



The effect of dietary sericite on growth performance, digestive enzymes activity, gut microbiota and haematological parameters of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings

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ABSTRACT

A feeding trial was conducted to assess the effect of dietary supplementation of sericite (silicate clay) on growth performance, digestive enzymes activity, immune parameters and gut microbiota, of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. Five isonitrogenous diets were formulated each diet supplemented with different levels of sericite 0 (control), 2.5, 5, 7.5 and 10 g/kg diet. After 70 days of feeding trial, supplemental sericite were quadratically improved the final body weight (FBW) ($P = 0.023$), weight gain (WG) ($P = 0.012$), specific growth rate (SGR) ($P = 0.023$) and feed conversion ratio (FCR) ($P = 0.023$) and protein efficiency ratio (PER) (quadratic, $P = 0.045$). However, the relationship between FCR and sericite levels was expressed by a broken-line model with an identified optimal breakpoint of 6.3 g/kg of sericite inclusion in the diets. Additionally, significant quadratic increase in alkaline phosphatase (ALP), amylase, trypsin, chymotrypsin and lipase enzymes were detected ($P = 0.026$; $P = 0.023$; $P = 0.013$; $P = 0.045$; $P = 0.023$) as the level of sericite increased in the diet. Furthermore, dietary sericite levels exhibited linear decreased in the total count bacteria, *E. coli* and Enterobacteriaceae of stomach and gut of experimental fish (linear, $P = 0.032$; $P = 0.024$; $P = 0.035$; $P = 0.023$; $P = 0.012$; $P = 0.039$, respectively). There was no effect of dietary sericite levels on hemoglobin (Hb), hematocrit (Htc) and red blood cells (RBCs), but a quadratic trend was observed in white blood cells (WBCs), monocytes and lymphocytes of fish (quadratic, $P = 0.036$; $P = 0.013$; $P = 0.034$), respectively. Increasing dietary sericite levels did not affect alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin and globulin but quadratically increased total protein (quadratic, $P = 0.012$) and IgM exhibited linear trend (linear, $P = 0.045$). Results in this study

Abbreviations: IBW, initial body weight (g); WG, weight gain (g); SGR, specific growth rate (%); t, period in days; FI, feed intake (g days⁻¹); PER, protein efficiency ratio; FCR, feed conversion ratio; ALP, alkaline phosphatase; Hb, hemoglobin; Htc, hematocrit; RBCs, red blood cells; WBCs, white blood cells; IgM, immunoglobulin M; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBC, total bacterial count; *E. coli*, *Escherichia coli*; CUF, colony forming unit; TAME, Na-p-toluenesulphonyl-L-arginine methyl ester; BTEE, N-benzoyl-L-tyrosine ethyl ester; EDTA, ethylenediaminetetraacetate; ELISA, Enzyme-Linked Immunoabsorbent Assay; ANOVA, analysis of variance; SEM, pooled standard error of the mean

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indicate that the addition of sericite as feed additive enhanced the growth, feed utilization, digestive enzyme activities, blood profile and gut biota of Nile tilapia.

1. Introduction

Sustainable aquaculture depends on a perfect balance between health and growth condition in fish. Using artificial feed that do not meet all nutrient requirements not only affect growth and feed efficiency but equally increase the susceptibility to induce the appearance of deficiency signs, including pathological changes (Ayoola, 2016). Likewise, the improvement of the health status is vital in fish industry (Harikrishnan and Balasundaram, 2008; Harikrishnan et al., 2010; Shahkar et al., 2015; Jawahar et al., 2016; Hassaan et al., 2018a). In this context, fish nutritionists have focused on the development of feeding strategies to maximize growth, nutrient digestibility and immunity (Roohi et al., 2017; Na-Phatthalung et al., 2018). One of these strategies encompasses the use of inert clays as an environment-friendly feed additive to enhance fish production (Murray, 2000; Kanyilmaz et al., 2015; Ghasemi et al., 2018). Many studies have investigated the benefits of various additives in aquafeeds to improve health of fish: organic acid (Ng and Koh, 2016; Khajepour and Hosseini, 2012; Hassaan et al., 2014, 2018a), nucleotides (Li and Gatlin, 2006; Ringø et al., 2011, 2012; Hossain et al., 2016; Hassaan et al., 2018b), synbiotics (Cerezuela et al., 2011; Hassaan et al., 2014; Ringø and Song, 2016), and clay minerals (Hu et al., 2008; Yildirm et al., 2009). However, more studies are required to develop aquafeed additives and their effects on growth and health status of fish. Silicate (clay minerals) such as sericite, zeolite, kaolinites, zeolites, clinoptilolite, bentonites, vermiculites, biotites and aluminosilicates, attapulgite, kaolinite, smectite, and sepiolite are non-toxic, cheap, ecologically advantageous and low-cost materials (Murray, 2000; Chen et al., 2005; Slamova et al., 2011). These minerals are crystalline, hydrated aluminosilicate molecules composed of alkali and alkaline earth cations along with small amounts of various other elements (De Souza et al., 2013). As an unconventional feed additive, previous studies indicated that the addition of clay minerals as clinoptilolite, bentonite and modernite can in fact improve the growth performance, feed utilization and blood profile of different fish species (e.g. European seabass (*Dicentrarchus labrax*) (Dias et al., 1998), rainbow trout (*Oncorhynchus mykiss*) (Obradović et al., 2006; Eya et al., 2008; Kanyilmaz, 2008), Nile tilapia (*Oreochromis niloticus*) (Hu et al., 2008), Tilapia (*Tilapia zillii*) (Yildirm et al., 2009), African catfish (*Clarias gariepinus*) (Ayoola, 2016)). On the other hand, Kanyilmaz and Tekelioğlu (2009) and Yiğit and Demir (2011) reported that the zeolite supplementation has no effect on growth performance of common Carp (*Cyprinus carpio* L., 1758) or rainbow trout (*O. mykiss*), respectively.

Sericite is a mica-based clay mineral, with a compact layered structure, generally recognized as a white fine powder of muscovite form (Lee and Tiwari, 2014). As fine-grained mica, sericite has multiple characteristics of mica and clay minerals due to the same chemical composition, structure and construction (Allaby and Allaby, 1999). To the best of our knowledge there are no available reports on the effects of natural "sericite" minerals on the growth performance, digestive enzymes, hematology, blood biochemical and gut microbiota in fish. Therefore, the present study aimed to evaluate the effects of sericite as unconventionally feed additives on growth, digestive enzymes, hematology, blood biochemical, stomach and gut bacteria count of Nile tilapia, *O. niloticus* fingerlings.

2. Materials and methods

2.1. Experimental fish and feeding trial

Nile tilapia, *O. niloticus* fingerlings were obtained from El-Kanater El-Khayria, Fish Research Station, National Institute of Oceanography and Fisheries (NIOF), Al Qalyubia Governorate, Egypt. Fingerlings were acclimated to the environmental conditions and fed with a commercial feed for 2 weeks prior to the start of the trial. The growth trial was conducted according to a complete randomized design, and three replicate tanks were assigned for each dietary treatment. 10 fish (initial average weight 1.53 ± 0.1 g) were randomly allocated into each of 15 glass aquariums (60 l for each). The aquaria were supplied with ground water and individually aerated to meet the oxygen requirements. Fish were fed four times a day to apparent satiation (at 9:00 am, 11:00 am, 13:00 pm and 15 pm) for a 70-day period. On a daily basis, one-third of the water volume in each aquarium was renewed with fresh water at flow-rate of 4 L/min up to the filling level after cleaning and removal of the accumulated excreta. Tanks were illuminated from above with fluorescent ceiling lights with photoperiod of 12- h light, 12- h dark (light period from 08:00 – 20:00 h). Water temperature was recorded daily with a mercury thermometer suspended at 15-cm depth. Dissolved oxygen (mg L^{-1}) was measured using oxygen meter (YSI model 56, YSI Company©, Yellow Springs Instrument, Yellow Springs, Ohio, USA) and pH was determined using a pH meter (Orion pH meter, Abilene, Texas, USA). Total ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, Co. USA). During the feeding trial, all water quality parameters (temperature, dissolved oxygen, pH value, ammonia-N and nitrite) were kept within the acceptable limits and presented in Table 2 for rearing Nile tilapia (Boyed, 1990) (average \pm SD); All fish sampled for blood sampling, organ collection and whole-body fish proximate analysis were humanely euthanized with excessive anesthesia with 3-aminobenzoic acid ethyl ester (MS 222, 100 mg L-1, Sigma, St. Louis, MO) according to the institution guidelines for animal welfare.

2.2. Experimental diet preparation

Five isonitrogenous (315.65 g/kg crude protein) and isocaloric (18.7 MJ/kg gross energy) diets were formulated to fill the nutritional requirements of Nile tilapia (Table 1). The first (control diet) was provided without sericite supplementation. The remaining four were respectively supplemented with 2.5, 5, 7.5 and 10 g/kg sericite. Sericite was provided by the Geophysics lab of the

Table 1
Formulation and proximate composition of the experimental diets (g/Kg diet, dry matter).

Ingredient	Control	2.5 g/kg Sericite	5.0 g/kg Sericite	7.5 g/kg Sericite	10.0 g/kg Sericite
Fish meal	100	100	100	100	100
Soybean meal	350	350	350	350	350
Corn gluten meal	30	30	30	30	30
Yellow corn	250	250	250	250	250
Wheat bran	100	97.5	95	92.5	90
Rice polishing	100	100	100	100	100
Fish oil	40	40	40	40	40
Premix	25	25	25	25	25
Vitamin C	5	5	5	5	5
Sericite	0	2.5	5	7.5	10
Chemical analysis %					
Protein	316.35	316.00	315.65	315.30	314.95
Lipid	70.3	70.425	70.55	70.675	69.9
Ash	56.69	56.643	56.595	56.548	56.05
Fiber	54	53.96	53.92	53.88	53
Nitrogen free extract ²	532.7	533.0	533.3	533.6	536.1
Gross energy ³ MJ Kg	18.64	18.65	18.65	18.65	18.65

¹Vitamin and mineral mixture kg⁻¹ of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B₁₂, 4.0 g Vit B₂, 6 g Vit B₆, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; D-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39 % Na, 61 % Cl), 3077 mg; ferrous sulfate (FeSO₄·7H₂O, 20 % Fe), 65 mg; manganese sulfate (MnSO₄, 36 % Mn), 89 mg; zinc sulfate (ZnSO₄·7H₂O, 40 % Zn), 150 mg; copper sulfate (CuSO₄·5H₂O, 25 % Cu), 28 mg; potassium iodide (KI, 24 % K, 76 % I), ²NFE (Nitrogen free extract) = 100-(crude protein + lipid + ash + fibre content). ³Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kJg⁻¹ for protein, fat and carbohydrate, respectively according to Brett (1973).

Inland and Freshwater Division of the National Institute of Oceanography and Fisheries (NIOF), Egypt. Sericite composition was 43.1–49.0 % SiO₂, 27.9–37.4 % Al₂O₃, 9–11 %, K₂O + Na₂O, and 4.1–6.1 % H₂O.

Diet ingredients were ground into fine powder through a 200 µm mesh and then thoroughly mixed with fish oil. The mixture was then passed through a laboratory pellet mill (2-mm die; California Pellet Mill, San Francisco, CA, USA). During the process, pellet temperature did not exceed 40 °C. After pelleting, diets were dried in open air, packed in cellophane bags and stored at –20 °C until used. The approximate chemical composition of formulated diets was determined according to standard methodology (AOAC, 1995) (Table 1).

2.3. Digestive enzymes activity

Fish were sampled from each replicate. After dissection, samples of middle intestine were immediately homogenized in 10 volumes (w v-1198) of ice-cold physiological saline solution and centrifuged at 5000 g for 15 min at 4 °C. The supernatant was then stored for endogenous enzymes activity analysis following the protocol designed by Furné et al. (2008). Chymotrypsin activity was estimated using the method of Hummel (1959) with N-benzoyl-L-tyrosine ethyl ester (BTEE) as substrate at 254 nm. In brief, 0.2 ml diluted sample solution was added to 6 ml of 0.0005 M BTEE in Tris buffer [10.55 g CaCl₂·2H₂O dissolved in 250 ml 0.2 M Tris (hydroxymethyl) aminomethane, adjusted to pH 7.8, diluted to 1 l, and 432 ml methanol added]. Trypsin activity was also measured using the method of Hummel (1959) using Na-p-toluenesulphonyl-L-arginine methyl ester (TAME) as substrate at 247 nm. 0.2 ml diluted sample solution added to 6 ml of 0.00104 M TAME in Tris buffer [1.47 g CaCl₂·2H₂O dissolved in 200 ml 0.2 M Tris (hydroxymethyl) aminomethane diluted to 1 l, pH 8.1]. Lipase activity was determined as described by Zamani et al. (2009) titration method. Amylase activity was estimated according to Bernfeld (1951) at 540 nm, using starch as substrate. 1 ml of diluted sample was incubated for 3 min with 1 ml of 1 % starch [1 g soluble starch and 0.035 g NaCl in 100 ml 0.02 M Na₃PO₄, pH 6.9]. After 3 min, the reaction was stopped by the addition of 2 ml 3, 5-dinitrosalicylic acid reagent. The solution was then heated for 5 min in boiling water, cooled, and then added to 20 ml distilled water. Intestinal alkaline phosphatase (ALP) activity was determined by the method of Wahlefeld et al. (1974) with 4-nitrophenyl phosphate as substrate at 405 nm.

Table 2
Mean value (± SE) of water quality parameters.

Temperature (°C)	26.30 ± 0.7
DO (mg/l)	5.95 ± 0.12
pH	8.13 ± 0.35
NH ₃ - N (mg/l)	0.16 ± 0.01
NO ₃ - N (mg/l)	0.03 ± 0.01

2.4. Intestinal total bacterial count

At the end of experiment, stomach and gut of fish from each replicate were aseptically excised. Each of them was individually washed three times with sterile distilled water to remove non-adherent surface bacteria. The stomach and gut were rinsed separately in 96 % ethanol and homogenized using a mortar. The homogenate samples were suspended in 10 ml of saline solution and diluted to 10^{-5} in 9 ml saline solution (Al-Harbi and Uddin, 2004). Stomach and gut sample bacterial counts were determined using the spread plate method. A 0.5 ml volume from each dilution was spread onto triplicate nutrient agar medium and standard nutrient agar medium. The plates were incubated (25 ± 1 °C) for 48 h. After incubation, the colonies in each plate were counted (only plates containing count between 15 and 300 colony were accepted). The count was then computed by multiplying the average number of colonies per plate by the reciprocal of the dilution used and then reported as colony forming unit (CFU) ml^{-1} . Total count bacteria and *E. coli* using standard techniques according to Refstie et al. (2006)

2.5. Hematological and biochemical blood indices

At the end of the experiment, blood samples were collected using clean syringes from the caudal vein of fish and divided into two portions. The first portion was collected with anticoagulant 10 % ethylenediaminetetraacetate (EDTA) to determine the hematocrit (Htc), hemoglobin (Hb), red blood cells (RBCs) and total count of white blood cells (WBCs) according to standard methods described by Rawling et al. (2009). For differential counting of leukocytes, the smears were stained by Giemsa / May- Grunwald (Rosenfeld, 1947) for the establishment of each cell count. The second portion of the blood sample was allowed to clot overnight at 4 °C and then centrifuged at 3000 rpm for 10 min. The non-hemolysed serum was collected and stored at -20 °C until use. Levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) were analyzed according to the method described by Reitman and Frankel (1957). Total serum protein and albumin were determined according to Henry (1964) and Wotton and Freeman (1974), respectively. However, the total serum globulin was calculated by subtracting the total serum albumin from the total serum protein according to Coles (1974). Serum total IgM levels were measured by an Enzyme-Linked Immunoabsorbent Assay (ELISA) assay using a kit (Cusabio, Wuhan, Hubei, China).

2.6. Proximate composition

Fish were sampled from each replicate and then oven-dried at 70 °C until constant weight and calculating weight loss. Samples were then stored at -20 °C until further analysis. All chemical analysis was conducted according to AOAC (1995). Proximate composition was analyzed on experimental diets and fish samples at the end of the experiment. Crude protein was estimated by micro-Kjeldahl method, $N \times 6.25$ (using Kjeltac auto analyzer, Model 1030, Tecator, Höganäs, Sweden). Lipid content was determined by Soxhlet extraction with diethyl ether ($40-60$ °C). Ash was determined using Barnstead/Thermo Scientific Benchtop 47900, Thermo Scientific, Massachusetts, United States by incineration at 550 °C for 12 h. Fiber content of the experimental diets was determined using the method described by Van Soest et al. (1991). Nitrogen-free extract was computed by taking the sum of values for crude protein, crude lipid, crude fiber and ash and by subtracting this sum from 100.

2.7. Growth and feed efficiency calculation

Weight gain (WG) was calculated as follow: $WG = \text{final weight (g)} - \text{initial weight (g)}$.

Specific growth rate (SGR) = $\ln W_2 - \ln W_1 / t$, where, \ln = the natural log; W_1 = initial fish weight, W_2 = the final fish weight in grams and t = period in days.

Feed conversion ratio (FCR) was calculated according to by the equation: $FCR = \text{Feed intake (g)} / \text{weight gain (g)}$.

Protein efficiency ratio (PER) = $\text{Weight gain (g)} / \text{protein ingested (g)}$.

2.8. Statistical analysis

Polynomial contrasts were used to detect linear and quadratic effects of various dietary sericite levels on the observed response variables. The level of significance adopted was 5 %. A statistical package SAS (Statistical Analysis System, 1993) was used for all statistical analysis response variables. All percentage data were arc-sin transformed prior to analysis (Zar, 1984); however, the data are presented untransformed to facilitate comparisons.

3. Results

3.1. Growth performance and feed utilization

Growth performance and feed utilization of tilapia is shown in Table 3 as affected by different level of sericite. Final body weight (FBW), weight gain (WG) and specific growth rate (SGR) were quadratically improved with the increasing of dietary sericite (quadratic, $P = 0.023 = 0.012$; $P = 0.023$). No significant ($P < 0.05$) differences were found for feed intake (FI) in response of dietary sericite. Dietary sericite had a significant improving effect on feed conversion ratio (FCR) (quadratic, $P = 0.023$) and protein efficacy ratio (PER) (quadratic, $P = 0.045$). At the highest desirability (0.95), maximum WG, SGR, PER and FCR values were 15.27,

Table 3
Growth performance and feed utilization of Nile tilapia fed the experimental diets for 70 days.

	Experimental treatments					P values	
	Control	2.5 g Sericite/kg diet	5.0 g Sericite/kg diet	7.5 g Sericite kg/diet	10.0 g Sericite/kg diet	Linear	Quadratic
Initial body weight (g fish ⁻¹)	1.52	1.52	1.53	1.54	1.52	0.523	0.639
Final body weight (g fish ⁻¹)	11.95	14.16	16.80	16.40	15.40	0.366	0.023 [†]
Weight gain (g fish ⁻¹)	11.86	12.64	15.27	14.86	13.88	0.456	0.012 [†]
Specific growth rate (% day ⁻¹)	2.94	3.18	3.42	3.38	3.31	0.062	0.023 [‡]
Feed intake (g fish ⁻¹)	21.37	22.03	23.26	23.33	22.62	0.562	0.456
Feed conversion ratio	1.80	1.74	1.53	1.57	1.63	0.456	0.023 [§]
Protein efficiency ratio	1.94	2.01	2.30	2.22	2.14	0.023	0.045 [#]

[†] y Final body weight = $-0.6757x^2 + 4.9683x + 7.47$; $R^2 = 0.9606$.

[‡] y Weight gain = $-0.4686x^2 + 3.4374x + 8.544$; $R^2 = 0.8372$.

[§] y specific growth rate (SGR) = $-0.07x^2 + 0.506x + 2.49$; $R^2 = 0.973$.

[§] y feed conversion ratio (FCR) = $0.035x^2 - 0.261x + 2.052$; $R^2 = 0.8345$.

[#] y protein efficiency ratio (PER) = $-0.0479x^2 + 0.3481x + 1.604$; $R^2 = 0.7937$.

3.42, 1.53 and 2.30, respectively and the estimated optimum sericite level was 5 g/kg diet. However, the relationship between FCR and sericite levels was expressed by a broken-line model with an identified optimal breakpoint of 6.3 g/kg of sericite inclusion in the diets (Fig. 1).

3.2. Digestive enzymes activities

Endogenous alkaline phosphatase, amylase, trypsin, chymotrypsin and lipase fed the experimental diets are presented in Fig. 2(a–e). Significant quadratic in alkaline phosphatase, amylase, trypsin, chymotrypsin and lipase ($P = 0.026$; $P = 0.023$; $P = 0.013$; $P = 0.045$; $P = 0.023$) were detected as the level of sericite increased in the diet. At the highest desirability (0.95), maximum alkaline phosphatase, amylase, trypsin, chymotrypsin and lipase values were 42.76, 684, 2.50 and 7.82, respectively and the estimated optimum sericite level was 5 g/kg diet.

3.3. Gut bacterial count

Total count bacteria, *E. coli* and Enterobacteriaceae of stomach and gut of experimental fish tended to decrease (linear, $P = 0.032$; $P = 0.024$; $P = 0.035$; $P = 0.023$; $P = 0.012$; $P = 0.039$), respectively in response to increasing dietary sericite levels (Table 4). At the highest desirability (0.95), lowest *E. coli* and Enterobacteriaceae values of stomach and gut were estimated in diet supplemented with 10 g/kg diet.

3.4. Hematological indices

Hematology indices of Nile tilapia fed the experimental diets are presented in Table 5. Increasing dietary sericite contents did not

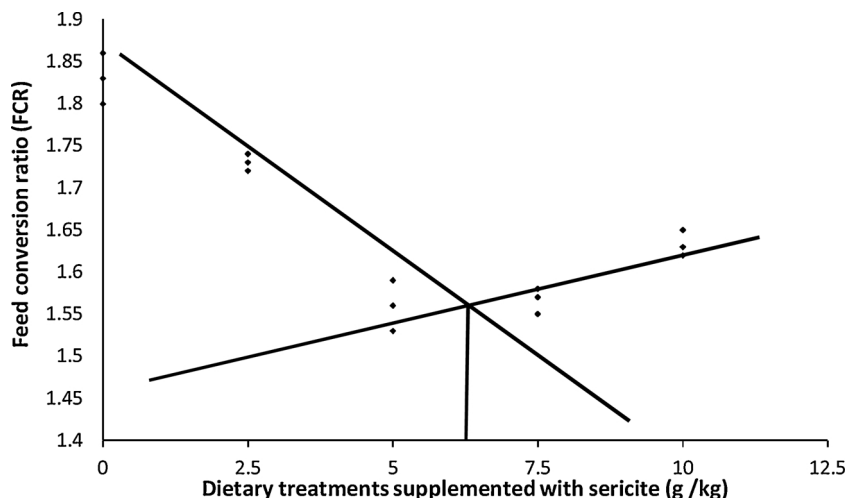


Fig. 1. Broken-line regression analysis of feed conversion ratio of Nile tilapia, *O. niloticus*, fed control and four diets supplemented with sericite g kg⁻¹ diet; $y = 1.85 - 0.089x + 0.0067x^2$; $R^2 = 0.921$; The broken-line is 6.3 g of sericite kg⁻¹ diet.

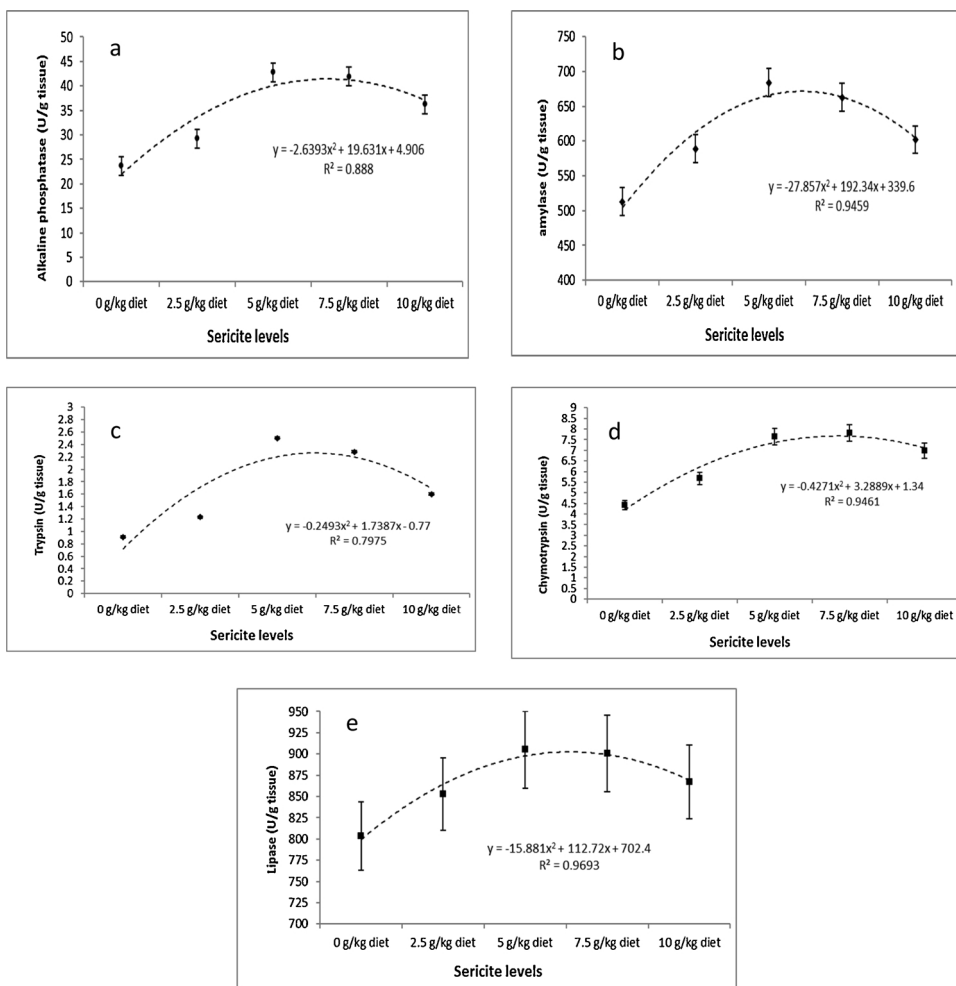


Fig. 2. Quadratic regressions of digestive enzymes; a) alkaline phosphatase (U/g tissue), b) amylase, c) trypsin, d) chymotrypsin, e) lipase activities of Nile tilapia fed 0, 2.5, 5, 7.5 and 10 g sericite kg⁻¹diet.

Table 4

Gut total bacterial count of Nile tilapia fed experimental diets for 70 days.

	Experimental treatments					P values	
	Control	2.5 g Sericite/kg diet	5.0 g Sericite/kg diet	7.5 g Sericite/kg diet	10.0 g Sericite/kg diet	Linear	Quadratic
Stomach							
Total count bacteria (log CUF/g)	7.58	7.51	7.45	7.32	7.29	0.032 [#]	0.568
<i>E. coli</i> ($\times 10^5$) CUF/g	7.51	7.44	7.40	7.29	7.26	0.024 [§]	0.233
Enterobacteriaceae (log CUF/g)	7.67	7.56	7.55	7.13	7.18	0.035 [*]	0.596
Gut							
Total count bacteria (log CUF/g)	7.62	7.61	7.59	7.50	7.47	0.023 [†]	0.523
<i>E. coli</i> (log CUF/g)	7.52	7.43	7.42	7.29	7.28	0.012 [‡]	0.231
Enterobacteriaceae (log CUF/g)	7.56	7.42	7.40	7.13	7.18	0.039 [¶]	0.335

[#] y Total count bacteria = -0.484x + 4.242; R² = 0.973.

[§] y *E. coli* = -0.356x + 3.528; R² = 0.9735.

^{*} y Enterobacteriaceae = -0.853x + 5.505; R² = 0.8795.

[†] y Total count bacteria = -0.336x + 4.676; R² = 0.905.

[‡] y *E. coli* = -0.353x + 3.557; R² = 0.9226.

[¶] y Enterobacteriaceae = -0.553x + 4.005; R² = 0.8817.

Table 5
Hematological parameters and differential white blood cells of Nile tilapia fed the experimental diets for 70 days.

	Experimental treatments					P values	
	Control	2.5 g Sericite/kg diet	5.0 g Sericite/kg diet	7.5 g Sericite/kg diet	10.0 g Sericite/kg diet	Linear	Quadratic
Hemoglobin (g/dl)	10.89	11.30	11.19	11.91	11.04	0.512	0.896
Hematocrit (%)	14.61	14.88	15.25	15.05	15.21	0.389	0.785
Red blood cell count $\times 10^6$ /cmm ¹	2.67	2.51	2.53	2.50	2.78	0.542	0.563
White blood cell count $\times 10^3$ /cmm ²	19.14	25.76	27.83	28.13	26.88	0.199	0.036 [†]
Monocyte (%)	4.33	6.33	5.67	6.33	5.67	0.563	0.013 [*]
Lymphocyte (%)	51.00	55.00	56.00	55.67	56.00	0.065	0.034 [†]

²WBCs, white blood cell count.

¹ RBCs, red blood cell count.

[†] y White blood cell count = $-1.2507x^2 + 9.2893x + 11.438$; $R^2 = 0.9819$.

^{*} y Monocyte = $-0.2857x^2 + 1.9823x + 2.862$; $R^2 = 0.6979$.

[†] y Lymphocyte = $-0.6193x^2 + 4.7827x + 47.198$; $R^2 = 0.9259$.

Table 6
Serum biochemical parameters of Nile tilapia fed the experimental diets for 70 days.

	Experimental treatments					P values	
	Control	2.5 g Sericite/kg diet	5.0 g Sericite/kg diet	7.5 g Sericite/kg diet	10.0 g Sericite/kg diet	Linear	Quadratic
ALT ¹ U/L	42.23	42.05	42.08	42.93	42.76	0.523	0.639
AST ² U/L	12.09	11.43	11.00	10.48	11.90	0.366	0.123
Total Protein U/L	2.91	3.53	3.60	3.48	3.50	0.456	0.012 [†]
Albumin U/L	1.42	1.68	1.68	1.62	1.64	0.332	0.323
Globulin U/L	1.50	1.85	1.92	1.86	1.86	0.562	0.456
IgM μ g/ml	141.67	153.00	157.00	157.67	159.00	0.045 [†]	0.523

¹ ALT = alanine aminotransferase.

² AST = Aspartate aminotransferase.

[†] y total protein = $-0.0993x^2 + 0.7087x + 2.37$; $R^2 = 0.848$.

[†] y IgM = $3.936x + 141.87$; $R^2 = 0.7739$.

affect Hb, Htc and RBCs but quadratically increased WBCs, monocytes and lymphocytes of fish (quadratic, $P = 0.036$, $P = 0.013$; $P = 0.034$), respectively were detected as the level of sericite increased in the diet.

3.5. Serum biochemical parameters

Estimated alanine aminotransferase, aspartate aminotransferase, total protein, albumin, globulin and IgM values are presented in Table 6. The ALT varied between 42.05 and 42.93 U/L and AST varied between 10.48 and 12.09 U/L among the treatments without significant effect of sericite. Increasing dietary sericite contents did not affect albumin and globulin, but significant quadratic increases total protein ($P = 0.012$) and linear IgM ($P = 0.045$) were detected as the level of sericite increased in the diet.

3.6. Proximate composition of whole-body fish

Chemical composition of Nile tilapia fed the experimental diets is showed in Table 7. Increasing dietary sericite contents did not affect dry matter and protein but linearly (0.035) reduced lipid content and linearly ($P = 0.012$) increased ash of fish.

4. Discussion

The present study has shown, for the first time, the effect of dietary natural minerals (sericite) supplementation in diets for Nile tilapia. Regardless, of the inclusion levels, the addition of sericite to the diets produced the best results in all the parameters analyzed. The significant increase ($P < 0.01$) in the growth performance and feed utilization of Nile tilapia fed diets with supplemented sericite compared to the control diet may be due to the presence of natural sericite elements such as aluminum, sodium and potassium which have the ability to change the pH buffering capacity of gastrointestinal glands and influence the transport through intestinal epithelium of fish (Yildirim et al., 2009). Still, the addition of natural minerals such as bentonite and zeolite may decrease the digesta viscosity and subsequently, to encourage the proper breakdown of feed materials by the digestive enzymes, which in turn might improve the fermentation process in the gut and enhances the nutrients absorption in fish (Eya et al., 2008; Steica and Morea, 2013).

Table 7

Proximate composition of whole body (g/kg) of Nile tilapia fed experimental diets for 70 days.

	Experimental treatments					P values	
	Control	2.5 g Sericite/kg diet	5.0 g Sericite/kg diet	7.5 g Sericite/kg diet	10.0 g Sericite/kg diet	Linear	Quadratic
Dry matter	216.80	217.50	228.90	256.50	243.30	0.523	0.639
Protein	556.60	560.70	574.40	572.60	571.60	0.366	0.323
Lipid	204.50	176.50	158.00	156.6	156.30	0.035 [†]	0.012
Ash	158.60	162.80	178.50	179.90	187.30	0.032 [‡]	0.323

[†] y lipid = $-11.63x + 205.27$; $R^2 = 0.776$.

[‡] y Ash = $7.45x + 151.07$; $R^2 = 0.9362$.

Oluwaseyi (2016) reported that bentonite clay enhanced nutrient utilization of catfish by reducing the flow rate of feed in the digestive tract, allowing for better digestion and absorption. Concordantly, the addition of montmorillonite improved growth performance and feed utilization for Nile tilapia (Hu et al., 2008). Likewise, dietary zeolite supplementation has also shown to enhance growth performance and feed utilization of rainbow trout (*O. mykiss*) (Lanari et al., 1996; Obradović et al., 2006; Danabas, 2009), gilthead sea bream (*S. aurata*) juveniles (Kanyılmaz et al., 2015), striped snakehead (*Channa striatus*) (Jawahar et al., 2016) and tilapia (*T. zillii*) (Eya et al., 2008; Yildirim et al., 2009). However, Kanyılmaz and Tekelioğlu (2009) and Yiğit and Demir (2011) found that zeolite supplementation have no effect on growth performance of common carp (*C. carpio*) and rainbow trout (*O. mykiss*). The reasons for the differences among these studies can however result from the differences in fish species, physiological conditions, source, type, properties and dietary levels of natural elements (Mumpton and Fishman, 1977; Papaioannou et al., 2005).

Endogenous enzymes play an important role in digesting nutrients in fish, directly enhancing the digestive ability (Adeoye et al., 2016; Wen et al., 2009). The activities of intestinal enzymes vary in their distribution and intensity according to feeding habits and intestinal morphology. In the present study, the activity of alkaline phosphatase, amylase, trypsin, chymotrypsin and lipase was higher with the addition of sericite. To the present, there is no information on the effect of sericite on the digestive enzyme activity, but Hu et al. (2008) reported that the addition of Cu^{2+} -exchanged montmorillonite in diets for Nile tilapia increased the activity of intestinal amylase, lipase, and alkaline phosphatase when compared to the control diet. The improvement in the intestinal enzymes may be explained by the attachment of natural minerals to the mucous, protecting and strengthening the intestinal mucosal barrier, thus helping in the regeneration of the epithelium (Albengres et al., 1985; Girardeau, 1987; Hu et al., 2008). Additionally, natural clays can elevate the pH of gastrointestinal fluids, thereby changing the enzymatic activities of gastrointestinal secretion in mice (Martin-Kleiner et al., 2001). In the present study, results help to conclude that the sericite addition improved the digestive enzyme activity, thus, enhancing the feed utilization of Nile tilapia (Table 3).

Clay minerals have geochemical properties, which make them to adhere to bacteria and/or have an antibacterial role (Williams et al., 2011). Activity as a physical bactericide (adsorption) can occur by surface interaction between clay minerals and bacteria, which on its turn can hamper passive, active uptake of essential nutrients and disrupt cell envelopes (Morrison et al., 2014). Until now, just a few clays minerals have been identified as antibacterial, completely killing a broad spectrum of pathogen bacteria (Williams et al., 2011). Pathogenic bacteria can destroy normal morphology of intestinal mucosa, causing a reduction in the secretion of intestinal enzymes (Han et al., 2012). In this study, total bacteria, *Escherichia coli* and *Enterobacteriaceae* counts in stomach and gut were gradually reduced ($P < 0.05$) with increasing addition of sericite to Nile tilapia diets. This reduction may be associated with a bactericidal process to buffer the aqueous pH and oxidation state to conditions that promote element solubility (Williams et al., 2004, 2011). In an *in vitro* study, Hu et al. (2002) noted that Cu^{2+} -montmorillonite adsorbed *E. coli*. Also, Cu^{2+} -montmorillonite has a strong antibacterial ability over aquaculture pathogenic bacteria, *A. hydrophila*, *P. fluorescens* and *V. parahaemolyticus* (Hu et al., 2005, 2008). The present results are consistent with those of Rivera-Garza et al. (2000) who reported that clays as montmorillonite have effective anti-bacterial materials. Present results are in line with previous studies, which noted improvements on disease resistance in Nile tilapia after the addition of natural minerals such as Macsumuk® to diets (Shahkar et al., 2015) and yellow loess to rainbow trout diets (Lee et al., 2015). In extension, the microbiological parameters in the water (e.g. *Salmonella*) in experimental recirculating aquaculture systems were observed to be significantly reduced by using natural zeolite compared to microbiological parameters in conventional recirculating aquaculture systems (Sirakov et al., 2015).

Hematological parameters are used as valuable biological indicators in response to dietary manipulations (Rey Vázquez and Guerrero, 2007; Hassaan et al., 2018a). A reduction in RBC and Hb values below the normal range in fish is assumed to be an indicator of anemia (Maita, 2007). In the present study, no significant differences were found in Hb, Htc and RBCs count levels among the fish groups fed diets with supplemented sericite. These results are consistent with Kanyılmaz and Tekelioğlu (2016) who found that the values of Hb and RBC of sea bream juveniles were not significantly affected by dietary zeolite. However, Hb, Htc and RBCs were significantly improved with zeolite supplementation in striped snakehead (Jawahar et al., 2016) and common carp (Kanyılmaz, 2008). In the present study, WBCs and their differential were increased by sericite supplementation in all supplemented diets compared to control diet. This increase can be related to the increase of non-specific or innate immunity, and their count can be considered as an indicator of the health status of fish. Similarly, WBCs was significantly higher in striped snakehead fed zeolite enriched diets (Jawahar et al., 2016).

The activity of liver enzymes; ALT and AST are considered as indicators for hepatotoxicity and histopathological changes (Sheikhzadeh et al., 2017; Hassaan et al., 2018b). In the present study, serum activity of ALT and AST were not significantly affected

by sericite supplementation, indicating that the different levels of dietary sericite had no harmful effect on Nile tilapia health, but further histological studies are necessary to confirm this assumption. Similar findings were previously quantified in common carp (Kanyılmaz and Tekelioğlu, 2016) and rainbow trout (Sheikhzadeh et al., 2017) fed diets with different levels of zeolite. Serum proteins are useful for the production of an exceeding amount of energy during stress conditions (detoxification of the toxicant) to overcome this stress exposure (Singh et al., 2010). Serum albumin contributes to the metabolic process and participates in the transport function of substances necessary via blood (Andreeva, 1999). Immunoglobulin can be considered a humeral element of serum protein; it plays an important role in innate immunity and is an indicator of the health status of fish (Tukmechi et al., 2011; Hassaan et al., 2015). Compared to the control diet, serum protein, albumin, globulin and IgM levels were significantly higher ($P < 0.05$) in Nile tilapia fed sericite supplemented diets which may be a result of the enhancement of the non-specific immune response of the species. To the best of our knowledge, there is no available information showing the effects of dietary sericite on biochemical blood parameters of fish species. However, the studies on the effects of zeolite on serum protein and albumin (Jawahar et al., 2016; Sheikhzadeh et al., 2017) are line with the present results. Conversely, zeolite and perlite supplementation did not affect the serum total protein contents in common carp (Khodanazary et al., 2013). The mechanism of how dietary sericite or any other natural clays effects the serum biochemical changes in fish is not well-known and it seem to be species and clay mineral dependent so further studies are needed.

In terms of the whole-body proximate composition, fish fed sericite supplemented diets had significantly lower lipid content and higher protein content ($P < 0.05$) compared to control diet. The increase in whole body crude protein may be associated with the participation of sericite in the nitrogen feedstuff conversion to animal protein as reviewed by Eya et al. (2008). On the other hand, Oluwaseyi (2016) found an increased fat content in whole body of African catfish, *C. gariepinus* with the increase in dietary bentonite compared to the control. In the present study, there was an increase in the ash content with the increasing levels of sericite. The increase in the ash content may be in direct relationship with the presence of mineral clay at high inclusion levels (Oluwaseyi, 2016). Again, and to the best of our knowledge, very few studies did evaluate the effect of clay minerals on whole-body fish composition, so present results lack comparison to other studies, but clearly point to a beneficial effect of specific clay mineral addition to fish diets, as they clearly improve growth, whole-body fish quality and overall health fitness.

5. Conclusion

The results in this study highlight the importance of using Sericite in Nile tilapia diets as follow: i) Sericite clay supplementation to Nile tilapia diets improved growth performance, digestive enzymes, hematological, and immunological parameters, ii) The optimum inclusion level of sericite clay to achieve the best feed conversion ratio is 5.5 g/kg, iii) The study was conducted on a laboratory scale with the ingredient processed under controlled conditions; there is an urgent need to scope for larger scale production to generate enough volumes to be used in the aquafeed industry.

In conclusion; as this was the first approach to the subject, more studies are warranted to reveal the mechanism of how sericite affect fish, feed durability and methods of administration to aquaculture species.

Ethics statements

Experiment was subjected to ethical reviewed and approved by the National Institute of Oceanography and Fisheries (NIOF) through the Animal and Welfare Ethical Review Body. This study was conducted under Aquaculture Division of NIOF and Benha University, Egypt with amended Animals Scientific Procedures Act implementing EU Directive 2010/63.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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This study was conducted under Aquaculture Division of NIOF and Benha University, Egypt with amended Animals Scientific Procedures Act implementing EU Directive 2010/63.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.anifeedsci.2020.114400>.

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