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EVALUATION OF NATURAL ZEOLITE (CLINOPTILOLITE) AS A FEED ADDITIVE IN NILE TILAPIA FISH DIETS AND ITS INFLUENCES ON GROWTH PERFORMANCE, GENETIC TRAITS AND FISH HEALTH

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Abstract

Natural zeolites are a group of minerals used as a source of feed and treatment of ammonia in fish culture ponds. The objective of this study was to assess the effect of natural zeolite (clinoptilolite) as a feed additive in fish diets on growth performance, genetic traits and health status of freshwater Nile tilapia fish (*Oreochromis niloticus* L.). Zeolite (Clinoptilolite) was added to Nile tilapia diet at concentrations of 1%, 2% and 3% per 1kg diet which represent three treatments. Eight ponds (½ feddan each) were stocked by *O.niloticus* in two replicates for each treatment (6000 fish/pond). The control is artificial feeding without zeolite, T1, T2, and T3 artificial feeding with 1%, 2%, and 3% zeolite, respectively. The experimental period lasted up to 20 weeks (140 dayes). In this work, the second treatment (2% zeolite) recorded the highest values for whole body weight (BW), body length (BL) and specific growth rate (SGR), while the third treatment recorded the highest values of condition factor (K). The highest values of daily weight gain (DWG) was recorded in both T2 and T3. The highest values for protein and fat contents were recorded in T2, while control recorded the highest values for dry matter and ash contents. Fish body weight and length have been evaluated also as an important economic traits through estimation of correlation factor between them. Therefore, the most important factor, that affects production in ponds culture after feeding and water quality, is the quality of fish genetic sources. Some specimens of the control group showed hyperplasia of the epidermis, torn fins and hemorrhagic dermis. Based on the obtained results, it can be concluded that feeding Nile tilapia on artificial feed containing crude protein (25%) contained 2% zeolite leads to an increase in growth performance of Nile tilapia and survival rates in addition to economic efficiency for total yeild. So, 2% zeolite could be add within the artificial feed for tilapia fish.

Keywords: Natural zeolite, Clinoptilolite, Nile tilapia fish, Oreochromis niloticus, Feed additive, Growth performance, Genetic traits, Fish health

INTRODUCION

Aquaculture is a fast growing industry, which represented one third of the world fisheries production (Lowther, 2005). In Egypt, the production of fish coming from aquaculture sources which represents about 70% (127632 tons) of total fish production (GAFRD, 2012). The total production of all sources in Egypt reached about one million tons (1.077) in 2012 (FAO, 2014). Tilapia species are the important group of cultured fish for several reasons e.g. rapid growth, high tolerance to low water quality, handling, efficiency food conversion, resistance to disease and good consumer acceptance (El-Wakil *et al.,* 2008). Natural zeolites represent a large and very diverse group of minerals such as water-silicates that are characterized by three-dimensional structure and belong to the class of aluminum silicates from a chemical point of view (Strakova *et al.*, 2008 and Waughan, 1988). Additionally, natural zeolites are porous and hydrated aluminum silicates that have ion-exchange and adsorption properties that due to their a large surface area (Xia *et al*., 2009). The dietary system use of naturally or synthetic zeolites has been reported to improve feed efficiency, thus leading to a beneficial growth response in animal farm. The numerous reference data about zeolite importance as a feed additive are referred to its usage in poultry, ruminants nutrition and agriculture (Loughbrough, 1993; Ramos and Hernandez, 1997; Polat *et al.,* 2004; Karamanlis *et al.*, 2008; Macháček *et al.*, 2010; Mallek *et al.*, 2012). In contrast, little reference data about zeolite applications have reported in fish aquaculture and breeding (Obradović *et al.,* 2006; Eya *et al.,* 2008; Paritova *et al.*, 2013). Zeolite has been recommended and used effectively in reducing toxic effects of feed materials infected with aflatoxins (Jand *et al*., 2005; Ortatatli *et al*., 2005). Moreover, zeolite can be used as an antimicrobial agent (Haile and Nakhla, 2010). Forms of zeolite have been used to remove ammonium from aqueous solutions by cation exchange capacity (Wen *et al*., 2010) and help in reducing ammonia production by pellets (Wu-Haan *et al*., 2007) becaue the zeolites have many important characters such as ion exchange, filtering, odour removal, chemical sieve, water softener, stabilization of soil and gas absorption (Kocakusak *et al.*, 2001).

Natural zeolites can play multiple roles in aquaculture; to remove ammonium from hatchery, transport, and aquarium waters; to generate oxygen for aeration systems in aquaria and transporting and to supplement fish rations. Thus, one of the best zeolites in ammonia removal is clinoptilolite (Bower and Turner, 1982). So, this study was conducted to evaluate the effect of zeolite clinoptilolite on some water quality parameters, fish performance for important traits and fish health when add to artificial feed in tilapia ponds.

MATERIALS AND METHODS

Experimental Design.

This study had been done in a private farm at Tollumbat No. 7 (agriculture drainage water) in Riyad City, KafrEl-Sheikh governorate, Egypt. The current experiment was conducted using eight earthen ponds (1/2 feddan each) represent four treatments; control and three zeolite treatments.

The experimental ponds were equal in water volume (2625 m^3) and dimensions (21x100 m) with the same average water depth of 125cm. The ponds were stocked with monosex male Nile tilapia, *Oreochromis niloticus*, fingerlings at an average initial length of 12.20, 11.70, 12.40 and 11.93cm and an average initial weight of 16.10, 15.43, 16.70 and 16.40g for control and three zeolite treatments, respectively. Each pond was stocked with 6000 fish/ pond. The experiment lasted for 20 weeks, started on the 10^{th} of June and harvested on 29^{th} October 2014 (140 days). At the end of the experiment, ponds were gradually drained and the fish (Nile tilapia) were washed, sorted and collectively weighed.

Commercial diet (25% protein) was manufactured by a local factory. The study includes four treatments; the first (control) includes a diet without zeolite; other three zeolite treatments were added at a percentage of 1, 2, and 3% per kg, respectively. Table 1 represents the components proportions of the artificial feed-based experiment.

Table 1. Components proportions of experiment diets for crude protein, lipid, crude fiber and ash in all treatments based on dry matter.

Items	**Experimental Diets**			
	Cont	**Diet1**	**Diet2**	**Diet3**
Crude protein (CP)*	22.68	22.66	22.71	22.80
Lipid (L)	4.44	4.23	4.87	4.20
Crude fiber (CF)	9.33	10.22	10.10	10.24
Ash	10.12	10.14	10.33	10.45
Wheat bran	10.5	10.4	10.3	10.2

* Nitrogen free extract (NFE) =100 - (CP + L + CF + Ash).

Samples of artificial fish food were collected and send for proximate analysis at the Central Laboratory for Aquaculture Research at Abbassa. The fingerlings were fed commercial diet contain 25% crude protein and fed six days per week at a daily feeding rate of 3% of the estimated fish-weight twice at 9.00am and 3.00pm during the experimental period.

Zeolite (Clinoptilolite) Properties.

Commercial natural zeolites are grayish white and porous granules of sedimentary rocks as shown in Figure 1. A zeolite is a crystalline, molecular sieve, hydrated aluminosilicate of alkali and alkaline earth cations having an infinite. It is further able to lose and gain water reversibly with great ability for ions exchange (Kocakusak *et al.*, 2001 and Xia *et al.*, 2009). Moreover, the high content of Si results in the chemical and thermal stability of the mineral structure (Barloková, 2008).

The major physical properties and average percent of quantitative chemical composition for crude material of natural zeolite (clinoptilolite) were analyzed by A & O Company for Trading® at both of Central Metallurgical Research & Development Institute (CMRDI), Central Lab for Elemental & Isotopic Analysis and National Research Center (Analysis & Consulting Unit), Cairo, Egypt as summarized in Table 2.

Figure 1. Commercial natural zeolite, clinoptilolite, granules.

Table 2. The major physical properties and quantitative chemical composition for crude material of natural zeolite (clinoptilolite) by some national Egyptian laboratories

Physical properties	Average	Chemical composition	
		Element	Average%
Bulk density (gcm^{-3})	1.83	Si	55.79
Porosity%	42.2	Al	8.41
Humidity%	6.75	Cu	§Nd
Solubility%	7.38	Mn	0.10
Hardness	4.0 hard	Cd	0.09
Grain size	<6mm	Pb	Nd
pH	7.2	Zn	0.05
CEC* (meq/g)	1.65	Fe	4.77
Water retention	high	K	4.08
Attrition	resistant	Ca	6.81

*CEC; cation-exchange capacity in milliequivalent/g (meq/g). §Not detected

Water Quality Parameters.

Water temperature, dissolved oxygen and pH were measured daily at 6am and 12pm using thermometer, dissolved oxygen meter (YSI model 57) and pH meter (model Corning 345), respectively. Transparency (cm) was measured by using a Secchi Disc of 20cm diameter. Determinations of the other water quality parameters (alkalinity, nitrate, nitrite and ammonia) were carried out every two weeks according to Boyd (1979).

Chemical Analysis of Fish.

At the end of the experiment, randomly three fish of Nile tilapia were chosen from each pond and exposed to the chemical composition of whole fish body according to the methods of Association of Official Analytical Chemists (AOAC, 1990).

Growth Performance.

The average body weight of fish was determined, biweekly where samples were taken by seining. 30 fishes from each pond were collected and then retaind again to the pond after individual measuring the body weight and length. Fish were observed daily for unusual behavior, morphological changes and mortality.

a) Condition factor (K) was determined according to (Busacker *et al.*, 1990) as the following equation;

$$K = \text{mean} \left[\text{body weight (g)} / \text{body length}^3 \text{ (cm)} \right] \times 100$$

Specific growth rate (SGR) was calculated according to Jauncey and Rose (1982) as the following equation;

$$\text{SGR} = \frac{Ln\text{W2} - Ln\text{W1}}{t} \text{ x } 100$$

b) Daily weight gain (DWG) was calculated as the following equation;

$$\text{DWG} = [\text{Average W2(g)} - \text{Average W1(g)}] / \text{time (days)}$$

Where W1 and W2 are the initial and final fish weight, respectively and *t* is the experimental period in days.

c) Correlation coefficient (*r*) was calculated as the following equation **(**see Bailey, 1995);

$$r = \frac{\sum XY - \frac{(\sum X)(\sum Y)}{n}}{\sqrt{\left(\sum X^2 - \frac{(\sum X)^2}{n}\right)\left(\sum Y^2 - \frac{(\sum Y)^2}{n}\right)}}$$

Where: r; Pearson's correlation coefficient for two variables, X; variable x, Y; variable y, n; number of alive fish samples, Σ; sum of values for x or y, √; The square root of variables.

Clinical and Histopathological Examinations.

Fish clinical investigations was done for infested fish according to (Stoskopf, 1993). The abdomen was examined for enlargement and or distention. Also, liver, spleen and muscliture were examined. Histopathological examination for infested or infected specimens were done according to Bullock, (1978).

Statistical Analysis.

One-way ANOVA and Duncan's multiple range tests were used to evaluate the significant difference of the data. The data were statistically analyzed according to Bailey (1995) using General Linear Models (GLM) procedure adapted by SAS (1996) program for user's guide. Significant differences are stated at $P<0.05$.

RESULTS AND DISCUSSIONS

With the usage of zeolite as a feed additive in fish there are numerous positive results, as a growth increase, the conversion nutrition improvement, mortality reduction, toxin elimination, as well as the improvement of common state of health of treated fish (Obradović *et al.,* 2006 and Khodanazary, 2013).

Water Quality Parameters of Fish Ponds.

The obtained results of water quality parameters of the experimental ponds during the experimental period (140 days) as averages are summarized in

Table 3. In general, water temperature averages were 27.63, 27.18, 26.48 and 27.13°C during the whole experiment period at the four treatments Cont, T1, T2, and T3, respectively. Water temperature not significantly varied among zeolite treatments throughout the experimental period while the control was significantly different (P<0.5). This is probably due to the high rate of phytoplankton metabolism and Co_2 in control ponds. It is already known that the optimal temperature for feed intake, growth and spawning of the Nile tilapia is 25 - 28 °C .

The data of dissolved oxygen (DO) in experimental ponds for all treatments where relativly good for Nile tilapia culture and have an averages of 4.77, 4.80, 4.85 and 4.70 mg /l for Cont, T1, T2 and T3, respectively. DO of T2 showed higher value (4.85) than other treatments while control showed relatively low (Table 3). The suitable levels DO concentration for tilapia as warm water species are 5.0 to 15.0 mg/l or greater to maintain good health and feed conversion (Boyd, 1998).

Data of pH in midday for different treatments showed significant difference at P<0.5. The highest value of pH was 8.65 in control ponds while T1, T2 and T3 recorded 8.10, 7.75 and 7.60, respectively. Thus, the growing algae and phytoplankton raise of pH and decrease of DO to become more alkaline as shown in control ponds. However, the desirable pH range for most fish species is 6 to 9 (Boyd, 1998; Barker *et al.*, 2009).

The average values of seechi disk readings were increased with increasing the proportion of zeolite in the fish diet as shown in Table 3. T3 recorded the highest value at 26.54cm, followed by T2, T1 and control at 20.20, 19.10 and 18.90cm, respectively. Due to its absorption/ adsorption properties and its ability to gain and lose water reversibly (Kocakusak *et al.*, 2001; Xia *et al.*, 2009), zeolite can be adsorbed the suspended and organic matters and play a role in leaching the water of ponds.

The average concentration of total ammonia (NH_4^+-N and NH_3^+-N) was 0.35, 0.25, 0.24 and 0.21 mg/l for Cont, T1, T2 and T3, respectively. The concentrations of nitrite (NO_2-N) were 0.033, 0.026, 0.023 and 0.022 mg/l during the whole experimental period wherase the concentrations of nitrate (NO_3-N) were 0.112, 0.098, 0.088 and 0.081 mg/l. In this context, Kamal *et al.* (2008) recorded similar data for nitrite (0.02 - 0.03 mg/L). Zeolite preference for larger cations, including NH_4^+, was exploited for removing NH_4-N from municipal sewage effluent and has been extended to agriculture and aquaculture applications (Mumpton and Fishman, 1977; Bruin *et al.*, 1980). It is clear that addition of zeolite to feed decrease the nitrogen components (Table 3) in water by ion exchange activity and also inside fish body by metabolism activation.

Meanwhile, the values of the total alkalinity [$CaCO_3$+$Ca(HCO_3)_2$] showed significant increase ($P<0.5$) in control with a total mean of (422.97 mg/l), while the values were decreased with increase zeolite levels in diet of T1 (413.55), T2 (402.63) and T3 (392.96) mg/l, respectively. On the other hand, the total hardness (Ca^{+2}+Mg^{+2}) showed also significant increase ($P<0.5$) in control with a total mean of (242.36mg/l), while the values were decreased with increase zeolite levels in diet (209.14, 205.98 and 201.66 mg/l) for T1, T2 and T3, respectively. On his part, Barker *et al.* (2009) indicated that the desirable range of alkalinity is 50-300 mg l^{-1}, but fish survive in waters up to 400 mgl^{-1}. This may be refer to the adsorption activity of zeolite for removing positive ions (Ca^{++}, Mg^{++} and/or Fe^{++}) from water, which was compatible with the interpretation of (Mumpton, 1999). Also, zeolite can be used to fixation of phosphates, cleanup of sewerage, and both heavy metal, and ammonium ion removal (Kocakusak *et al.*, 2001). Obradović *et al.* (2006) stated that zeolite proved itself as reliable corrector of environmental conditions in removal of ammonia NH_3 and decreased other water parameters from water. Accordingly, the above results revealed that all parameters of water quality for zeolite treatments were in the suitable ranges and good for tilapia culture than control.

Table 3. Some water quality parameters of earthen ponds stocked with Nile tilapia treated with zeolite.

Variable	NO	Control	T1	T2	T3
Temperature (°C)	6	27.63±0.14^{a}	27.18±0.05^{b}	26.98±0.06^{b}	27.13±0.06^{b}
DO oxygen	6	4.77±012^{b}	4.80±0.17^{ab}	4.85±0.15^{a}	4.70±0.12^{c}
PH	6	8.65±0.17^{a}	8.10±0.29^{b}	7.75±0.23^{c}	7.60±0.16^{d}
Seechi disk (cm)	6	18.90±0.05^{c}	19.1±0.06^{c}	20.20±0.07^{b}	26.54±0.07^{a}
T. ammonia-N mg/l	6	0.35±0.001^{a}	0.25±0.002^{b}	0.24±0.006^{c}	0.21±0.001^{d}
NO_2-N mg/l	6	0.033±0.001^{a}	0.026±0.002^{b}	0.023±0.007^{b}	0.022±0.001^{b}
NO_3-N mg/l	6	0.112±0.002^{a}	0.098±0.001^{b}	0.088±0.001^{c}	0.081±0.006^{d}
Total alkalinity (mg/l)	6	422.97±1.74^{a}	413.55±2.33^{b}	402.63±1.96^{c}	392.96±1.88^{d}
Total hardness (mg/l)	6	242.36±1.69^{a}	209.14±1.33^{b}	205.98±1.22^{ab}	201.66±1.77^{c}

$^{a-d}$ Means with the same letter in each row are not significantly different at P≥0.05 level.

Fish Chemical Composition.

Chemical body composition of fish could illustrate its health status. Both condition and quality of fish are affected by the environmental conditions (Ibrahim *et al*., 2008) Chemical analysis at the end of a feeding trial is frequently used to determine the influence of feed on fish body composition. According to Hanley (1991), endogenous factors (size, sex and stage of life cycle) and exogenous factors (diet composition, feeding frequency, temperature etc.) affect the body composition of fish.

Table 4. Effect of zeolite treatments on chemical composition % of Nile tilapia (DM basis).

Treatments	NO	Dry Matter%	Crude Protein%	Lipid Extract%	Ash%
Cont	3	32.71±0.57^{a}	57.54±1.10^{c}	22.53±0.78^{a}	13.14±0.46^{a}
T1	3	28.79±0.58^{c}	63.78±1.12^{b}	22.80±0.72^{a}	12.11±0.38^{b}
T2	3	29.01±0.52^{c}	65.85±1.17^{a}	21.55±0.74^{b}	11.41±0.52^{c}
T3	3	30.07±0.54^{b}	65.81±1.15^{a}	21.20±0.70^{b}	10.18±0.48^{d}

$^{a-d}$ Means with the same letter in each column are not significantly different at P≥0.05 level.

The results in Table 4 indicated that control had the highest dry matter (DM) content with total mean (32.71) and ash content with total mean (13.14) compared to other treatments. Crude protein content in a whole fish body showed significantly increased ($P<0.05$) with increasing zeolite levels and the highest protein content was observed at T2 (65.85%). The corresponding values of protein for fish in the control which have fed basal diet without zeolite showed the lowest value of other treatments and was significantly different ($P<0.05$). Similarly, Ayadi *et al.* (2009) studied zeolite addition in Turkey's feed and its impact on the functional properties of turkey meat products and found that the proteins content increased in poultry meat with zeolite treatment.

With regard to the effect of zeolite treatments on lipid content of Nile tilapia fish, Table 4 showed that lipid content at the end of this experiment were 22.53, 22.80, 22.55 and 21.20 % for control and the three zeolite treatments T1, T2 and T3, respectively. The results observed that lipid content of fish at T1 was higher than those obtained at other treatments.

The average of ash values for Nile tilapia at the end of the experiment were 13.14, 12.11, 11.41 and 10.18%, respectively. However, ash percentage for control was the highest one. Accordingly, increase the percentage of ash content of tilapia in Table 4 mainly indicate the increase of elements content (heavy metals) and add zeolite to tilapia food was the cause of the low proportion of elements and metals subsequentially in fish which fed on zeolite treatments. This may be due to that zeolites have a relatively high Si/Al compositional ratio which gives them the special ion-exchange reactions for large cations and ease adsorb metal ions. So, the natural zeolites, clinoptilolite may be useful as metal scavengers in metal-rich sludges to its ability to take up heavy metals (Jain, 1999 and Zorpas *et al.,* 2000). In contrary study, Hsu and Chung (2001) observed an increase in fat, and ash parameters when adding up to 2% or more zeolite (j-carrageenan). Thus, it should be noted that within endogenous factors, the composition of the feed is only the factor which could have influenced the chemical composition of fish body. However, zeolite (clinoptilolites) are shown to be highly effective regarding the metabolic utilization of nitrogen in fish body because of its ability to adsorption of positive ions such as nitrogen in fish artificial food and water.

Fish Growth Performance.

The growth in the fish can be readily monitored by measuring the increase in live fish weight and length. In the present study, the average initial body weight (BW) of *Oreochromis niloticus* were 16.1, 15.43, 16.70 and 16.40g (Table 5). After 140 days of the experimental start (end of the experimental period), the final BW were 204.30, 235.10, 264.60 and 263.50g for Cont, T1, T2 and T3, respectively. And there were differences in final BW among the different treatments ($P<0.05$). The results indicated that the addition of zeolite increase body weight rate of T2 (2% zeolite/kg basal diet) resulted in the highest final BW (264.60g) compared to the control fish (204.30g) and showed significant difference ($P<0.05$).

Table 5. Means of initial and final whole body weight (g) and length (cm) of 60 Nile tilapia for control and zeolite treatments.

Treatments	NO	Initial weight	Final weight	Initial length	Final length
Cont	60	16.10 ± 0.59^{a}	204.30 ± 1.52^{c}	12.20 ± 0.80^{a}	25.30 ± 1.70^{b}
T1	60	15.43 ± 0.62^{a}	235.10 ± 1.63^{b}	11.70 ± 0.89^{a}	26.60 ± 1.91^{a}
T2	60	16.70 ± 0.59^{a}	264.60 ± 1.81^{a}	12.40 ± 0.86^{a}	26.80 ± 1.82^{a}
T3	60	16.40 ± 0.60^{a}	263.50 ± 1.80^{a}	11.93 ± 0.126^{a}	26.70 ± 1.77^{a}

$^{a-c}$ Means with the same letter in each column are not significantly different at $P\geq0.05$ level.

Average initial body length (BL) of *Oreochromis niloticus* were 12.20, 11.70, 12.40 and 11.93cm, (Table 5). At the end of this experiment BL increased and recorded 25.30, 26.60, 26.80 and 26.70cm for the Cont, T1, T2 and T3, respectively. The differences in final BW values among the different treatments were significant ($P<0.05$), while the BL values among the different zeolite treatments were non-significant ($P\geq0.05$). As shown in Table 5, T2 also showed the highest value of BL compared to control and other treatments.

In similar study, Bower and Turner (1982) reported that average growth rates were significantly ($P<0.05$) different between Nile tilapia of different treatments, where Nile tilapia that were fed on the 'zeolite diet' were growing faster ($P<0.05$) compared with that of the control fish. The differences were more evident after 98 days. The improved performance is likely to be associated with the improved utilization of nutrients (Olver, 1989) and/or detoxifying effects of zeolites (Harvey *et al.,* 1993 and Rizzi *et al.,* 2003), which has been documented repeatedly.

The efficacy can be explained by slower passage of pre-digested food through the intestine which leads to the improved utilization of nutrients from the feeding dose, particularly nitrogen (Mumpton and Fishman, 1977; Dias *et al.,* 1998). Obradović *et al.* (2006) consolidated that 1% zeolit as a food additive, had a positive effect on all analysed morphometrical indices of speed of fish growth, increase the final weight and final length measures in rainbow trout for a period of 150 days. The stimulating effect of zeolite is manifested in respect of the daily feed consumption and feed conversion ratio and nutrition.

In aquaculture, there are contrary results on the physiological benefits of adding zeolites to fish feeds. However, Reinitz, (1984) demonstrated that dietary inclusion of sodium bentonite, at 5, 10 and 15% adversely affected weight gain in rainbow trout, while Eya *et al.* (2008) concluded that the inclusion of bentonite at 5% and mordenite at 2.5% were the optimum levels for maximum percent weight gain, specific growth rate and feed efficiency for rainbow trout. In addition, whole body composition of rainbow trout was not adversely affected by the addition of bentonite or mordenite to diets.

Fish Condition Factor (K).

The condition factor of fish based mainly on both of weight and length traits and can be easily calculated from routinely collected length and weight data by Eq. (1). Average initial condition factor (K) of studied fish (*Oreochromis niloticus*) were 0.90, 0.96, 0.88 and 0.97 for Cont, T1, T2 and T3, respectively as shown in Table 6. At the end of the experiment the averages of K were 1.49, 1.53, 1.63 and 1.65 for control and zeolite treatments, respectively. The differences in K between the control and different zeolite treatments were significant ($P<0.05$). The obtained results herein indicate that the addition zeolite increase fish condition factor and its rate of 3%/kg basal diet resulted in the highest K (1.65) compared to the control group (1.49). Similar findings by Emadi *et al*. (2001) who reported that the addition of zeolite in the fish feed leads to the increase condition factor where the highest K values (1.87) were recorded by the fish which was fed on basal diet contained 2% zeolite/kg feed.

Condition factor may be used as index of growth and provides a measure of “fatness” of fish and food conversion efficiency (Power, 1990). Furthermore, the condition factor (K) is estimated for comparative purposes to assess the impact of environmental alterations on fish performance (Clark and Fraser, 1983). The condition factor also reflects the nutritional state or well-being of an individual fish, and is sometimes interpreted as an index of growth rate, (Bagenal and Tesch, 1978). Condition factor can be measured to the “robustness” in fish. However, condition is frequently assumed to reflect not only characteristics of fish such as health, reproductive state and growth but also characteristics of the environment such as habitat quality, water quality (Liao *et al*., 1995).

Fish Daily Weight Gain (DWG).

Table 6 shows means of daily weight gain (DWG) during the experimental period as affected by different levels of zeolite. As described in this Table, the averages of (DWG) of *Oreochromis niloticus* were 1.03, 1.21, 1.36 and 1.36g/fish for Cont, T1, T2 and T3, respectively. The results indicated that the addition of zeolite form 2-3%/kg basal diet increase DWG of fish at T2 and T3, where recorded the highest DWG value (1.36g/fish) compared to the control group (1.03g/fish) and showed highly significance ($P<0.05$). In other study, the application of clinoptilolite (0.5 and 1% of the commercial additive zeolite feed) in the feed mixtures for the fish in the experimental ponds was positively reflected in performance indicators. So, Nile tilapia fish which fed commercial food with 1% zeolite diet were significantly higher (P< 0.01) than those fed the basal diet without zeolite (Landau, 1992). Hence, by addition of zeolite in fish diets, the weight gain and growth rate were increased. Thus, there is a positive impact for zeolite on weight trait and subsequently fish production and this could be due to its ability to increase the stomach's ability to absorb nutrients (Bower and Turner, 1982). The present study agree with the finding mentioned by latter author.

Table 6. The effect of zeolite levels on condition factor (K), daily weight gain (DWG) and specific growth rate (SGR) of Nile tilapia fish.

Treatments	NO	Initial K	Final K	DWG g/fish	SGR/day
Cont	60	0.90 ± 0.15^{b}	1.49 ± 0.11^{b}	1.03 ± 0.032^{c}	1.40 ± 0.054^{c}
T1	60	0.96 ± 0.18^{a}	1.53 ± 0.10^{ab}	1.21 ± 0.034^{b}	1.50 ± 0.059^{b}
T2	60	0.88 ± 0.13^{b}	1.63 ± 0.11^{a}	1.36 ± 0.042^{a}	1.53 ± 0.027^{a}
T3	60	0.97 ± 0.19^{a}	1.65 ± 0.12^{a}	1.36 ± 0.033^{a}	1.52 ± 0.028^{a}

$^{a-c}$ Means with the same letter in each column are not significantly different at $P \geq 0.05$ level.

Fish Specific Growth Rate (SGR).

Specific growth rate (SGR) is an important index for fish growth and can be calculated by Eq. (2). Table (6) shows means of (SGR) during the experimental period as affected by different levels of zeolite. As described in this Table, the averages of (SGR) of *O.niloticus* were 1.40, 1.50, 1.53 and 1.52% /day at Cont, T1, T2 and T3, respectively. The SGR data showed significant differences (P<0.05) between control and zeolite treatments. The highest value of SGR was found at treatment T2 (2% zeolite) and the lowest was found in control fish. Leornard (1979) added the same percent, 2% clinoptilolite, to trout diets and found a significant improvement in weight gain after a 64-day feeding period. In other study, the use of natural zeolite (clinoptilolite) at 5 and 10% levels did not affect the growth of coho salmon (Edsall and Smith, 1989). In opposite, dietary inclusion of bentonite, at 5, 10 and 15% adversely affected weight gain in rainbow trout (Reinitz, 1984).

In freshwater fish, Bower and Turner (1982) found that average growth rates of Nile tilapia were significantly ($P<0.05$) different among Nile tilapia of different zeolite dites. So, Nile tilapia that were fed on the zeolite diet were growing to a faster rate compared with those of the control fish. In recent study, Stetca and Morea (2013) concluded that using natural zeolite as a feed additive for carp fish diet in a ratio of 3-7%, increased the growth between 25-30% and weight gain about 43% compared to the control as well as improved fish meat quality.

The data in the present study complies with conclusion of Xia *et al*. (2009) who reported that a natural zeolite helps to prevent the occurrence of disease and enables to promote growth and survivability and higher profits. Mumpton and Fishman (1977) noted that the growth-enhancing effect of dietary zeolites may be related to the type, properties and their incorporation levels. The improved performance is likely associated with an improved nutrient utilization (Olver, 1989) and detoxifying effects of zeolite (Harvey *et al*. 1993). The better nutrient utilization can be explained by a slower passage of pre-digested food through the intestine, leading to the improved nutrients absorption, particularly nitrogen in diet (Dias *et al*., 1998).

Evaluation of Phenotypic and Genetic Traits.

The most important factor that affects fish production in ponds culture after feeding and environmental quality, is the quality of genetic sources of fish stocked. It does not refer to the genetic resources of the fish but also refers to the general health, relative size and other physical and physiological characteristics of the fish. The length and weight measurements of fish are one of the most studied biological traits of fish biology. With small numbers of fish, it is more precise to measure weight as well as length to estimate growth rates (Bagenal and Tesch, 1978). In fish, changes in weight are relatively greater than changes in length. Increased aquaculture production is clearly needed to meet the increased demand for high-quality protein, especially from aquatic sources. Increased demands for aquaculture production mean increasing pressure for development of more efficient production systems. Major improvements have already been achieved through enhanced management, nutrition, disease diagnostics and therapeutics, water quality maintenance and genetic improvement of production traits. A common theme through all these is genetics, which, actively and passively, has been used to meet many production challenges, such as disease resistance, tolerance of handling, enhanced feed conversion and spawning manipulation (Dunham *et al.,* 2001).

Growth is an important trait in animal and fish breeding. However, the genetic effects underpinning fish growth variability are still poorly understood (Liu *et al.,* 2014). In this regard, sustainable use of genetic resources with recognized traits of fish species or strains, good water quality and effective natural food additives were to become the bases for improvement and increasing the fish production in the field of aquaculture genetics and breeding.

According to data in Tables 5 and 6, significant differences were found in the present study for final weight, length, DWG, SGR, survival and condition factor between control and three zeolite treatments. The weight and length traits together are frequently assumed to reflect not only characteristics of fish such as health, reproductive state and growth but also characteristics of the environment such as habitat quality and water quality (Liao *et al*., 1995). Therefore, the fluctuation in these parameters may reflect the health condition of the fish. So, weight, length, environment tolerance, diseases resistance and survival of fish species were very important phenotypic and genetic traits for fish breeding programs and selection to increasing marketable fish production, especially when significant differences are occurrence or detecting among these traits.

Analysis of variance for final body weight and length traits of Nile tilapia with all treatments were shown in Table (7). There was a high significant difference ($P< 0.05$) between body weight trait and four different treatments while the body length trait only with different treatments showed non-significance. It is known that the performance of length trait in fish differed from weight traits where the length slowly increase than weight when fish grow-up or advanced in age even stop growing (very extreme slowly), where growth is heading towards the weight.

Table 7. Analysis of variance for final weight and length traits of Nile tilapia with all treatments.

	Final Weight		
Source of variance	**d.f**	**Mean Squares**	**F**
Among treatments	3	48836.95	2270.323***
Residual error	236	21.511	
	Final Length		
Source of variance	d.f	Mean Squares	F
Among treatments	3	29.80	1.4232 ns
Residual error	236	20.939	

*** Highly significant at the P< 0.05 level, ns = non-significant

Phenotypic correlation coefficient (*r*) between weight and length traits within and among treatments were estimated as shown in Table 8 by Eq. (4). There was also high significant differences (P<0.05) between body weight/length traits together and all different treatments using Duncan's test. According to correlation data in Table 8, perfect positive correlation (*r*=1.0) of weight/length was noticed in three zeolite treatments; T1, T2 and T3, indicating improving in growth and health of Nile tilapia which were fed on zeolite diets than the control (*r*=0.95). The performance of length trait only with all different treatments showed the lowest correlation (0.11) with lower significance while the performance of weight trait showed the higher correlation (0.92) with significance at (P< 0.05).

Table 8. Pearson correlation coefficient(*r*) between final weight and length traits of Nile tilapia for each treatment.

Traits	**Treatments**	**NO**	**Correlation Coefficient (*r*)**	**P**
weight & length	Cont	60	0.95	***
weight & length	T1	60	1.00	***
weight & length	T2	60	1.00	***
weight & length	T3	60	1.00	***
final weight	all treatments	240	0.92	**
final length	all treatments	240	0.11	*

*** Correlation is highly significant at the P<0.05 level.

Charo-Karisa *et al.* (2006) quantified the environmental and genetic effects on early growth of Nile tilapia, *Oreochromis niloticus*, in hapa-in-two earthen ponds in low-input environments. They found significant positive spatial autocorrelation indicating resemblance in growth of fry in neighboring hapas. The low genetic correlation between body weights of fry in both ponds might therefore suggest genotype by environment interactions for tolerance to low dissolved oxygen in Nile tilapia juveniles. Also, the latter authors showed that the correlations between hapa effects and body weight as well as with water quality parameters were low and non-significant. On the other hand, they found also highly significant correlations between mean body weight per hapa and DO per hapa.

It is seems that when fish reared in different environments, genotypes may exhibit differences in growth performance. In recent study, Liu *et al.* (2014) studied the genetic variation and associations between genotypes and phenotypes of two tilapia species (Nile tilapia and Mozambique tilapia) and their red tilapia hybrids by conducting a genome-wide scan for quantitative trait loci for growth traits. They found that the correlation coefficients were 0.98 between body weight and total length at 140 days post hatch in red tilapia hybrid family named OmR1. The correlations between paired traits were all significant ($P<0.01$).

Fish Survival Rate and Total Yield.

Averages of survival rate% at the end of the experiment were listed in Table 9. As described in this Table, *Oreochromis niloticus* gained the highest survival rate 96.3% at T2 compared with Cont, T1 and T3 which were 89.8%, 92.6% and 95.8%, respectively. These results indicate that the addition of zeolite to the artificial feed increased the rates of survival for Nile tilapia fish. The low survivability in the control group may be due to the state of ponds water, relatively low in DO and low in viability of fish than those were fed on zeolite diet. Moreover, zeolite addition to artificial diets may be reduced the bioavailability of mycotoxins. In this regards, Sarr *et al.* (1995) reported that the hydrated sodium calcium alumino-si1icate (HSCAS) from zeolite has been shown to prevent aflatoxicosis in farm animals by reducing the bioavailability of aflatoxin and determined the effects of HSCAS on the metabolism of aflatoxin B1 in an aflatoxin-sensitive species. So, natural zeolites have proven to be effective in protecting animals against mycotoxins (Dvorak, 1989 and Schell *et al.,* 1993). The apparent ability of clinoptilolite and other zeolites to absorb aflatoxins that contaminate animal feeds has resulted in measurable improvements in the health of swine, sheep, and chickens (Kovac *et al.,* 1995).

Table 9. Survival rate (SR) percentages and total yield of different treatments.

Treatments	SR %	Yield (kg)
Cont	89.8%	2201.50
T1	92.6%	2612.40
T2	96.3%	3057.70
T3	95.8%	3029.20

Averages of total yield at the end of the experiment were listed in Table 9. As described in this Table, *Oreochromis niloticus* gained the highest yield 3057.70 tons/feddan for T2 compared with 2201.50, 2612.40 and 3029.20kg for Cont, T1 and T3, respectively. Similarly, Bower and Turner (1982) reported that the average total yield of Nile tilapia was significantly different among Nile tilapia of different zeolite livels; this means that Nile tilapia that fed on the artificial feed with zeolite showed higher total yield compared with those of basal feed only. The stimulating effect of zeolite is manifested in respect of the daily feed consumption and feed conversion ratio and nutrition. This positive effect of zeolite on growth performance and production could be due to its ability as a corrector of environmental conditions and to reduce toxic effects of materials such as aflatoxins as has been reported in several studies (Emadi *et al.,* 2001; Erdem *et al.,* 2004; Jand *et al*., 2005 and Obradovic *et al.,* 2006). To improve the overall nutrient use efficiency of fish in fertilized ponds and reduce cost of production, efficient breeding programs are crucial.

Fish Clinical and Histopathological Examinations.

Some fish of the control group showed skin darkening, discoloration, frayed fins, detached scales and haemorrhagic areas. Enlarged and distended abdomen was noticed. On the other hand, liver showed pale and enlarged in some fishes and congested with necrotic patches in others. Also, spleen showed congested and enlarged in most examined fishes (Figure, 2). In infested fish, gall- bladder was enlarged and engorged with bile. Such signs may be due to the stress of aquaculture in non-treated agriculture drainage waste water. These findings and interpretation came similar to that mentioned by Gatesoupe (2008) and Haile and Nakhla (2010) who indicated that there is an evidence that zeolite can be used as an antimicrobial agent and improves fish viability and health. In addition, zeolite can be reduced the toxic effects of many fish farms pollutants (Jand *et al*., 2005).

Moreover, Nik-Khan and Sadeghi (2002) applied natural clinoptilolite-tuff in feeding of neonatal calves and found that 1g clinoptilolite per kg of body weight per day had the best effects on increasing serum immunoglobulins, vit A adsorption and reduction of mortality.

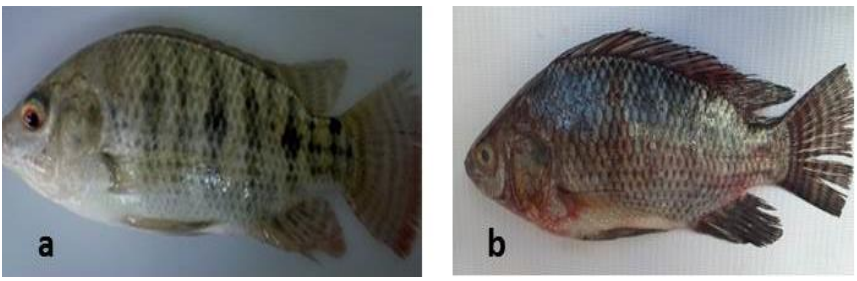

Figure 2. Treated groups show healthy Nile tilapia; **"a"**., control group shows skin darkening, frayed fins, detached scales and difused haemorrhage of unhealthy Nile tilapia; **"b"**.

Histopathological examinations for some specimens of untreated fish (control) showed hyperplasia of the epidermis, congested and hemorrhagic dermis with lymphocytes aggregations and hyaline degeneration of melanomacrophage cells from skin (Fig. 3). Some liver samples from the control group suffered atrophy together with necrosis, congestion and marked fatty changes (Fig. 4). While spleen samples showed marked lymphoid depletion and inhibition of MMC (Melanomacrophage center, excessive pigmentation and cellular density (Fig. 5). These pathological changes were more or less similar to the alterations described by De Smet and Blust (2001) and Mela *et al*. (2007). The alterations were overcomed in the treated groups as zeolite have the ability to adsorbe the toxic effects of many fish and broilers farms pollutants (Jand *et al*., 2005).

Al-Nasser Afaf *et al.* (2011) evaluate the zeolite as a feed additive to reduce *Salmonella* and found that adding zeolite in the broiler diet significantly reduced *Salmonella* levels in the chicken body and carcass, as compared to the control with a positive effect on the production parameters.

The natural zeolite structure is polar and effectively attracts the complex and polar mycotoxins molecules such as aflatoxins. The internal CEC of zeolite binds positively charged ions such as the ammonium cation when excess ammonia builds up in the digestive tract thereby reducing the toxic effects of excess ammonia **(Mumpton, 1999 and Tomasevic-Canovic *et al.*, 2003)**.

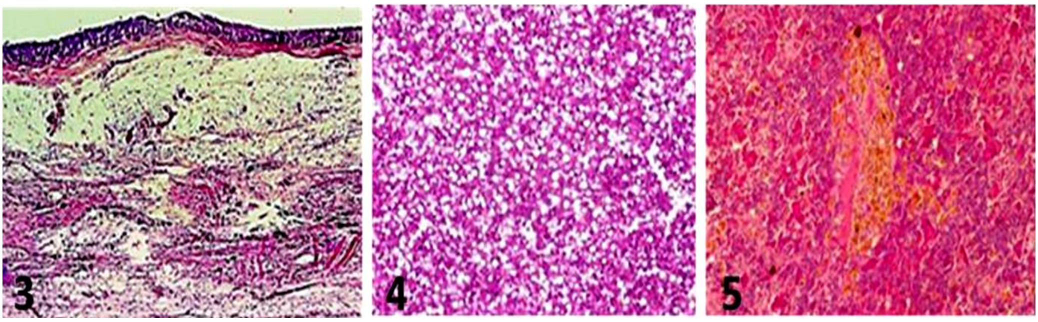

Figure 3. Skin suffering hyperplasia, congestion and hemorrhagic dermis with lymphocytes aggregations and hyaline degeneration of melanomacrophage cells.

Figure 4. The liver of infested fish showed atrophy and marked fatty change.

Figure 5. The spleen of infested fish with marked lymphoid depletion, inhibition of MMC (Melanomacrophage Center), pigmentation and cellular density.

Economic Evaluation of Fish Production.

The results of costs including variable, fixed and interest on working capital for the treatments applied are shown in Table 8. The results of this Table revealed that costs of fingerlings of Nile tilapia are similar in all treatments applied, however the feed costs differed according to body weight, and zeiolite addition.

As shown in Table 10, the differences for total costs were attributed to the differences in feed costs, and zeolite addition. These results indicated that, when *Oreochromis niloticus* fed on artificial feed containing 25% crude protein with 2% zeolite resulted in best economic efficiency compared to the other treatments. In this regards, studies in Japan using zeolite as a dietary supplement for animals showed that the test animals generally grew faster than control groups with a simultaneous decrease in the amount and cost of the feed (Mumpton and Fishman, 1977).

Table 10. Evaluation and estimation of the economic efficiency under experimental conditions.

Item	Cont	T1	T 2	T3
A-Variable costs (EP/Feddan)				
1-Fish production				
a. *O. niloticus* fingerlings	2220	2220	2220	2220
b. Feeds	17310.66	20205.25	22801.8	22728.26
c. Zeolite	0	51.81	116.93	174.83
Total variable costs (EP/Feddan)	17310.66	20257.06	22918.73	22903.09
B- Fixed costs (EP/Feddan)				
a. Depreciation (materials & others) 10%	400	400	400	400
b. Taxes	500	500	500	500
Total fixed costs (EP/Feddan)	900	900	900	900
Total operating costs (variable & fixed)	18210.66	21157.06	23818.73	23803.09
Interest on working capital *	1047.74	1217.26	1370.39	1369.49
Total costs	19258.40	22374.32	25189.12	25172.58
% of the smallest value	100	116%	131%	131%
Returns				
Total return (EP) **	23776.60	28214.26	34246.44	339270
Net return (EP/Feddan)	4518.20	5839.94	9057.32	8754.42
% of the smallest value of net return	100%	129.25%	155.09%	96.66%
% Net returns to total costs	23.46%	26.10%	35.96%	34.78%

* 15% × total operating costs × 140/365 days.

** The economical evaluation of results was carried out according to market prices in 2014 in EP (Egyptian Pound).

O. niloticus = EP 185 /1000 fry. Zeolite= 3900/1000Kg Artificial feed (25% protein) = EP 3650 /1000 Kg.

However, the present study is consent the findings of many other studies that zeolite (Clinoptilolites) are shown to be highly effective regarding the metabolic utilization of nitrogen in poultry and animals in addition to its ability to improvement of fish meat quality and growth (Mumpton and Fishman, 1977; Shurson *et al.,* 1984; Polat *et al.,* 2004; Strakova *et al.,* 2008; Stetca and Morea, 2013). Therefore, aquaculture requires high-quality feeds, which should contain not only necessary nutrients but also complementary feed additives to keep organism's healthy, better growth and keeping environment-friendly aquaculture (Robertson *et al.*, 2000). In addition, good water quality of fish culture ponds and aquatic environment play a critical role for successful fish production (Saeed, 2013).

CONCLUSION

Based on the obtained results in this study and the economic evaluation, it can be concluded that feeding Nile tilapia fish (*Oreochromis niloticus*) on artificial feed containing crude protein, 25% with 2% zeolite leads to better growth performance and health status of fish, in addition to that was the best in terms of economic efficiency as compared with other treatments. However, good health of fish stock, genetic traits of species, water quality, feed additives like natural zeolite, aquaculture management are very important for successful and improvement fish production. In Egypt, till now, there were no enough experimental data about zeolite applications and need more studies as a feed additive for fish breeding and aquaculture practices.

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تقييم الزيوليت الطبيعى كإضافة غذائية لعلائق اسماك البلطي النيلي وتأثيراته على آداء النمو والصفات الوراثية والحالة الصحية

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الملخــص العربـــى

تهدف هذه الدراسة الى تقييم تأثيرالزيوليت الطبيعي كإضافة لعلائق الأسماك على أداء النمو والانتاج الكلى لأسماك البلطى النيلى. تمت إضافة الزيوليت (كلينوبتيلولايت) لعلائق أسماك البلطي النيلى بتركيزات ١، ٢ و ٣٪ لكل ١كجم عليقة لتشمل ثلاث معاملات بالاضافة إلى عليقة الكونترول الخالية من الزيوليت كمقارنة لتقييم فعاليتها لتحسين جودة الغذاء وتعظيم الاستفادة منه وتقليل مخاطر زيادة الأمونيا والمعادن الثقيلة في الغذاء والمياه وانعكاس ذلك على حيوية أسماك البلطى وإزالة السمية من جسم الأسماك وأثر ذلك على أداء النمووصفتى الوزن والطول. وقد تم استخدام ثمانية أحواض نصف فدان (مكررتين لكل معاملة)، بكل منها ٦٠٠٠ سمكة /حوض. واستمرت التجربة ٢٠ أسبوع، وتتلخص أهم النتائج التي تم الحصول عليها فيما يلي:

سجلت المعاملة الثانية (٢% زيوليت) أعلى معدل لوزن الجسم ، طول الجسم ومعدل النموالنوعى ، وسجلت المعاملة الثالثة (٣% زيوليت) أعلى معدل لعامل الحالة K–factor في حين سجلت كلا من المعاملات الثانية والثالثة على أعلى معدل للوزن اليومي المكتسب. كما سجلت المعاملة الثانية أيضا أعلى معدل للبروتين والدهون على التوالى، بينما سجلت أسماك المقارنة أعلى معدل للمادة الجافة والرماد على التوالى. بالنسبة للأعراض المرضية وصحة الأسماك ، أظهرت بعض عينات أسماك المقارنة التى لم تتغذى على الزيوليت تضخم ونزيف دموى فى السطح الخارجى لطبقة الجلد، وتمزق فى الزعانف الذيلية والصدرية.

تم أيضا تقييم صفتى الوزن والطول كصفات وراثية هامة لدراسة سلوك النمو من خلال معامل الارتباط بين الصفات والمعاملات المختلفة حيث أظهرت النتائج وجود ارتباط موجب قوى (+١) ومعنوية عالية للصفات المظهرية مع معاملات الزيوليت عن المقارنه التى انخفضت بدرجة طفيفة. كما أوضح

تحليل التباين لصفتى الوزن والطول معنوية عالية للوزن مع المعاملات المختلفة بينما لم تظهر صفة الطول أية فروق معنوية مع المعاملات المختلفة وربما يرجع ذلك الى اختلاف طبيعة صفة الطول عن صفة الوزن فى الأسماك. ومن ذلك يتضح أن الشكل المظهرى والتركيب الوراثى يستجيب لظروف البيئة المائية ولمعاملات التغذية المضاف إليها الزيوليت، وظهور تباين فى سلوك النمو لأسماك البلطى النيلى موضع الدراسة.

بناء على النتائج التي تم الحصول عليها في هذه الدراسة والتقييم الاقتصادي، فإنه لوحظ أن تغذية أسماك البلطي النيلي على أعلاف صناعية تحتوي على ٢٥٪ بروتين خام مع ٢٪ من الزيوليت الطبيعى يؤدي إلى تحسن فى الصفات الوراثية الاقتصادية وخاصة زيادة الوزن ومعدلات البقاء ، بالإضافة إلى ذلك أعطت هذه الاضافة الغذائية أفضل معدل من حيث الكفاءة الاقتصادية مقارنة مع المعاملات الأخرى.

وعلى ما تقدم فإن التأثيرات الايجابية لمادة الزيوليت الطبيعى على تحسين جودة الاعلاف وبعض الصفات الوراثية وأداء النمو وصحة الاسماك لكونها تعمل كمضادات للأكسدة، بالإضافة الى خواص المسامية وإدمصاص السموم والعناصرالثقيلة وخاصية التبادل الأيونى ، تحتاج إلى مزيد من الدراسة والبحث من خلال مزيد من التجارب البحثية من أجل زيادة وتحسين كفاءة الانتاج السمكى فى مصر.