) / energy intake in diet (g)].

Fulton condition factor (FQ) = 100 [fish weight / fish length $(cm)^3$]

Liver somatic index (LSI) = 100 (liver weight / fish weight)

Viscera somatic index (VSI) = 100 (viscera weight without liver / fish weight).

Preparation of Blood Samples:-

Fish were not fed in the 24 hour immediately prior to sampling. Fish were anaesthetized with buffered MS222 (30 Mg/L) and blood was collected with a hypodermic syringe from the caudal vein. The blood collection lasted less than 3 min in order to avoid cortisol rise induced by the manipulation during sampling. The extracted blood was divided in two sets of Eppendorf tubes. One set contained heparin, used as an anticoagulant, for hematology (hemoglobin, haematocrit and red blood cell counting). The second set, without anticoagulant, was left to clot at 4°C and centrifuged at 5000 rpm for 5min at room temperature. The collected serum was stored at -20°C for further assays.

Determination of Serum Parameters:

Total lipids content was determined as described by Frings (Frings *et al.,* 1972). Total serum protein was determined following the protocol of Lowry (Lowry *et al.,* 1951) using standard protein estimation kit., albumin and globulin were determined colorimetrically (Wotton and Free man, 1982). The albumin/globulin ratio was obtained by dividing the calculated albumin value by the calculated globulin value. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using spectrophotometric methods (Reitman and Frankel, 1957) and glucose was determined coloremetrically using glucose kits (Trinder, 1969). Serum samples were subjected also to determine urea (Chaney and Marbach, 1962) and Cratinine (Bowers and Wong, 1980). All kits reagents used in our study were supplied by Egyptian American Company for Laboratory Services, Egypt.

Haematological Studies:

1-Blood cell count:

Total erythrocytic were performed simultaneously using the improved neubauer chamber and Natt Herrick,s solution as diluting fluid (Natt and Herrick, 1952).

2-Determination of hemoglobin(Hb)) gm%):

Hemoglobin levels (Hb, g/dL) were determined calorimetrically by measuring the formation of cyanomethaemoglobin using a commercial kit.

3-Packed Cell Volume (PCV):

The packed cell volume was determined using the microhaematocrit method (Schalm, 1975). The blood was drown into the capillary tube (75 mm X 1.0 mm) by capillary attraction to three-fourth its length, and the index finger was placed over the moist end to hold the column of blood in place as the opposite dry end was forced into the sealing material to form a tight plug. The capillary tubes were centrifugated in International Haematocrit centrifuge for 15 minutes at 2500 r.p.m. with the sealed end pointing outward. The volume of the red cells as a percentage of the total volume of the blood was recorded as PCV.

Economical Evaluation:

The cost of feed to raise unit biomass of fish was estimated by a simple economic analysis. The estimation was based on local retail sale market price of all the dietary ingredients at the time of the study. These prices (in LE/kg) were as follows: herring fish meal,12; soybean meal,2.0; corn meal, 1.5; wheat bran, 1.25; starch, 3.0; fish oil, 7.0; corn oil, 5.0; vitamin premix, 7.0; mineral mixture, 3.0; and fenugreek, 4.0 .

Challenge test:

After 90 days of feeding a different fenugreek diets, the fish of each group were divided into two subgroups; the first subgroup was challenged I/P with pathogenic Aeromonas hydrophila (0.2 ml of 5 x 105 CFU), which was obtained from Fish Disease Department, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. The second subgroup was injected I/P with 0.2 ml of saline solution as a control. Both subgroups kept under observation for 10 days to record the daily mortality rate.

Statistical Analysis:

The obtained data of fish growth, feed utilization, survival rate and proximate chemical composition were subjected to one-way ANOVA. Differences between means were tested at the 5% probability level using Duncan test. All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, USA).

RESULTS AND DISCUSSION

The water samples were collected for analysis before changing water. The most important physico-chemical parameters of tap water used in the experiment are shown in Table 3 as overall ranges during the experimental period. The chemical water analysis showed no apparent fluctuation during the experimental period, however, water quality was found to be within the acceptable range for tilapia growth.

Initial body weight at all experiment groups did not differ significantly as in Table 2. The present study of in Table 2 shows that the growth performance (final body weight, weight gain, weight gain % and specific growth rate) of Nile tilapia fingerlings fed diets containing different levels of fenugreek. The maximum growth performance was obtained at group fed 1 % fenugreek (26.96 g), while the lowest one obtained at control group (21.41 g). Nile tilapia fed diets containing 1 % Fenugreek had significantly higher ($P< 0.05$) body weight, weight gain, weight gain % and specific growth rate. The survival rate was slightly enhanced due to the inclusion of fenugreek in fish diets with non-significant difference ($P> 0.05$; Table 2).

Data of feed intake, FCR, FER, PER, APU % and EU % were shown (Table 4). It was found that feed intake significantly increased ($P < 0.05$) with increasing fenugreek level in fish diet, and the maximum feed consumption was significantly greater with fish fed 1 % and 1.5 % fenugreek diet. Contrarily, FCR value decreased significantly ($P<0.05$) at fish groups fed 1 % and 1.5 % (1.90 and 1.94 recpictively), while the highest FCR values were obtained at control and 0.5 % fenugreek diet (2.46 and 2.39 recpictively).

Diet containing 1 % fenugreek was the best supplemented level for FER%, PER, APU and EU in comparison to the control and other treatment (test fenugreek levels). The lowest PER was obtained at control control group. Similarly, APU and EU increased significantly due to the increase fenugreek in fish diet up to 1 %.

Data in Table 6 show the proximate chemical composition of whole body of fingerlings Nile tilapia fed diets containing different levels of fenugreek. Results in Table 6 indicated significantly (P<0.05) equal or higher values as percentages of dry matter, crude protein and fat compared to values of fish fed the control diet.

At the end of the study, the examined K-factor and somatic indices of monosex-Nile tilapia in Table 7 fed the experimental diets were all normal with no observable irregularity, therefore indicating that the fenugreek no overt physiological abnormalities. K-factor and somatic indices were not significantly (P>0.05) affected by the different levels of fenugreek in the diets. Results in somatic indices measurements for Nile tilapia of K-factor, LS-index and VS-index similar or little lower than those of fish fed the control diet as illustrated in Table 7.

The economical evaluation of the experimental diets contained different fenugreek levels 0.0, 0.5 %, 1 % and 1.5 % are shown in Table 8. The highest reduction in feed cost compared with control diet showed to produce one kg fish gain of treatment containing 1 % Fenugreek.

The results of fish challenge against *A. hydrophila* for 10 days is shown in Table 8 .Fish mortality not observed in fish fed diet containing different level of fenugreek , While mortality percentage in fish fed control diet was 89 %.

Haematological Indices of the Growing Fish:

Data present in Table 10 indicate that, the response of haematological indices of growing and fish healthy to the dietary treatments containing varied levels of fenugreek however, a significant difference (P < 0.05) were found between the treatments means of other haematological parameters. A marked increase in the Haemoglobin (Hb), red blood cells (RBCs) and PCV as a result of increase concentration of fish fed diets from 0.5 to 1% fenugreek.

Serum Biochemical Parameters:

The results obtained from fed Nile tilapia by fenugreek in serum biochemical parameters as shown in Table 10, no significant differences (P >0.05) were recorded for AST and glucose when fish fed during all time period for experiments. The serum proteins of growing fish were significantly influenced (P<0.05) by dietary treatments. Serum total protein of fish fed diets 0.5, 1.5% lower than those fed diet 1%, but still higher than control. Otherwise, serum albumin directly related with dietary fenugreek percentage. The highest content of serum globulin level was obtained at fish group fed 1% content of diet (2.67 g/dl). ALT enzyme activity examined was significantly increase particularly, at fed diet 0.5%, and the lowest activity for AST and ALT those fed diet 1% fenugreek. Total lipids are significantly decrease at fed diet 1% and 1.5% compared with control otherwise, A/G ratio apparently decrease less than control in all fenugreek level in the diet percentage. The biochemical results, detected in Table 10 showed significant increase in urea and creatinine in fed diet (1.5 and 0.5%) respectively, but in case of 1% fish fed diet, no significant differences were observed for them.

Discussion:

The chemical analysis of the locally produced fenugreek is presented in Table 1.Chemical analysis of fenugreek was crude protein, ether extract, crude fiber and ash, similar results were obtained by shalaby (Shalaby, 2004). FKSM was found to be rich in protein ,fat, total carbohydrates and minerals such as calcium, phosphorous, iron, zinc and magnesium (Gupta *et al.,* 1996).

In the present study fish fed diets activity and grow efficiently without external sign of nutritional deficiency. Nile tilapia fed diet containing 1 % FKSM had significantly higher (P<0.05) growth parameters (body weight, weight gain, weight gain % and specific growth rate) than those fed the control diet. These results agreed generally with those reported by Shalaby (24) who found that Nile tilapia fed diets containing 2 % FKSM had significantly higher (P<0.05) body weight, weight gain and SGR.

The improve in live body weight, body weight gain, weight gain % , SGR and survival rate may be due to antibacterial related to flavonoids in FKSM (Bhatti *et al.,* 1996). Also, These results agreed with those reported by Abd - El-Maksoud (Abd El-Maksoud *et al.,* 2002) fed Nile tilapia fingerlings (10.3 g / fish) a basal diet containing 0.0, 0.5, 1.0, 2.0 and 3.0 % marjoram leaves at a feeding rate of 3 % of their body weight for 90 days period. Their data suggested the use of 1 % level.

Survival rate in fish fed all the experimental diets were statistically comparable to that to the of fish fed the control diet and ranged from 93.33 % to 100 %.Shalaby (24) reported that the survival rate of Nile tilapia fed diets containing different levels of fenugreek (0.0, 2.0, 4.0, 6.0 and 8 % was within normal range .It recorded 100% for all fish groups except those fed diets containing 6 and 8 % FKSM levels which gave 95

% survival rate for both. Based on the previous results reported by Muralidhara (Muradlidhara *et al.,* 1999) the suitable doses of fenugreek seeds powder fed to weanling rates at dietary doses of 1, 1, 5, and 10 % in a pure diet had no toxicity.

Feed intake was increased significantly ($P<0.05$) in diet containing 1 and 1.5 % FKSM as in Table 5 .The diet containing 1 % was the best supplemented level for FCR,FER and PER in comparison to the control and other diets FKSM levels. These results are in agreement with those of Abd El-Maaksoud (2002) and El-dakar., (El-Dakar *et al.,* 2004) who showed similar results with different spices e.g. marjoram, basil, peppermint in addition, El-dakar (2004) working on *Oreochromis niloticus* x *Oreochromis auraus* tilapia hybrid (13 g / fish) fed diets containing caraway seeds at level 0, 0.5, 1, and 2 % from the diet. Fish were reared in glass aquaria for 112 days. He found that the 0.5 % level of caraway seed was the best followed by the 2 % level. The high values of APU and EU observed in diets containing 1% FKSM. These results in agreement with obtained of shalaby (Shalaby, 2004; Shalaby *et al.,* 2003) and El-Dakar (2004). FKSM may have a negative impact on the environment and parasite resistance to poisonous chemicals can develop after repeated applications (Magi and Sahk, 2003).

Dry matter, crude protein, fat and ash in fish body did not be affected by different FKSM levels. These results are in agreement with those obtained by (Abd El-Maksoud *et al.,* 2002), Abd elmonem *et al.,* (2002), Shalaby (Shalaby, 2004; Shalaby *et al.,* 2003; Gamlath (2009) suggested that the functional, nutritional and therapeutic characteristics of fenugreek polysaccharide can be exploited further in the development of healthy extruded products. Due to the distinct bitter flavor of fenugreek flour it is difficult incorporate more than 2% level in extruded chickpea based products.

In this study, we assessed some of haematological and biochemical parameters and their effect in the normal health status of Nile tilapia during experiment period.

The blood and biochemical parameters were affected by fenugreek seeds meal as natural feed additives, in diets for fingerlings monsex Nile tilapia .In our study we set out to determine which percentage fed fenugreek is suitable for fish health. Blood and biochemical studies were done to understand the changes in the protein, lipid levels after feeding.

Haematological indices are an index and a reflection of the effects of dietary treatments on the animal in terms of the type, quality and amounts of the feed ingested and were available for the animal to meet its physiological, biochemical and metabolic necessities (Ewuola *et al.,* 2004), in this regard, all haemotological parameters analyzed (Hb, PCV and RBCs) for Nile tilapia were significantly increased as shown in Table 10.This could be attributed to the increase in the blood parameters to shift of water from the plasma to the muscle cells, thereby increasing the hemoconcentration Wilson and Taylor (1993).

The concentration of total protein in blood plasma is used as a basic index for the health status of brood fish (Mulcahy, 1971; Svobodova and Parova, 1977; Hille, 1982; Rehulka, 1996) as measurement of serum or plasma albumin is of considerable diagnostic value in laboratory animals as it relates to general nutritional status, the integrity of the vascular system and liver function. In our study, total protein level increased significantly ($p<0.05$) as a result increases protein in diet (Vasudeva *et al.,* 2004).

Fish fed on 1% and 1.5 % fenugreek in diet exhibited the highest values of albumin and globulin respectively both increased significantly but A/G ration is non-significantly decreased, these might be because fenugreek is a rich source in selenium that aid increase production of albumin and globulin in liver (Ahmed *et al.,* 2006; Mohsen *et al.,* 2007) moreover albumin and globulin concentrations are commonly used for evaluating the effect of nutrients on the fish immunity. Low albumin may result from impaired synthesis, loss though urine or feces or increased catabolism (Nguyen, 1999).

The increase in the serum protein, and globulin levels is thought to be associated with a stronger innate response in fish (Wiegertjes *et al.,* 1996) and the decrease in the A/G ratio is indicative of better immunity of the animal, which may occur due to an increase in the globulin level compared with albumin. The increase in total serum protein and globulin indicates that fish are immunologically strong (Nayak *et al.,* 2004).

ALT and AST belong to the non-plasma specific enzymes which are localized within tissue cells of liver, heart, gills, kidneys, muscle and other organs (Bell, 1968; Gaudet *et al.,* 1975) and in blood plasma they may give specific information about organ dysfunction (Casillas *et al.,* 1983), moreover AST and ALT are transferases concerned with nonessential amino-acid metabolism and gluconeogenesis (Peter and Peter, 2007).

The Significant difference ($p<0.05$) in the activity of ALT (only in one case at 0.5% fenugreek in fed diet),and non-significantly difference in AST activity in experimental fish compared with the control may related to a number of chemical stressors (may be in fenugreek) have been shown to inhibit AST and stimulate ALT activates (Gill *et al.,* 1990; Khalaf, 1999).

The quality and quantity of protein in the diets have a direct effect on the levels of cholesterol. Generally plant protein appears to lower cholesterol level (James, 2004), in the present work total lipid is significantly decreased by increase fenugreek in fish diet, because fenugreek seed would be considered as effective agent for lipid lowering purposes (Abu *et al.,* 2026) and it is in rich protein (26%) has added advantage in that it is a good source of protein as well as fiber (48%) and it might exert a lipid lowering effect (Sharma, 1986) moreover, the ability of fenugreek alkaloids treatment to reduce blood serum lipids including total lipids (Sauvaire *et al.,* 1991; West *et al.,* 1982).

Urea is present in all fish, the liver being the primary organ of production and the gills appearing to be the main organ of excretion (Walsh *et al.,* 2003) and the low-protein diet group, the serum urea levels were significantly lower than in the other groups, indicating depressed ureagenesis (Divino *et al.,* 1999). Our study showed that fed diet containing 0.5 and 1.5 % FKSM had significantly higher urea, and creatinine level than control. Therefore an elevated BUN is probably not indicative of renal disease as it might be in humans but is more likely associated with gill or liver disease or as a result of increase in higher protein intake that caused higher serum urea concentration.

Fenugreek seeds have been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal values in various animal model systems (Raju *et al.,* 2001; Vats *et al.,* 2003).

Our results showed that, no significant differences in plasma glucose due to the variation in fenugreek levels in fish diet, because of presence steroid saponins in fenugreek seeds (Patit *et al.,* 1995; Yoshikawa *et al.,* 1997). Saponin compounds diasgenin, alkaloids (Neveen *et al.,* 2007) and trigonelline inhibit intestinal glucose uptake in vitro (Al-Habori *et al.,* 2001) Fenugreek has a hypoglycemic effect on the body due to its mucilaginous fibers, known as galactomannan. While the fiber may help lower blood sugar levels (Ribe *et al.,* 1986) and the non-significant increase in plasma glucose, may be due to the enhancement of fish health.

The mortality rate of fish challenged A. hydrophila for 10 days was high in fish fed control diet, but not mortality in fish fed other diets. These results indicated that fenugreek is essential for maintaining the normal function, increasing disease resistance to etiological agent. Studies in rodents indicate that fenugreek has immune stimulating, antioxidant and anti-tumor properties, and protects the liver against alcohol toxicity. Also, fenugreek seeds exhibited appreciable antioxidant property in vitro which was comparable with that of reduced glutathione and vitamin E. Further, examination of liver and brain revealed that, extract of fenugreek seeds could offer a significant of fenugreek seeds could offer a significant protection against ethanol toxicity. Fenugreek also has anti-ulcer properties (Sahalian, 2004).

It is concluded that the using FKSM as natural feed additives in fish diet, especially at 1 % can improve the growth performance, feed utilization and immune response.

Table 1: Proximate chemical analysis fenugreek seed meal(FKSM), herring fish meal (HFM), soybean meal (SBM), wheat bran (WB) and corn meal (CNM) (%; on dry matter .

Items	FKSM	HFM	SBM	WB	NM
Chemical analysis					
Dry matter	91.21	92.42	93.80	91.01	90.37
Crude protein	29.11	72.21	44.03	14.98	9.55
Total lipids	6.22	11.42	1.31	4.41	3.98
Ash	4.50	11.14	5.95	3.34	1.50
*NFE		4.68	43.09	66.43	79.83
Crude fiber	9.31	0.55	5.62	10.84	5.14
** Digestible energy (DE)		480.21	372.76	347.10	362.98

*Nitrogen-Free Extract (calculated by difference) = 100 – (protein + lipid + ash + fiber).

**Digestible energy was calculated as 5; 9; and 3.5 kcal/g for protein, lipid, and carbohydrates respectively.

Table 2: Ingredients and chemical analysis of the experimental diets (on dry matter basis) containing different levels of fenugreek.

Ingredients	Fenugreek levels % in the diet			
	Control	0.5%	1%	1.5%
Fish meal	10.4	10.4	10.4	10.4
Soybean meal	42.98	42.98	42.98	42.98
Ground corn	20.32	20.32	20.32	20.32
Wheat bran	15.49	15.49	15.49	15.49
Cod fish oil	2.31	2.31	2.31	2.31
Corn oil	1.50	1.50	1.50	1.50
Vitamins premix	1.0	1.0	1.0	1.0

Table 2: Continue

Minerals Premix	2.0	2.0	2.0	2.0
Starch	4.0	3.5	3.0	2.5
Fenugreek	0.0	0.5	1.0	1.5
Total	100	100	100	100
Chemical analysis %)				
Dry matter	91.23	91.75	91.91	91.49
Crude protein	30.19	30.27	30.39	30.69
Crude fat	7.18	7.19	7.22	7.31
Ash	8.18	8.15	8.21	8.18
Fiber	5.31	5.33	5.54	5.35
NFE	49.40	49.06	48.64	48.47
GE(Kcal/100 g)	441.45	440.62	439.84	441.69
P/E ratio	68.39	68.70	69.09	69.48

1-Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; a-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

2- Mineral premix (g/kg of premix): CaHPO4.2H2O, 727.2; MgCO4.7H2O, 127.5; KCl 50.0; NaCl, 60.0; FeC6H5O7.3H2O, 25.0; ZnCO3, 5.5; MnCl2.4H2O, 2.5; Cu(OAc)2.H2O, 0.785; CoCl3..6H2O, 0.477; CaIO3.6H2O, 0.295; CrCl3.6H2O, 0.128; AlCl3.6H2O, 0.54; Na2SeO3, 0.03.

3 -Nitrogen-Free Extract (calculated by difference) = 100 – (protein + lipid + ash + fiber).

4- Gross energy (GE) was calculated from (NRC, 1993) as 5.65, 9.45, and 4.1 kcal/g for protein, lipid, and carbohydrates, respectively.

Table 3: Physical – Chemical parameters of water measured

Temperature (°C)	DO2 (mg / L)	pH	Hardness (mg / L)	Alkalinity (mg / L)	NO3 (mg/L)	NO2(mg / L)
26 – 29	5.4 – 6.3	7.6 – 8.2	340-350	140 – 150	1.11 – 1.90	0.12 – 0.15

Table 4: Effect of the experimental diets on Growth performance (Initial weight, Final weight, Weight gain, Relative weight gain and Specific growth rate) of *Tilapia Nilotica* during the experimental period.

Items			Fenugreek	
	Control 0.0	0.5%	1%	1.5%
Initial weight (g)	9.95±0.05a	9.90±0.03a	9.87±0.02a	9.80±0.06a
Final weight (g)	21.41±0.35b	22.52±0.19b	26.96±0.58a	25.81±1.23a
Weight gain (g)	11.46±0.34b	12.62±0.21b	17.09±0.55a	16.01±1.26a
Weight gain%	115.18±3.3b	127.47±2.5b	173.15±5.1a	163.36±13.2a
SGR (% / day)	0.851±0.02b	0.913±0.01b	1.116±0.02a	1.075±0.06a
Survival rate %	93.3±3.85a	95.6±2.22a	95.6±2.22a	95.6±2.22a

Means the same letter in the same row is not significantly different at p<0.05.

Table 5: Feed intake, feed conversion ratio (FCR), Feed efficiency ratio (FER), Protein efficiency ratio(PER), Apparent protein utilization (APU) and Energy utilization (EU) of fingerlings Nile tilapia *O. niloticus* fed diets containing different levels of fenugreek.

Items	Control		Fenugreek	
		0.5%	1%	1.5%
Feed intake	28.21±0.11c	30.22±0.21b	35.51±0.82a	31.10±0.56b
FCR	2.46±0.069a	2.394±0.03a	1.90±0.15b	1.941±0.11b
FER	40.638±1.12b	41.760±0.49b	52.568±3.56a	51.495±3.11a
PER	1.476±0.05b	1.493±0.02b	1.88±0.02a	1.88±0.11a
APU (%)	28.84±0.88b	33.99±0.66c	37.71±1.45a	33.41±1.23b
EU (%)	18.80±0.59c	22.98±1.04c	30.62±0.91b	25.59±61b

Means the same letter in the same row is not significantly different at p<0.05.

Table 6: Proximate chemical analysis (% on dry matter basis) of whole body of fingerlings Nile tilapia *O. niloticus* fed diets containing different levels of fenugreek.

Items	Initial	Fenugreek levels in the diet			
		Control	0.5%	1%	1.5%
Dry matter	22.03±0.20c	26.36±0.42b	28.87±0.32a	28.68±0.74a	26.23±0.70b
Crude protein	54.26±0.72c	60.75±0.24a	60.15±0.25ab	59.60±0.12ab	59.29±0.11b
Total Lipids	21.93±1.16a	20.03±0.18b	20.88±0.20ab	21.56±0.19ab	21.86±0.88a
Ash	23.80±0.47a	16.79±0.12b	16.93±0.22b	16.58±0.25b	16.87±0.20b

Means the same letter in the same row is not significantly different at p<0.05.

Table 7: Changes in fulton condition factor (FQ), liver somatic index (LSI), and viscera somatic index (VSI) (mean ± SE) of fingerlings Nile tilapia O. niloticus fed diets containing different levels of fenugreek.

Items	Control		Fenugreek levels 5 in the diets	
		0.5%	1%	1.5%
K-factor	1.626±0.06a	1.533±0.07a	1.542±0.08a	1.410±0.24a
Ls-index	1.179±0.10a	0.977±0.17a	1.158±0.18a	1.144±0.06a
Vs-index.	5.459±0.31a	4.770±0.27ab	4.773±0.50ab	5.036±0.44ab

Means the same letter in the same row is not significantly different at p<0.05.

Table 8: Mortality rate (%) of fingerlings Nile tilapia *O. Niloticus* fed diets containing different levels of fenugreek for 90 days and challenged by *A. hydrophyla* for 10 days.

Items	Control	Fenugreek levels 5 in the diets		
		0.5%	1%	1.5%
No. injected fish	10	10	10	10
Bacteria dose (5 x 10 CFU)	0.2 ml	0.2 ml	0.2 ml	0.2ml
Injection route	I / P	I / P	I / P	I / P
Mortality rate (%) after 10 days of injection	89	0.0	0.0	0.0

Table 9: Economic efficiency for production of one Kg gain of fingerlings Nile tilapia *O. niloticus* fed diets containing different levels of fenugreek.

Items	Fenugreek levels			
	Control	0.5 %	1 %	1.5 %
Price/ kg feed P.T	3.09	3.09	3.10	3.11
FCR (kg feed/kg gain)	2.46	2.39	1.90	1.94
Feed cost / kg gain P.T	7.60	7.39	5.89	6.03
Reduction cost in kg gain	100	6.46	22.50	20.66

Table 10: Some haematological and biochemical parameters in Nile tilapia *O. niloticus* (L) fingerlings fed on different levels of dried fenugreek seeds as natural feed additives.

Parameters	Control 0.0	Fenugreek levels % in the diet		
		0.5%	1%	1.5%
HB	3.05±0.13c	3.36±0.12b	3.78±0.19a	3.35±0.14b
RBCs	1.113±0.032d	1.285±0.052c	1.452±0.042a	1.301±0.035b
PCv	10.08±0.05c	11.12±0.04b	12.63±0.06a	11.32±0.04b
T.Protein (g/dl)	3.04±0.11c	3.35±0.14b	3.92±0.17a	3.41±0.11b
Albumin(g/dl)	1.27±0.06b	1.32±0.05a	1.25±0.8b	1.35±0.06a
Globulin (g/dl)	1.87±0.03c	2.03±0.04b	2.67±0.02a	2.06±0.03b
A/G ratio %	0.679±0.013a	0.650±0.012a	0.468±0.013b	0.655±0.012a
AST (GOT) U/L	30.12±3.52a	31.35±3.25a	28.52±4.25a	35.25±4.52a
ALT (GPT) U/L	26.52±2.52b	35.12±2.15a	26.31±2.31b	28.23±3.21b
T.Lipids(mg/dl)	730.5±25.3a	720.3±20.3a	638.5±22.3b	638.5±22.3b
Urea(mg/dl)	20.05±0.52c	25.41±1.02a	19.82±0.63c	22.03±0.81b
creatinine (mg/dl)	0.682±0.031b	0.728±0.021a	0.678±0.022b	0.725±0.023a
Glucose(mg/dl)	62.81±2.25a	64.81±2.15a	65.35±3.25a	63.62±4.21a

** Data are presented as mean ±SE. Means in the same row with different superscript a, b and c are not significantly different (p>0.05)*

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