Egyptian J. Nutrition and Feeds (2024), 27 (3):415-430

EVALUATION OF SYNTHETIC ZEOLITE AND MICROALGAE AS ADSORPTION ADDITIVES FOR HEAVY METALS ON PRODUCTIVITY, EGG QUALITY AND BLOOD METABOLITE TRAITS OF LAYER HENS DRANK EL-SALAM CANAL WATER AT NORTHERN SINAI, EGYPT

Safaa, A. M. Ali

Animal and Poultry Physiology Department, Desert Research Center, Egypt.

(Received 30/9/2024, accepted 10/10/2024)

SUMMARY

This study aimed to evaluate the effect of dietary inclusion with 2% synthetic zeolite (SZ) or 2% *Nanochloropsis sp*. (NC) as adsorption substances for heavy metals of old layer hens drank El-Salam Canal water (SCW) for 12-weeks period during winter season. A total of 160 Lohmann brown laying hens (62-weeks old) with an initial body weight of 1846 ±17.02 g, were randomly allocated to equally 4 experimental groups, with 4 replicates of each group. The first group were drank tap water (TW) and fed basal diet without any supplementation and served as control group; the second group were drank El-Salam Canal water (SCW) and fed basal diet without any supplementation (treated group, T1); the third group drank El-Salam Canal water (SCW) and fed basal diet supplemented with 2% microalgae (*Nanochloropsis sp*.) (treated group, T2); the fourth group drank El-Salam Canal water (SCW) and fed basal diet supplemented with 2% synthetic zeolite as treated group (T3). Egg production, feed intake, feed conversion ratio, egg weight, egg mass, egg quality indices were recorded. Liver and kidney functions were estimated. The results indicated dietary *Nanochloropsis sp*. and synthetic zeolite. improved productive and reproductive performance and mitigating the adverse effect of heavy metals (cadmium and lead) against oxidative stress of laying hens by increasing serum total antioxidant capacity ($P<0.05$). Dietary inclusion with *Nanochloropsis sp.* and synthetic zeolite at 2% improved laying performance and egg quality indices. Serum concentrations of total protein (TP) and globulin (G) increased significantly ($P<0.05$) on hens fed diets supplemented with 2%NC or 2%SZ, respectively. In conclusions, Dietary inclusion with synthetic zeolite or microalgae (*Nanochloropsis sp.*) at 2% evidenced their superiority in protection of layer hens from the adverse effects of Pb and Cd induced liver and kidneys damage, in addition improved productive and reproductive performance.

Keywords: *Heavy metals, adsorption additives, zeolite, microalgae, laying performance.*

INTRODUCTION

Optimizing the egg production requires having efficient hens breed and as the same time to apply an adequate management, good health practices and feeding programs. The inclusion of diets with different types of feed additives may improve nutrient utilization. One of these types of additives is the natural zeolite, which can be used in farms of animal production due to its unique antibacterial properties, safety and efficacy (Abdulkarim, 2021). Zeolite has specific physical structure, which provides it with special properties, and so it is able to adsorb gases and toxins, mycotoxins, ammonia, water, and eliminate heavy metals and radioactive elements, which might be harmful to animals (Zhou, 2008).

The absorptive characteristics of zeolite are due to their high cation-exchange capacity, which affects tissue uptake and utilization of $NH4+$, $Cu2+$, $Pb2+$, $Cd2+$, $Cs+$, and other cations in animals (Oğuz, 2011). Additionally, zeolite has the ability to selectively exchange its own cations for ions from the environment. Therefore, zeolite appears to be stable in gastrointestinal tract of animals and as unique selective adsorbers. Many experiments showed that the dietary inclusion of zeolites improved the performance and egg quality by layer hens in early or late egg production periods (Abdulkarim, 2021). Several types of natural zeolites are produced worldwide, e.g., zeolite, chabazite, heulandite, modernite, phyllite, silicate, silicalite, and heroinite. However, the zeolite most used in animal research is zeolite (Mastinu *et al*., 2019) which can adsorb harmful substances in the gastrointestinal tract such as mycotoxins, ammonia, and heavy metals (Pavelić *et al*., 2018).

Another type of these additives is Algae including macro-algae and microalgae, offer a rich source of proteins, lipids, vitamins, and minerals, making them potentially valuable feed ingredients for farm animals. The nutritional profile of microalgae is remarkable, often described as a rich source of proteins, lipids, vitamins, and essential minerals. The mineral content in microalgae includes calcium, magnesium, phosphorus, potassium, and trace elements such as iron, zinc, and selenium, which are crucial for animal health (Becker, 2007). In addition to their nutritional value, algae have been recognized for their potential for removing heavy metals from the low water quality [Leong and Chang., (2020) & Uguz and Sozcu., (2024)]. It is known that microalgae have the capability to absorb potentially harmful heavy metals through different pathways, including biosorption, biotransformation, or bioaccumulation (Leong and Chang., 2020).

There are three types of remediation of wastewater, physical, chemical and bio-sorption methods. Recently, synthetic zeolite and some micro-organisms which are described as eco-friendlier became the common applicable methods for bio-remediation of low-quality water. The use of biological organisms as an adsorbent defined as biomaterials against heavy metals such as bacteria, fungi, yeast and algae (Mustapha and Halimoon, 2015).

Silent toxicity and oxidative stress induced by heavy metals especially cadmium and lead elements occurring in microorganisms, plants, animals and human (Ceramella *et al*., 2024). Lead metal (Pb) has the capacity to cause oxidative stress and serves as a catalyst for oxidative processes of biological molecules by generating free radicals (Mateo *et al*., 2014). Depending on the degree of exposure, the negative consequences of Pb can range from minor physiological or biochemical abnormalities to significant pathologic illnesses, in which various organs and systems may be harmed or their functions altered (Mateo *et al*., 2014).

Recently, Yuan *et al*. (2013) and Rahman *et al*. (2023) evaluated the effect of microalgae (*spirulina platensis*, *Sp*) as feed additives on removal of heavy metals of laying hens and reported that *spirulina* can potentially be used as a natural and effective dietary supplement to mitigate the heavy metals burden in bone and tissues of the internal organs of birds.

The physiological mechanism of preventing heavy metal toxicity of algae due to the antioxidant and detoxifying properties where the presence of many flavonoids compounds in algae cells which may chelate transition metal ions, this is due to the ability of the many hydroxyl groups to form complexes with metals, preventing their gastrointestinal absorption and accelerating their elimination through urine (Borowska *et al*., 2018). Today, scientists have investigated and applied a variety of nutritional tactics to lessen the harmful effects of oxidative stress on animal health brought on by heavy metals especially lead and cadmium in low quality water as source of drinking water (Ebrahimi *et al*., 2023).

Therefore, the present study designed to evaluate using both synthetic zeolite and microalgae (*Nanochloropsis sp*.) as an adsorbent additive for layer hens drank from El-Salam canal water at northern Sinai, Egypt to mitigate the negative consequences associated with water quality on productive performance, egg quality indices and some blood metabolites of Lohmann brown layer strain during winter season.

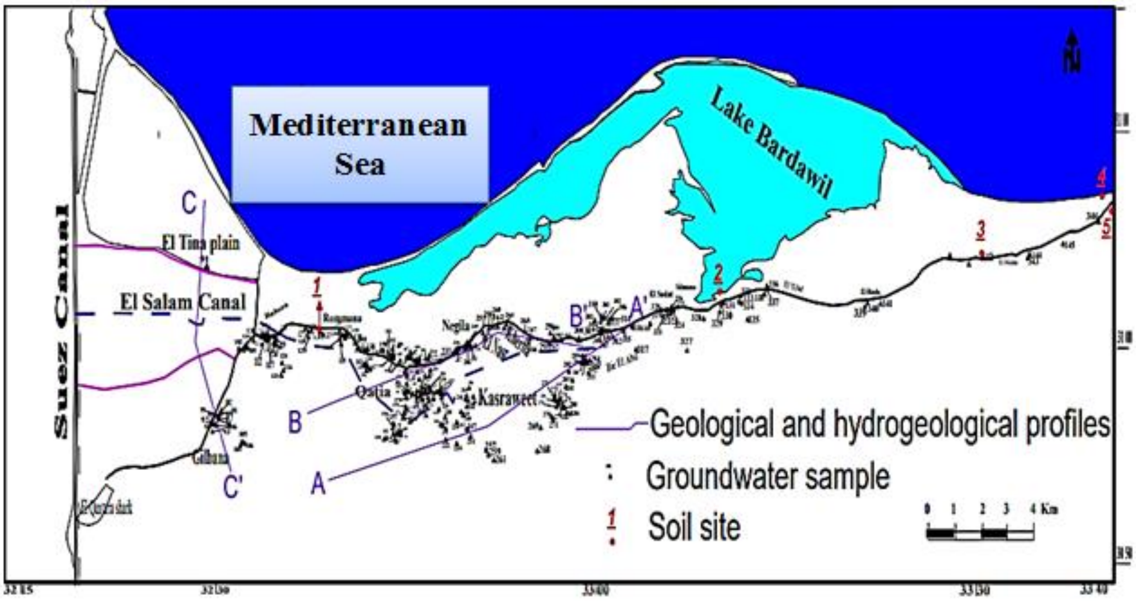
MATERIALS AND METHODS

Location of El-Salam Canal:

El-Salam Canal is surrounded the land of Sahel El-Tena (Tina plain) region and located at North-East of Egypt. It supplies water for the reclamation of the new lands east and west of Suez Canal. The total length of the Canal is about 220-km. This Canal passes through five Governorates: Damietta, Dakahliya, Sharkiya, Port-Said,Ismailia and North Sinai. El-Salam canal water is a mixture of Nile and drainage waters (such as El-Serw and Hadous drains).

According to El-Amier *et al*., (2021), the sequence of average concentration of cations in El-Salam Canal water were $Na^{+} > Ca^{2+} > Mg^{2+} > K^{+}$, the major cations constitute around 60% of the total dissolved salts. While the sequence of major anions in water was $SO_4{}^{2-} > HCO3^{-} > Cl^{-} > CO_3{}^{2-}$. The order of heavy metals was Zn < Cd < Cr <Ni < Fe < Mn < Co < Cu < Pb.

According to the WHO (2011) guidelines, the levels of Fe and Zn in the El-Salam Canal are within the permissible limits for drinking and irrigation purposes, while Mn, Pb, Cu, Co, Ni, Cr, and Cd were detected at higher concentrations than those recommended for irrigation and drinking purposes. The general layout of the Canal is shown in the flowing Figure.



Heavy metals assessment in El-Salam Canal water (SCW):

According El-Amier *et al*. (2021), the mean values concentrations ±SE in mg/L^{-1} of Fe, Mn, Pb, Cu, Co, Ni, Cr, Cd, and Zn in the surface water samples collected along the canal during winter season are shown in Table (1). The values of Fe and Zn levels were within the permissible limit recommended for drinking and irrigation purposes, while Mn, Pb, Cu, Co, Ni, Cr, and Cd were found in higher concentrations of the permissible limit and recommended for drinking and land irrigation purposes according both to the European Standard (ES) and United States Environmental Protection (USEP) Agencies.

Table (1): Mean values concentrations (mg/L^{-1}) in El-Salam Canal water at northern Sinai, Egypt.

Items	Fe	Mn	Pb	Cu	Co	Ni	Cr	Cd	Zn
	1.50± 0.08	1.67± 0.18	5.58± 0.25	4.56± 0.30	3.63± 0.40	1.40± 0.13	0.86± 0.09	0.58± 0.17	0.26± 0.03
	The permissible limits and recommended for drinking and irrigation according ES and USEP								
					ES				
	0.20	0.05	--	0.2	--	0.02	0.05	0.005	--
					USEP				
	5.0	0.20	0.20	0.20	0.05	0.20	0.10	0.01	2.0

Laboratory analysis for tap water (TW) and El-Salam Canal water SCW:

Representative periodical monthly (February, March and April 2021) water samples were collected (5 liter each) from El-Salam Canal water (SCW) as well as tap water (TW) for laboratory analysis. About 100 ml of each water sample were filtered through nitrocellulose filter membrane of 0.45µm pore size prior dried in 105^{0}C for 2 hours. The filtrate and unfiltered water samples were preserved in 2 ml concentrated nitric acid (HNO_3) to prevent precipitation of metals and growth of algae. Samples were collected according to international standard methods for examination of water and wastewater (APHA, 2005) to evaluate the concentrations of selected heavy metals. Samples were collected in stopper polyethylene plastic bottles, stored in an iced cooler box and delivered immediately to the laboratory for analysis. The plastic bottles were cleaned by soaking in 10% HNO_3 and the procedural blanks of standard solutions were prepared under clean laboratory environment. All reagents were used for analytical grade and deionized water was used for all the prepared reagent solutions. Heavy metals (Fe, Cu, Zn, Mn, Cd, Pb, Cr and Ni) were determined using the model 2380 atomic absorption spectrometer according to Shkinev *et al*. (1989).

Table (2): Monthly element analyses of tap water (TW) and El-Salam Canal water (SCW) for three months period (from February till April 2021).

Month	Water	Heavy metals concentrations (mg/L^{-1})							
		Fe	**Cu**	**Zn**	**Mn**	**Cd**	**Pb**	**Cr**	**Ni**
February	**TW***	0.02	0.03	0.41	0.09	0.07	0.58	0.05	0.06
	SCW	2.40	4.59	5.10	4.28	0.35	0.80	51.00	0.44
March	**TW***	0.01	0.05	0.49	0.03	0.07	0.52	0.05	0.04
	SCW	1.05	5.70	5.30	4.88	0.44	0.98	55.00	0.55
April	**TW***	0.08	0.45	0.39	0.03	0.09	0.61	0.06	0.06
	SCW	2.10	6.10	5.90	5.70	0.51	1.05	56.00	0.45
Overall mean	**TW***	0.03	0.17	0.43	0.05	0.07	0.57	0.05	0.06
	SCW	1.85	5.46	5.43	4.95	0.43	0.94	54.00	0.48

***** *= all determined values were within the permissible and recommended limits by WHO (2011) and Domo et al. (2024); the determined elements are in µg/L with exception Fe element (mg/L).*

Monitoring of potential toxic elements PTEs in El-Salam Canal water:

To explore the degree of pollution in El-Salam Canal water, potential toxic elements (PTE's) were analyzed periodically for three months (from February 2021 till April 2021) for El-Salam Canal water. The results for the concentrations of eight elements are presented in Table (2) The obtained results indicated that during different months, all the PTE's were under the toxic levels with the exception of Cd and Pb elements where the numerical values showed that in April month the concentration of these elements recorded 0.5 and 1.05 mg/L^{-1}, whereas the save levels for these PTE's were 0.01 and 0.2 mg/L^{-1}, respectively (Table 1).

Site of the study:

This study was conducted at private poultry farm (Latitude 31º 29 N; Longitude 32º 34 E), North Sinai Governorate, in cooperation with Animal and Poultry Production Division (Department of Animal and Poultry Physiology), Desert Research Center, Ministry of Agriculture and Reclamation, Cairo, Egypt, Systel Telecom Company and Egyptian Center of Excellence for Bio-Saline Agriculture.

Animals, housing and diet:

A total of 160 commercial Lohmann brown layer hens at 62-weeks of age and with an initial body weight of 1846.103±17.02g were used in this study. The hens were divided into equally four experimental groups and each group consisted of four replicates (10 hens replicate^{-1}). The 1st experimental group (Control group) drank tap water (TW) containing 265 ppm total dissolved solids (TDS) and fed on basal diet free from any sorption additives. The 2nd experimental group (T_1) drank El-Salam Canal water (SCW) and was fed basal diet free from any sorption additives. The 3rd experimental group (treatment, T_2) drank El-Salam Canal water (SCW) and fed basal diet supplemented with 2% *Nanochloropsis oculata* (*N. oculata*). The 4th experimental group (treatment, T_3) drank El-Salam Canal water (SCW) and fed basal diet supplemented with 2% synthetic zeolite meal. Birds were housed in 16 cages (10-hens cage^{-1}) with dimensions 150 × 55 × 45 cm. length, width and height, respectively. Each cage was equipped with 1.5 m of linear feeder at the cage front, and 5 nipple drinkers and all birds received feed and water *ad libitum*. All experimental diets were formulated based on NRC requirements (NRC, 2014). The house was provided with open side curtain ventilation with a circulation fan. The lighting program used was 14 h of light at the beginning of the trial, with light increasing by 15 min. weekly to 17 h of light. Synthetic zeolite powder and *Nanochloropsis oculata* (*N. oculata*) meal was added to the basal diet according to the design of the experiment as shown in Table (3).

Microalgae (Nanochloropsis oculata, N. oculata) and synthetic zeolite:

Synthetic zeolite are hydrated Aluminosilicate minerals. They belong to the mineral class known as "tectosilicates." Synthetic zeolite has a lot of potential to be one of the best and most cost-effective ways to remove ammonia and some heavy metals from water. This is because it has special molecular sieve, sorption, and ion exchange properties. Clinoptilolite is the most prevalent natural zeolite. Synthetic zeolite powder is a non-dusting, hard and resistance to attrition, non-clouding in liquids (due to absence of clays), good permeability, relatively high density and high-water retention according to Metallurgical Research and Development Center. The chemical composition of the Synthetic zeolite (Clinoptilolite) is presented in Table (4).

The microalgae (*N. oculata*) used in the current study was prepared and kindly provided by the Biotechnology Microalgae Culture Unit, National Research Center (NRC), Egypt. Microalgae were maintained in standard F/2 Guillards media (Guillard and Ryther, 1962). The collected microalgae were stored in the refrigerator at 4ºC until the culture period was finished and then harvested by centrifugation. The chemical composition (g/100g) of microalgae *(Nannochloropsis oculata*) and quantitative constituents of minerals and amino acids profiles (mg/100g) are presented in Table (5).

Table (3): Composition and calculated analysis of the experimental layer diets.

Ingredients	Experimental groups			
	C	**T_1**	**T_2**	**T_3**
Crushed Corn yellow	46.00	46.00	45.00	45.00
Soybean meal (48% crude protein)	21.00	21.00	20.00	20.00
Wheat	7.00	7.00	7.00	7.00
Barley	3.00	3.00	3.00	3.00
Wheat bran	8.75	8.75	8.75	8.75
Molasses	9.00	9.00	9.00	9.00
Limestone	0.40	0.40	0.40	0.40
Nanochloropsis oculata meal*	0.00	0.00	2.00	0.00
Synthetic zeolite powder**	0.00	0.00	0.00	2.00
Salt	0.25	0.25	0.25	0.25
Di-calcium phosphate[1]	2.00	2.00	2.00	2.00
Vitamin-mineral premix[2]	0.40	0.40	0.40	0.40
DL- Methionine	0.15	0.15	0.15	0.15
L-Lysine HCl	0.15	0.15	0.15	0.15
Ethoxyquin`	0.15	0.15	0.15	0.15
Analysis (g/kg dry matter basis)				
Dry matter, %	89.00	89.00	89.00	89.00
Crude protein, %	16.70	16.70	16.50	16.20
Crude fiber, %	3.60	3.60	3.60	3.60
Ether extract, (%)	3.16	3.16	3.16	3.16
Ash (%)	10.40	10.40	10.40	10.40
Ca (%)	2.65	2.65	2.65	2.65
Av. Phosphorus (%)	0.71	0.71	0.71	0.71
ME (Kcal/kg)	2690.00	2690.00	2680.00	2650.00

[1] each kilogram contained Ca 24% and P 17.5%, [2] each kilogram contained vitamin A 15000IU; cholecalciferol 15000IU; DL-α-tocopheryl acetate 30IU; menadione5.0mg; thiamine 3.0mg; riboflavin 6.0mg; niacin 20.0mgpantothenic acid 8.0mg; pyridoxine 5.0mg; folic acid 1.0mg; vitamin b1 15µg; M 80.0mg;Z 60.0mg; F 30.0mg; Cu 5.0mg and Se 0.15mg.

**Microalgae (Nanochloropsis oculata) meal was supplemented by 2.0 kg/100kg basal diet (T_2)*

***Synthetic zeolite powder was supplemented by 2.0 kg/100kg basal diet (T_3)*

Table (4): Chemical composition of synthetic zeolite (Clinoptilolite).

Cations	%	Major Mineral salts	%	Other Mineral salts	%
K^+	3.27	**SiO_2**	62.22	**Cl**	0.05
Ca^{++}	3.58	**Al2O3**	11.09	**BaO**	0.08
Na^+	0.78	**Na_2O**	0.78	**P2O5**	0.03
--	--	**MgO**	0.59	**ZnO**	0.02
--	--	**CaO**	3.58	**SrO**	0.08
--	--	**Fe_2O_3**	4.03	**PbO**	0.002
--	--	**K2O**	3.27	**MnO**	0.12
--	--	**TiO_2**	0.34	**SO3**	0.03
--	--	**ZrO_2**	0.11	--	--

Table (5): Chemical composition (g/100g) of microalgae *(Nannochloropsis oculata*) and quantitative constituents of minerals and amino acids profiles (mg/100g).

Chemical composition (g/100g)		Minerals profile (mg/100g)		Amino acids profile (mg/100g)			
Moisture	7.15	Fe	19.35	Methionine	69.52	Tyrosine	87.69
Crude protein	55.78	Zn	1.02	Cysteine	17.30	Threonine	39.21
Fat	6.61	Sodium	1862.70	Phenylalanine	16.24	Valine	50.36
Ash	12.29	Calcium	229.00	Lysine	15.20	Serine	11.64
Total carbohydrates	18.17	Potassium	798.00	Isoleucine	55.95	Glycine	9.98
--	--	Magnesium	173.00	Leucine	65.11	Proline	31.52
--	--	--	--	Aspartic acid	30.16	Alanine	20.24
--	--	--	--	Glutamic acid	15.07	Arginine	8.56
--	--	--	--	Histidine	13.22	--	--

Productive performance parameters:

At the beginning and end of the study, live body weight (LBW) was weighed at early morning (0800h.am) on a digital scale to determine the changes in body weight throughout the experimental period (100-days). Total weight gain was calculated as the difference between the initial and final live body weight. The growth indices were calculated according to the following equations:

- Weight gain (WG, g) = final live body weight (g) - initial live body weight (g)
- Average daily gain (ADG, g/hen/d) = total weight gain / number days of study
- Feed intake (FI, g/hen) = total diet supplied - remaining diet
- Feed Conversion ratio (FCR) = feed intake (g) / weight gain (g).

Daily egg production as daily laying rate and egg weights for each experimental group were recorded. Egg production percent, average egg weight and egg mass per hen per day were calculated. Feed intake for each experimental group (feed left in the feeder at the end of the week was weighed and subtracted from the total amount supplied throughout the week). Average daily feed intake (ADFI, g) was calculated for individual hens as feed offered minus feed unconsumed throughout on a weekly basis and divided by 7. Total egg mass (TEM, g) per hen was calculated as (average number of eggs laid over 100-days × average egg weight). Feed conversion ratio (FCR) for each experimental group was expressed as total kilograms of feed consumed per total kilograms of egg produced.

A total of 100 eggs were randomly collected from the four experimental groups (25 eggs per experimental group) to assess separately egg quality indices. Prior to egg breaking, egg weight (EW, g) was recorded using an electronic weighing scale. Using a plastic scraper, the albumen was separated from the yolk, then albumen weight (AW, g) and yolk weight (YW, g) were individually weighed using an electronic weighing scale. The eggshells were carefully washed separately, air dried, and weighed with a digital scale (S_hW, g). Then, eggshell membranes were removed, and separately eggshell thickness (S_hT, mm $\times 10^{-2}$) was measured at three regions (top, equator, and base) in micrometer unit using a caliper according to Yoruk *et al*. (2004).

Eggs from each replicate were collected, counted, and weighed daily for the calculation of egg production (EP %) ratio, egg weight and egg mass (EM) using following equations:

- EP (%) = number of collected eggs / (number of hens × days) ×100
- EM (g/d/hen) = EP (%) × mean of egg weight (g)

Egg quality indices:

At the end of the study, five eggs from each replicate (20 eggs per experimental group) were collected (total number was 80 eggs) for determination of egg quality indices. The following parameters were determined: egg weight, egg white weight, yolk weight, eggshell weight, eggshell weight ratio and egg white and yolk ratio. To determine, the egg white, yolk and eggshell, the eggs were broken, and the yolk were separated handily and weighed. Individual shell (with membrane) was cleaned from adhering albumen and dried at room temperature overnight. The albumen (egg white) was determined by difference. The ratio of albumen (AR), yolk (YR) and shell (SR) were determined using the following equations:

- YR (%) = yolk weight (g) / egg weight (g) ×100

- AR (%) = albumen weight (g) / egg weight (g) ×100
- SR (%) = shell weight (g) / egg weight (g) ×100

Blood biochemical estimations:

Individually blood samples were collected from wing vein for 10 hens from each experimental group into 5ml tubes without any anticoagulant material. Serum was collected by centrifugation for 15 minutes at 3500 rpm and it stored at –20 °C until biochemical analyses. Serum total proteins (TP), albumin (A), uric acid (UA) and creatinine (CRE) concentrations as well as the activities of aspartate transferase (AST) and alanine transferase (ALT) enzymes were measured using an automated scanning spectrophotometer (CE1010, Cecil Instruments Limited, Cambridge, UK) and commercial colorimetric kits (BioDiagnostic, Giza, Egypt). Serum globulin concentrations (G) were calculated according to the formula: Globulin (g/dl) = total protein (g/dl) – albumin (g/dl), then A/G ratio was calculated. The full protocol description for each analysis is presented in the supplementary materials. Total antioxidant capacity (TAC) of Amphora acetone extract was spectrophotometrically estimated according to method described by Koracevic *et al*. (2001) at wavelength 505 nm.

Statistical analyses:

SAS (Statistical Analysis System - version 9.1) was used to statistically analyze the data. To evaluate significant differences between means, a one-way ANOVA and least significant differences (LSD) post hoc test were used. Statistics are considered significant at $P < 0.05$. Means in the same column with different small letters are significantly ($P<0.05$) different (SAS, 2004). Duncan's multiple range test was used to compare the individual variation among the means of different treatments (Duncan, 1955).

RESULTS AND DISCUSSION

Growth indices and laying performance responses:

In terms of growth indices, results in Table 6 illustrate the effect of dietary inclusion of 2% synthetic zeolite powder (2% SZ) or 2% microalgae (*Nannochloropsis oculata*) meal (2% NC) as sorption additives for heavy metals on body weight changes and growth rate after 12-weeks of treatments. The obtained results revealed that average daily gain and total weight gain for group fed basal diet free from any sorption additives and drank El-Salam Canal water (T1) were significantly decreased ($P>0.05$) compared to control group and groups fed diets supplemented with 2%NC or 2%SZ (T2 and T3, respectively). On the other hand, dietary supplementation of 2% NC (T2) and 2% SZ (T3) improved significantly ($P<0.05$) both of average daily gain (1.34 and 1.36 g/d, respectively) and total weight gain (134.06 and 136.59 g, respectively) compared to group fed basal diet free from any sorption additives and drank El-Salam Canal water (T1). These findings indicated that the dietary inclusion of 2% synthetic zeolite powder or 2% microalgae meal as adsorbents materials likely reflects the dynamics of the metabolism of nitrogen compounds and other nutrients in digestive system of birds where zeolite and algae eliminate a series of toxic substances such as salts, heavy metals, nitrates, mycotoxins, radionuclides, and ammonia as metabolism products. The numerical similarity for average daily gain and total weight gain in both T2 and T3 may be referring to the similarity between both NC and SZ in the sorption capacity for removal of heavy metals during metabolism process. As demonstrated from the obtained results in Table (6), birds fed the basal diet free from any sorption additives and drank tap water which served as control group were superior and have the best of average daily gain (2.07 g) and mean of total weight gain (207.174 g) compared to the other experimental groups (T1, T2 and T3). These results are in harmony with those showed by Abd El-khalek *et al*. (2019) & AL-musawy *et al*., (2023). Also, the present results agreed with Bilal *et al*., (2009) who found that broiler fed diets containing 2% Zeolite can lead to significant increase ($P<0.05$) in body weight and weight gain but on the contrary, Jarosz *et al*., (2017) found no significant effect ($P>0.05$) on body weight gain by addition of natural Zeolite.

Regarding laying performance traits, daily feed intake/hen, total feed intake/hen, feed conversion ratio (FCR), egg weight, egg number per hen and total egg mass per hen are summarized in Table 6. The results indicated that daily feed intake and total feed intake has significant differences among experimental groups where hens fed the basal diet free from any sorption additives (TW and SCW groups) had significantly higher ($P<0.05$) daily feed intake (110.53 and 106.40g, respectively) and total feed intake (11053 and 10640g, respectively) compared to hens fed the basal diet supplemented with

2%NC (103.54 and 10354g, respectively) or 2% SZ (105.09 and 10559g, respectively). However, hens fed diets supplemented with 2%NC or 2%SZ tended to have the best values of egg weight, total egg mass and feed conversion ratio compared to hens fed the basal diet free from any sorption additives (TW and SCW groups). These findings revealed the positive effect of NC and SZ on enhancing feed utilization efficiency subsequently improved laying performance (egg production rate, egg weight and egg mass).

Table (6): Effect of dietary inclusion with synthetic zeolite or microalgae *(Nannochloropsis oculata)* on laying performance of brown Lohmann laying hens drank El-Salam canal water during winter season.

Parameters	Experimental groups				±SE	P value
	TW	SCW	SCW+NC	SCW+SZ		
Initial body weight (g)	1849.17[a]	1843.35[a]	1846.05[a]	1845.82[a]	34.82	0.999
Final body weight (g)	2056.35[a]	1890.0[b]	1980.11[a]	1982.41[a]	33.67	0.010
Total weight gain (g)	207.17[a]	46.65[c]	134.06[a]	136.59[b]	18.60	0.001
Daily feed intake/hen (g/day)	110.53[a]	106.40[ab]	103.54[b]	105.59[ab]	1.78	0.060
Total feed intake/hen (g)	11053.00	10640.00	10354.00	10559.00	178.86	0.065
Egg number/hen	77.59[a]	59.00[d]	74.53[b]	71.91[c]	0.57	0.001
Egg weight (g)	70.10[b]	66.98[c]	74.83[a]	72.42[ab]	0.89	0.001
Total egg mass/hen (g)	5439.5[a]	3952.0[c]	5576.8[a]	5208.8[b]	76.48	0.001
Feed conversion ratio	2.04[b]	2.69[a]	1.86[c]	2.03[b]	0.05	0.001

TW = hens drank tap water and fed basal diet free from any sorption materials (control group), SCW= Hens drank El-Salam Canal water and fed basal diet free from any sorption materials.; SCW+NC = hens drank El-Salam Canal water and fed basal diet supplemented with 2% microalgae (Nanocloropsis Oculata) meal; SCW+SZ= hens drank El-Salam Canal water and fed basal diet supplemented with 2% synthetic zeolite powder.;
[a-c] Means followed by different letters in superscript in the same row differ significantly (P<0.05).

Our findings agree with previous studies of El-Sherbeni *et al*. (2024) who reported that adding zeolite to diet of laying hens significantly improved final BW and BW change, egg number, egg weight, egg mass, lay-ingrate, feed consumption (FC), and feed conversion ratio (FCR), as well as egg quality criteria when compared to the un-supplemented group.

The improved FCR due to synthetic zeolite administration may be related to the reduced rate of passage of food across the gut, which in turn decreases the feed consumption and increase nutrient digestibility (Prasai *et al*., 2016). Furthermore, the reduced rate of passage of food across the gut increases nutrient exposure to digestion and absorption (Khambualai *et al*., 2009). So, the enhancement in FCR is perhaps related to the effects of synthetic zeolite in stimulating the digestive system and improving the nutrients digestibility.

Shanmugapriya and Saravana Babu (2014) suggested that the improvement in FCR in chickens treated with Spirulina may be due to the balanced bacterial population in gastro-intestinal tract that play a role in boosting minerals and vitamins absorption and enhance performance.

Also, supplemented broiler diets with zeolite improved feed consumption and FCR (Alharthi *et al*., 2022; AL-musawy *et al*., 2023 and Pavlak *et al*., 2023). According to Sharvadze *et al*. (2022) found that layers fed on zeolite-enriched feeds (50g/kg diet) had considerably better laying rate (LR), egg number (EN), egg mass (EM), and egg weight (EW) than those fed on control diet. Hanusova *et al*. (2021) stated that Japanese quail egg productivity and egg weight were improved by adding 10 and 20g zeolite/kg diet.

the present study explains the beneficial effect of dietary inclusion with microalgae on laying performance. However, microalgae were reported to contain beneficial components such as essential amino acids, vitamins, carotenoids, antioxidants, antimicrobial factors and unsaturated fatty acids (Fields, 2020 and Costa *et al*., 2024). Furthermore, the positive effect of feeding microalgae on caeca microbiota profiles of layers has been reported (Kim and Kang, 2015). Thus, the mentioned advantages could be included in the metabolism regulating egg production of layers.On the contrary, Mens *et al*. (2022) reported that the inclusion of 2 and 3% microalgae (*Nannochloropsis limnetica*) in diets of laying hens not affected significantly (P>0.01) body weight, average egg weight, and feed conversion ratio. Also, Abouelezz (2017) reported that, supplementation of 1.0% of *Spirulina platensis* in feed or 0.25% in drinking water did not show significant (P≥0.05) effects on the egg laying rate, egg weight, daily egg mass, feed intake, feed conversion ratio and egg quality of Japanese quail as compared with the control group. Vieira *et al*. (2023) reported that the inclusion of different levels of zeolite in the diet of laying hens did not influence (P>0.05) the performance variables of laying percentage, average egg weight, egg mass, feed intake, or feed conversion ratio (FCR).

External and internal egg quality indices response:

Regarding the effect of dietary inclusion with 2%SZ powder or 2%NC meal on external (shell weight, shell percentage and shell thickness) and internal (albumin, yolk weights and their percentages) egg quality indices are presented in Table 7. In respect to external egg quality traits, the obtained results indicated that hens fed diet supplemented with 2% NC meal or 2% SZ powder as adsorbent additives had significantly affected (P<0.05) on shell weight and shell thickness, where shell weight values recorded 7.8 and 8.0g compared to hens fed basal diet free of any adsorption additives (7.5 and 7.4g for TW and SCW experimental groups, respectively). The corresponding values for shell thickness recorded 0.44, 0.43 for groups fed basal diet supplemented with 2% NC meal or 2% SZ powder, respectively compared to hens fed basal diet free from any adsorption additives (0.37 and 0.45g for SCW and TW experimental groups, respectively).

Our findings demonstrated significant effects of zeolite administration on the measurements of egg quality. These findings have been supported in laying hens by El-Sherbeni *et al*. (2024); Juzaitis-boelter *et al*. (2021) and Sharvadze *et al*. (2022) on shell thickness, Berto *et al.* (2013) on yolk relative weight. Also, Hanusova *et al*. (2021) found a significant impact on the egg yolk index of zeolite supplementation in Japanese quails' feeds. Also, the current results revealed an agreement with those obtained by Nys (1999) who noted that synthetic zeolites with a high cation exchange capacity slightly improve egg shell quality because they form complexes with Ca. On the contrary, several studies demonstrated an insignificant effect of adding zeolite in poultry feeds at various concentrations on egg production rate (Berto *et al*., 2013; Vasiljevic *et al*., 2021 and Vieira *et al*., 2023).

In respect to the effect of dietary microalgae, Park *et al*. (2015) found that supplementation of marine microalgae (*Schizochytrium*) meal at a level 0.5–1% of the diet had a beneficial effect on the egg shell thickness. The influence of marine algae on shell thickness may be attributed to its mineral content (Park *et al*., 2015). However, the mechanisms underlying this effect still remain unclear, and there was no scientific evidence explaining how algae affect the egg shell thickness. The obtained results showed that no effects of El-Salam Canal water as source of drinking water for laying hens on mortality were detected in this study.

Table (7): Effect of dietary inclusion with synthetic zeolite or microalgae *(Nannochloropsis oculata*) on egg quality indices of brown Lohmann laying hens drank El-Salam canal water during winter season.

Parameters	Experimental groups				±SE	P value
	TW	SCW	SCW+NC	SCW+SZ		
Egg weight (g)	70.36^{b}	67.20^{c}	74.30^{a}	71.50^{b}	0.74	0.001
Shell weight (g)	7.50^{b}	7.36^{b}	7.80^{ab}	8.00^{a}	0.15	0.015
Shell thickness (mm)	0.45^{a}	0.37^{b}	0.44^{a}	0.43^{a}	0.008	0.001
Shell (%)	10.68	10.98	10.51	11.26	0.24	0.130
Albumin weight (g)	45.20^{bc}	43.46^{c}	48.66^{a}	47.20^{ab}	0.81	0.001
Albumin (%)	64.11	64.63	65.45	65.75	0.60	0.198
Yolk weight (g)	17.66^{a}	16.36^{b}	17.83^{a}	16.30^{b}	0.27	0.001
Yolk (%)	25.20^{a}	24.03	24.38^{ab}	22.97^{b}	0.48	0.013

TW = hens drank tap water and fed basal diet free from any sorption materials (control group), SCW= Hens drank El-Salam Canal water and fed basal diet free from any sorption materials.; SCW+NC = hens drank El-Salam Canal water and fed basal diet supplemented with 2% microalgae (Nanocloropsis Oculata) meal; SCW+SZ= hens drank El-Salam Canal water and fed basal diet supplemented with 2% synthetic zeolite powder.; $^{a\text{-}c}$ *Means followed by different letters in superscript in the same row differ significantly (P<0.05).*

Internal egg quality indices:

In terms of egg quality, the variables of albumen weight, yolk weight and percentages of albumen and yolk as indicators of egg quality were used in the present study to elucidate the effect of dietary inclusion with adsorption additives on egg quality (Table 7). Regarding to these parameters, the obtained results in this table indicated that 2%NC group improved albumin weight and albumin percentage where albumin weight recorded 48.7, while albumin percentage recorded 65.45% compared to hens in other experimental groups (TW, SCW and SCW+2%SZ groups). The same trend was observed on yolk weight and yolk percentage where laying hens in groups supplemented 2%NC group improved yolk weight (17.83g) and yolk percentage (24.38%) compared to hens in in other experimental groups (SCW and SCW+2%SZ groups). The significant effect of dietary supplementation with 2% synthetic zeolite on albumin weight were in contrast with the previous study performed by Amad (2021) who reported that hens fed with natural zeolite at level of 0.5 and 1% showed insignificant effect (P>0.05) on the internal egg quality parameters compared to control group. On contrary, Abouelezz (2017) noted that adding 0.25% spirulina to drinking water and 1% in the feed yielded no noticeable improvements in egg quality traits. The same trend reported by Abd El-hack *et al*., (2024) when supplemented laying Japanese quail with different levels of microalgae. Selim *et al*. (2018) found that the addition of different levels of *S. platensis* to the diet did not affect the shape index, eggshell percentage, albumen weight, albumen index, yolk weight, and Haugh unit (p>0.05) of laying hens.

Liver and kidney functions:

The liver and kidneys are crucial for detoxification and the excretion of hazardous substances in both human and farm animals (Hassan *et al*., 2023). Lead (Pb) and cadmium (Cd) are the most poisonous of the most common heavy metals to accumulate in the water and food chain. Following absorption, these metals are predominantly dispersed across several tissues, mainly the kidneys and liver, the continuous exposure even at low levels of these metals can causing dysfunctions and damage of these organs (Aljohani (2023); Singh *et al*., 2023 and Verma *et al.*, 2023).

The obtained results in Table (8) indicated that serum total proteins (TP) deceased significantly (P<0.05) on SCW group while decreased insignificantly on the experimental groups (SCW+2%SZ and SCW+2%NC) compared to control group (TW). However, there was insignificant effect (P<0.05) with similar values (3.7: 3.9g/dl) in serum albumin (A) on all experimental groups. This result are agree with Abd El-Khalek *et al*.,(2019) Who found that dietary supplementation of *Spirulina platinsis* decreased significantly (P<0.05) plasma protein concentration while plasma albumin had insignificant effect (P>0.05) compared with control group of El Gimmizah layer hens breed while this result are disagreed with those reported by Elsherbeni *et al*., (2022) who found that dietary supplementation with different levels of zeolite caused significantly increase in plasma TP,A and G concentrations of silver Gimmizah layer hens strain compared to control group.

Table (8): Effect of dietary inclusion with synthetic zeolite or microalgae *(Nannochloropsis oculata*) on Serum total proteins (TP,g/dl), albumin (A, g/dl), globulin (G,g/dl), A/G ratio and total antioxidant capacity (T-AOC, mmol/ml) indicators of Brown Lohmann Laying hens drank El-Salam canal water during winter season.

Experimental groups	TP	Albumin	Globulin	A/G	T-AOC
TW	6.42^a	3.82	2.59^a	1.59^b	0.71^a
SCW	4.95^b	3.93	1.01^b	5.29^a	0.32^c
SCW+NC	5.71^a	3.73	1.97^a	2.35^b	0.56^b
SCW+SZ	5.77^a	3.77	2.00^a	2.11^b	0.53^b
±SE	0.26	0.17	0.23	0.76	0.04
P value	0.005	0.869	0.006	0.008	0.001

TW = hens drank tap water and fed basal diet free from any sorption materials (control group), SCW= Hens drank El-Salam Canal water and fed basal diet free from any sorption materials.; SCW+NC = hens drank El-Salam Canal water and fed basal diet supplemented with 2% microalgae (Nanocloropsis Oculata) meal; SCW+SZ= hens drank El-Salam Canal water and fed basal diet supplemented with 2% synthetic zeolite powder.; $^{a-b}$ *Means followed by different letters in superscript in the same column differ significantly (P<0.05).*

Serum globulin (G) concentration significantly elevated in groups fed diets supplemented with sorption additives (SCW+2%NC and SCW+2%SZ) with values 1.97 and 2.0 g/dl, respectively compared to hens drank SCW and fed basal diet free from any sorption additives (1.01g/dl) while hens in control group recorded the highest value of globulin concentration (2.59g/dl). This result indicated that there was an improving in immunity which perhaps due to the effects of dietary inclusion with synthetic zeolite or microalgae *(Nannochloropsis oculata*). The albumin/globulin ratio (A/G) significantly decreased (P<0.05) with 2%NC and 2%SZ treatments (2.35 and 2.11%, respectively) compared to SCW group (5.29%), these results referring to both NC and SZ treatments has a tendency to enhancing serum concentration of globulin (G). Vieria *et al*. (2023) reported that the inclusion of different levels of zeolite in the diet of Isa brown layer hens did not significantly affect (P>0.05) plasma biochemical traits (TP,A,G, ALT,AST and urea concentrations).

Antioxidant stress status:

Lead (Pb) and cadmium (Cd) are two examples of heavy metals that are harmful for chicken health. They can cause oxidative stress by increasing the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and blocking antioxidants from protecting cells due to increased amounts of free radicals (Ebrahimi *et al*., 2023).

The obtained results in Table (8) indicated that T-AOC increased significantly (P<0.05) in groups fed basal diet supplemented with 2% NC or 2 %SZ and drank El-Salam Canal water (0.56 and 0.53 mmol/ml) compared to group drank El-Salam Canal water and fed diet free from any sorption additives group (0.32 mmol/ml).These findings indicated that both microalgae and synthetic zeolite has an essential role for enhancing antioxidant enzymes activity to scavenge oxygen free radicals. Numerous studies (Stunda-Zujeva *et al*., 2023 and Panaite *et al*., 2023) have highlighted the higher antioxidant capacity and beneficial fatty acid profiles, including higher concentrations of omega-3 polyunsaturated fatty acids in microalgae. Moreover, recent studies have focused on examining chelating activity for Spirulina (*Spirulina platensis*) against toxicity induced by heavy metals (Cadmium, Mercury, and Lead) and antioxidant properties, especially as a candidate for protection against toxicity caused by heavy metals (Mallamaci *et al*., 2023). Also, several studies have successfully demonstrated that Spirulina has a crucial role in the prevention of oxidative stress (Sorrenti *et al*., 2021 and Caetano *et al*., 2022). Based on our discussion of antioxidant capacity status, it can be said that each of microalgae *(Nannochloropsis oculata*) and synthetic zeolite as sorption additives at 2% in the basal diet improved the antioxidant status of laying hens with good efficiency as observed in the control group (TW).

Liver enzymes activity:

The results regarding effect of dietary inclusion with sorption additives on hepatic enzymes activity (ALT and AST) are presented in Table (9). As shown in this table, the abnormality of serum ALT (80.00 iu/L) and AST (263.88 iu/L) levels for hens drank El-Salam Canal water and fed diet free from any sorption additives (SCW) compared the other experimental groups could be a signal to hepatocyte or muscle cell damage. In parallel, the abnormal levels of serum CRE (1.44 mg/dl) and SU (37.05 mg/dl) for the same experimental group could be markers to renal dysfunction. These findings stated that laying hens in SCW group were exposed to Lead (Pb) and Cadmium (Cd) metals in drinking water even at low levels for prolonged period (three months), these heavy metals may accumulate in the liver and kidneys tissues subsequently caused or induced oxidative stress by generating reactive oxygen species (ROS) and impairing the body’s antioxidant defense systems compared the other experimental groups.

Table (9): Effect of dietary inclusion with synthetic zeolite or microalgae *(Nannochloropsis oculata*) on Serum alanine amino transferase (ALT, iu/L), aspartate amino-tranferase (AST, iu/L), creatinine (CRE, mg/dl) and serum urea (SU, mg/dl) of Lohmann brown Laying hens drank El-Salam canal water during winter season.

Experimental groups	ALT	AST	CRE	uric acid
TW	32.63^{b}	150.38^{c}	0.86^{b}	25.61^{b}
SCW	80.00^{a}	263.88^{a}	1.44^{a}	37.05^{a}
SCW+NC2%	51.50^{b}	205.00^{b}	1.03^{b}	29.07^{b}
SCW+NZ2%	50.38^{b}	216.50^{ab}	1.04^{b}	28.97^{b}
±SE	9.02	18.39	0.07	2.02
P value	0.008	0.001	0.001	0.003

TW = hens drank tap water and fed basal diet free from any sorption materials (control group), SCW= Hens drank El-Salam Canal water and fed basal diet free from any sorption materials.; SCW+NC = hens drank El-Salam Canal water and fed basal diet supplemented with 2% microalgae (Nanocloropsis Oculata) meal; SCW+SZ= hens drank El-Salam Canal water and fed basal diet supplemented with 2% synthetic zeolite powder.; $^{a-c}$ *Means followed by different letters in superscript in the same column differ significantly (P<0.05).*

Our results are in accordance with previous studies performed by Vahdatpour and Nikpiran (2011) and AbdEl-Hack *et al*., (2024) who reported high levels of blood circulatory ALT, AST, CRE, and SU indicating an increase in incidence in the liver, kidney, and muscular tissues, which may be brought on by severe stress. A protective impact of NS or SZ on hepatocytes and renal function may be inferred from the significantly decrease ($P<0.05$) in serum ALT (51.50 and 50.38), AST (205.0 and 216.5), CRE (1.03 and 1.04, mg/dl) and urea (SU, 29.07 and 28.97 mg/dl) concentrations versus the high blood circulatory of both creatinine (1.44, mg/dl) and urea (37.05, mg/dl) which determined for hens drank El-Salam Canal water and fed the basal diet free from any sorption additives (SCW group) after three months period probably due to the direct toxic effect of Pb and Cd metals on renal tubules and glomerular filtration of kidneys to excrete pb and Cd metals in causing further damage and intoxication (nephrotoxicity). In accordance, nephrotoxicity in the present study is consistent with the previous studies performed by (Ibrahim *et al*., 2018; El-Sheshtawy *et al*., 2019 and Berbesh *et al*., 2022). However, in the present study, all determined values for liver and kidneys functions test were within the typical range despite a modest drop-in liver enzyme activity, indicating good liver function according to Abdel-Daim *et al* (2015) and AbdEl-Hack *et al*., (2024) who reported that the *spirulina* supplementation may have a hepato-protective impact by reducing ALT and AST enzymes activities of laying hens. This may help regulate increased enzyme activity and improve liver health in general. Therefore, dietary inclusion with microalgae *(Nannochloropsis oculata*) and synthetic zeolite at level 2% had a hepatocyte protective and nephroprotective effects against Pb and Cd induced renal injury in our experiment evidenced by restoration of hepatic enzymes and renal functions protected against oxidative stress and renal dysfunction.

CONCLUSION

Obtained results indicate that the application of biological resources (Microalgae) for mitigating any adverse effect of heavy metal, those microalgae as bio-sorption technique showed have many advantages compared to zeolite such as low cost, availability, profitability, ease of operation, and high efficiency, especially when dealing with low concentrations. In addition, the direct effect of microalgae for removal of heavy metals from drinking water, microalgae successfully used as a feed ingredient and are sustainable source of bioactive compounds, such as essential amino acids, polyunsaturated fatty acids, and antioxidant compounds, that have been documented to have more beneficial effects on nutrition and health of animal, poultry and human.

REFERENCES

Abd El-Hack Mohamed E., KamlahA.Majrashi, KhloudG.Fakih, MohamedRoshd, MahmoudKamal, RashaM.Saleh, AsmaaF.Khafaga, SarahI.Othman, HassanA.Rudayni, AhmedA.Allam , Mahmoud Moustafa, Guillermo Tellez-Isaias, and Mahmoud Alagawany (2024) Effects of varying dietary microalgae levels on performance, egg quality, fertility, and blood biochemical parameters of laying Japanese quails (Coturnix coturnix Japonica) Poultry Science 103:1-9.

Abd El-khalek Enass; Abaza, I. M and Asmaa, A. Saad (2019) Effect of dietary supplementation of spirulina platensis and organic selenium on some biochemical traits of local laying hen under heat stress conditions. Egyptian Poultry Science Journal Vol. (39)(I): (207-217).

Abdel-Daim, M.M., S.M.Abuzead, and S.M.Halawa (2015) Protective role of *Spirulina platensis* against acute deltamethrin-induced toxicity in rats.Plosone (9):72991.

Abdulkarim, A. (2021) The Effect of Natural Zeolite as Feed Additive on Performance and Egg Quality in Old Laying Hens. Journal of Poultry Research, 18(1): 13-18.

Abouelezz, F.M.K. (2017) Evaluation of spirulina algae (Spirulinaplatensis) as a feed supplement for Japanese quail: nutritional effects on growth performance, egg production, eggquality, blood metabolites, sperm-egg penetration and fertility. Egypt. Poult. Sci. J.3:707–719.

Alharthi, A.S., A.R. AlSulaiman, R.S. Aljumaah, A.A. Alabdullatif, G. Ferronato, A.H. Alqhtani, and A.M. Abudabos (2022) The efficacy of bentonite and zeolite in reducing aflatoxinB1 toxicity on production performance and intestinal and hepatic health of broiler chickens. Ital.J.Anim.Sci.21:1181–1189.

Aljohani ASM (2023) Heavy metal toxicity in poultry: a comprehensive review. Front. Vet. Sci. 10:1-12.

AL-musawy Sarah Ahmed Abd-alzahra, Mayada Fadil Mohammed, Mohammed Khadim Thamer and Ahmed Abd-alzahra AL-musawy (2023) Effect of adding Zeolite powder in broiler diet on performance, some physiological and biochemical traits. Acta Biomed 2023; Vol. 94, N. (2):1-8.

APHA (2005) Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC Vahdatpour,T., and H.Nikpiran (2011).Effects of Protexin ; Fermacto and combination of them on blood enzymes and performance of Japanese quails (Coturnix japonica). Ann.Biol.Res.2:283–291.

Becker E.W. (2007) Micro-algae as a source of protein. Biotechnol. Adv. (25):207–210.

Berbesh Sara A., Ragab M. El-Shawarby, Elham A. El-Shewy , Sahar M. El-Sheshtawy and Said M. Elshafae (2022) Ameliorative Effect of Spirulina Platensis against Cadmium Toxicity in Broiler Chickens. Benha Veterinary Medical Journal (42): 51-55.

Berto, D.A., E.A.Garcia, K.Pelicia, F.Vercese, A.D.B.Molino, A.F.daSilveira, and E.S.F.Murakami. (2013) Effects of dietary clinoptilolite and calcium levels on the performance and egg quality of commercial layers. Braz. J. Poult. Sci.15:263–268.

Bilal T, Kaygisiz F, Eseceli H and Altiner A. (2009) Effect of vitamin D3 and/or zeolite supplementation to laying hen rations added microbial phytase on performance and enterprise income. J Anim Vet Adv. 8(5): 1013-1020.

Borowska, S.; Brzoska, M.M. and Tomczyk, M. (2018) Complexation of Bioelements and Toxic Metals by Polyphenolic Compounds— Implications for Health. Curr. Drug Targets (19), 1612–1638.

Caetano, P.A.; do Nascimento, T.C.; Fernandes, A.S.; Nass, P.P.; Vieira, K.R.; Maróstica, J.M.R.; Jacob-Lopes, E. and Zepka, L.Q. (2022) Microalgae-based polysaccharides: Insights on production, applications, analysis, and future challenges. Biocatal. Agric. Biotechnol. 2022, 45, 102491.

Ceramella, J.; De Maio, A.C.; Basile, G.; Facente, A.; Scali, E.; Andreu, I.; Sinicropi, M.S.; Iacopetta, D. and Catalano, A. (2024) Phytochemicals Involved in Mitigating Silent Toxicity Induced by Heavy Metals. Foods, 13, 978.

Costa, M.M.; Spinola, M.P.; Prates and J.A.M. (2024) Microalgae as an Alternative Mineral Source in Poultry Nutrition. Vet. Sci., (11), (44):1-14.

Domo´n, A.; Kowalska, B.; Papciak, D.; Wojta´s, E. and Kami ´nska, I. (2024) Safety of Tap Water in Terms of Changes in Physical, Chemical, and Biological Stability. Water, (16):1-15.

Duncan, D. B. (1955) Multiple ranges and multiple Ftestes. Biometrics 11: 1-42.

Ebrahimi, R.; Ebrahimi, M. and Shakeri, M. (2023) Mitigating the Adverse Effects of Lead and Cadmium Heavy Metals-Induced Oxidative Stress by Phytogenic Compounds in Poultry. Poultry (2): 235–251.

El-Amier, Y.A.; Kotb, W.K.; Bonanomi, G.; Fakhry, H.; Marraiki, N.A. and Abd-El Gawad, A.M. (2021) Hydrochemical Assessment of the Irrigation Water Quality of the El-Salam Canal, Egypt. Water 2021, (13):1-18.

Elsherbeni Ahmed, Islam M. Youssef, Reda E. Hamouda, Mahmoud Kamal, Gaafar M. El-Gendi, Osama H. El-Garhi, Haifa E. Alfassam , Hassan A. Rudayni, AhmedA.Allam , Mahmoud Moustafa , Mohammed O. Alshaharn, and Mohamed S. ElKholy (2024) Performance and economic efficiency of laying hens in response to adding zeolite to feed and litter. Poultry Science 103:1-9.

Elsherbeni, A. I. ; EL-Gendi, G. M. ; El-Ggarhy , O. H. and Okasha, H.M (2022) Effect of Zeolite Addition to Diets and Litter on Carcass Traits and Blood Biochemistry Parameters of Laying Hens (Silver Montazah). Annals of Agric. Sci., Moshtohor ISSN 1110-0419 Vol. 60(4), 1051 – 1062.

El-Sheshtawy, S.M., Al-Shap, N.F., Nada, M., Shawky, N.A. (2019) Case history; Acute toxicity of cadmium in quail and treatment with N-Acetyl cysteine. Animal Health Research Journal (7): 182-199.

Fields, F.J. (2020) Biofortification of Microalgae for Nutritional Enhancement: A Review. Algal Res. 45, 101748.

Guillard, R.R.L. and Ryther, J.H. (1962) Studies on Marine Planktonic Diatoms I. Cyclotella nana Hustedt and Detonula confervacea (Cleve) Gran. Canadian Journal of Microbiology, 8, 229-239.

Hanusova, E., M. OravcoveA, €A.Chrastinove A, M.Rajske A, M.Chrenkove A, and A.Hanus (2021) The effect of natural zeolite dietary supplementation on the egg quality of Japanese quails (CoturnixJaponica) during the laying period. Microbiol.Biotech-nol. FoodSci.10:4631.

Hassan NH, Mehanna S, Hussien AM, Ibrahim MA and Hassanen EI. (2023) The potential mechanism underlying the hepatorenal toxicity induced by hymexazol in rats and the role of NF-κB signaling pathway. J Biochem. Mol. Toxicol. e23304.

Ibrahim, M.A., Almaeen, A.H., Abd El Moneim, M., Tammam, H.G., Khalifa, A.M. and Nasibe , M.N. (2018) Cadmium-induced hematological, renal, and hepatic toxicity: the amelioration by Spirulina platensis. The Saudi Journal of Forensic Medicine and Sciences 1(1), 5-13.

Jarosz L, Stepien-Pysniak D and Gradzki Z (2017) The effect of feed supplementation with Zakarpacki zeolite (clinoptilolite) on percentages of T and B lymphocytes and cytokine concentrations in poultry. Poultry Science 2017; 96:2091-7.

Juzaitis-Boelter, C.P., A.P.Benson, M.U.Ahammad, M.K.Jones, J.Ferrel, and A.J.Davis. (2021). Dietary inclusion of AZOMITE improves feed efficiency in broilers and egg production in laying and broiler breeder hens.Poult.Sci.100:101144.

Khambualai,O., J.Ruttanavut, M.Kitabatake, H.Goto, T.Erikawa, and K.Yamauchi.(2009).Effects of dietary natural zeolite including plant extract on growth performance and intestinal histology in Aigamo ducks. Brit.Poult.Sci.50:123–130.

Kim, C.H., H.K. Kang (2015) Effect of dietary supplementation with a Chorella by-product on the performance, immune response and metabolic function in laying hens. Europ. Poult. Sci. 79.108.

Koracevic, D.; G. Koracevic; V. Djordjevic; S. Andrejevic and V. Cosic (2001) Method for the measurement of antioxidant activity in human fluids. J Clin Pathol l54(5):356–361.

Leong, Y.K. and Chang, J.-S. (2020) Bioremediation of Heavy Metals Using Microalgae: Recent Advances and Mechanisms. Bioresour. Technol. 303.

Mallamaci, R.; Storelli, M.M.; Barbarossa, A.; Messina, G.; Valenzano, A. and Meleleo, D. (2023). Potential Protective Effects of Spirulina (Spirulina platensis) against In Vitro Toxicity Induced by Heavy Metals (Cadmium, Mercury, and Lead) on SH-SY5Y Neuroblastoma Cells. Int. J. Mol. Sci., 24, 17076

Mastinu A, Kumar A and Maccarinelli G (2019) Zeolite clinoptilolite: therapeutic Virtues of ancient mineral. Molecules 2019; 24:1517-1530.

Mateo R, Vallverdú-Coll N, López-Antia A, Taggart MA, Martínez-Haro M and Guitart R, (2014) Reducing Pb poisoning in birds and Pb exposure in game meat consumers: the dual benefit of effective Pb shot regulation. Environ Int. 63:163–168.

Mens A. J. W., M. M. van Krimpen,, S. K. Kar , F. J. Guiscafre , and L. Sijtsma (2022) Enriching table eggs with n-3 polyunsaturated fatty acids through dietary supplementation with the phototrophically grown green algae Nannochloropsis limnetica: effects of microalgae on nutrient retention, performance, egg characteristics and health parameters. Poultry Science 101:1-14.

Mustapha MU and Halimoon N (2015) Microorganisms and biosorption of heavy metals in the environment: a review paper. J Microb Biochem Technol 7:253–256.

N.R.C. (2014) Subcom, National Research Council (U.S.). Subcom, Nutrient Requirements of Poultry, Nabu Press.

Nys Y (1999) Nutritional factors affecting eggshell quality. Czech J Anim Sci 44: 135-143.

Oguz, H., (2011) A review from experimental trials on detoxification of aflatoxin in poultry feed. Eurasian. J. Vet. Sci. 27: 1–12.

Panaite, T.D.; Cornescu, G.M.; Predescu, N.C.; Cismileanu, A.; Turcu, R.P.; Saracila, M. and Soica, C. (2023) Microalgae (Chlorella vulgaris and Spirulina platensis) as a Protein Alternative and Their Effects on Productive Performances, Blood Parameters, Protein Digestibility, and Nutritional Value of Laying Hens' Egg. Appl. Sci. (13):1-21.

Park, J.H., S.D. Upadhaya, I.H. Kim, (2015) Effect of dietary marine microalgae (schizochytrium) powder on egg production, blood lipid profiles, egg quality, and fatty acid composition of egg yolk in layers. Asian-Australasian. J. Anim. Sci. 28, 391-397.

Pavelic KS, Medica JS and Gumbarevic D (2018) Critical Review on Zeolite Clinoptilolite Safety and Medical Applications in vivo. Frontiers in Pharmacology 2018; 9:1350-1365.

Pavlak, M.S., C. Kaufmann, C. Eyng, P.L. Carvalho, P. Pozza, F.M. Vieites, and R.V. Nunes. (2023) Zeolite and corn with different compositions in broiler chickens feeding. Poult.Sci.102:102494.

Prasai,T.P., K.B.Walsh, S.P.Bhattarai, D.J.Midmore, T.T.Van, R.J.Moore, and D.Stanley.(2016) Biochar, bentonite and zeolite supplemented feeding of layer chickens alters intestinal microbiota and reduce scampylobacter load.PLoSOne11:e0154061.

Rahman MM, Islam MS, Hasan R, Saha P and Alam MS. (2023) Spirulina supplementation to alleviate negative effects of lead in layer chicken. J. Adv.Vet.Anim.Res. 2023; 10(4):794–800.

SAS, (2004). SAS User's Guide: Statistics. Release 9.1. SAS Institute Incorporated., Cary, North Carolina.

Selim,S., E.Hussein, and R.Abou-Elkhair. (2018) Effect of Spirulina platensis as a feed additive on laying performance, egg quality and hepato-protective activity of laying hens. Europ.Poult.Sci.82:1–13.

Shanmugapriya, B. and Saravana Babu, S. (2014) Supplementary effect of Spirulina platensis on performance, hematology and carcass yield of broiler chicken. Indian Streams Research Journal. 4, 1-7.

Sharvadze, R, S. Sukhanova, and K. Babukhadia (2022) Influence of zeolites of different depositson egg production of chickens. Pages 70‒78 in: International Scientific Conferenceon Agricultural Machinery Industry "Inter-agro-mash". Springer International Publishing Cham.

Shkinev VM, Gomolitskii VN, Spivakov BY, Geckeler KE and BayerE. (1989) Determination of trace heavy metals in waters by atomic-absorption spectrometry after pre concentration by liquid-phase polymer-based retention. Talanta. 36(8):861–863.

Singh AD, Khanna K, Kour J, Dhiman S, Bhardwaj T and Devi K (2023) Critical review on biogeochemical dynamics of mercury (hg) and its abatement strategies. Chemosphere. (319).

Sorrenti, V.; Castagna, D.A.; Fortinguerra, S.; Buriani, A.; Scapagnini, G. and Willcox, D.C. (2021) Spirulina Microalgae and Brain Health: A Scoping Review of Experimental and Clinical Evidence. Mar. Drugs 2021, 19, 293. [CrossRef] [PubMed] Int. J. Mol. Sci. 2023, 24, 17076 12 of 13

Stunda-Zujeva, A.; Berele, M.; Lece, A.; Šķesters, (2023) A Comparison of antioxidant activity in various spirulina containing products and factors affecting it. Sci. Rep., (13): 4529.

Uguz, S. and Sozcu, A. (2024) Pollutant Gases to Algal Animal Feed: Impacts of Poultry House Exhaust Air on Amino Acid Profile of Algae. Animals. 14, 754.

Vahdatpour,T. and H.Nikpiran.(2011)Effects of Protexin Fermacto and combination of them on blood enzymes and performance of Japanese quails (Coturnixjaponica).Ann.Biol.Res.2:283–291.

Vasiljevic, M.,D.Marinkovic, D.Milicevic, J.Pleadin, S.Stefanovic, S.Trialovic and J.N.Trialovic (2021) Efficacy of a modified Clioptilolite based adsorbent in reducing detrimental effects of Ochratox in a inlaying hens.Toxins13:469.

Verma N, Rachamalla M, Kumar PS and Dua K. (2023) Assessment and impact of metal toxicity on wildlife and human health. Metals Water :93–110.

Vieira RB, Ludke MCMM, Ludke JV, Lopes CC, Oliveira EF, Santos ACA, Coldebella A and Rabello CBV (2023) Effect of Dietary Zeolite Supplementation on Production, Egg Quality, Ammonia Volatilization, Organ Morphometry and Blood Parameters in Brown Laying Hens. Brazilian Journal of Poultry Science Vol.25No.4:1-10.

WHO. (2011) Guidelines for Drinking Water Quality, 4th ed.; World Health Organization: Geneva, Switzerland; Volume 38, pp.104–108.

Yoruk M.A., Gul M., Hayirli A., Macit M. (2004): The effects of supplementation of humate and probiotic on egg production and quality parameters during the late laying period in hens. Poultry Science, 83, 84–88.

Yuan C. , H. H. Song , Y. J. Jiang , M. M. M. Azzam , S. Zhu , and X. T. Zou (2013) Effects of lead contamination in feed on laying performance, lead retention of organs and eggs, protein metabolism, and hormone levels of laying hens J. Appl. Poult. Res. 22 :878–884.

Zhou, Y.M., (2008) Mechanism of nutritional effect and harmful agent reduction of natural zeolite in broilers. PhD Thesis, Nanjing Agricultural University, China

تقييم كل من الزيولايت الإصطناعي وطحلب النانوكلوروبسيس أوكيولاتا كإضافات إدمصاصية للعناصر الثقيلة على الإنتاجية وأدلة جودة البيض وبعض صفات الدم الكيميائية للدجاج البياض الذي يشرب مياه ترعة السلام بشمال سيناء – مصر

صفاء علي مصطفى

قسم فسيولوجيا الحيوان والدواجن – شُعبة الإنتاج الحيواني والدواجن – مركز بحوث الصحراء – مصر

أجريت هذه الدراسة بهدف تقييم الأداء الإنتاجي والتناسُلي للدجاج البياض الذي يشرب من مياه ترعة السلام ويتغذى على العلائق المُدعمة بنوعين من الإضافات الإدمصاصية للعناصر الثقيلة (مسحوق طُحلب النانوكلوروبسيس أوكيولاتا وبودرة الزيولايت الإصطناعي بمُعدل 2% لكُلٍ منهُما) حيث تم إخضاع عدد 160 دجاجة من سُلالة اللوهمان البُني التُجاري عند عُمر 62 أسبوع وحتى عُمر 74 أسبوع وتوزيعهم عشوائياً إلى أربعة مجموعات تجريبية متساوية العدد كل منها فى 4 مكررات وكانت المجموعات التجريبية كالتالي:

المجموعة الأولى: وتُمثل مجموعة المُقارنة – تشرب الطيور ماء الصنبور وتتغذى على عليقة البياض الخالية من أي إضافات(TW) .

المجموعة الثانية: وتُمثل مجموعة المُعاملة الأولى – تشرب الطيور ماء ترعة السلام وتتغذى على عليقة البياض الخالية من أي إضافات (SCW).

المجموعة الثالثة: وتُمثل مجموعة المُعاملة الثانية – تشرب الطيور ماء ترعة السلام وتتغذى على عليقة البياض المُدعّمة بمسحوق طُحلب النانوكلوروبسيس أوكيولاتا بمُعدل 2 كجم/ 100 كجم عليقة (2%) (SCW+2%NC)

المجموعة الرابعة: وتُمثل مجموعة المُعاملة الثالثة – تشرب الطيور ماء ترعة السلام وتتغذى على عليقة البياض المُدعّمة ببودرة الزيولايت الإصطناعي بمُعدل 2 كجم/ 100 كجم عليقة (2%) .(SCW+2%SZ)

وكانت أهم النتائج كالتالي: أظهرت مجموعتي الطيور (SCW+2%SZ & SCW+2%NC) تحسُناً ملموساً إحصائياً (P<0.05) في الوزن النهائي للجسم و أفضل مُعدل تحويل غذائي و أعلى وزن للبيضة و تحسُناً ملموساً في وظائف الكبد والكلية وتركيز مُضادات الأكسدة الكُلية T-AOC مُقارنة بمجموعة الطيور (SCW) مما يُشير إلى إمتلاك كُلٍ من مسحوق طُحلب النانوكلوروبسيس أوكيولاتا وبودرة الزيولايت الإصطناعي الآلية الفسيولوجية لإدمصاص العناصر الثقيلة وبالتالي منع حدوث إجهاد تأكسُدي والحماية من إمكانية إنتاج الشوارد الحُرة .