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Biochemical Blood, Immunological Parameters and Bacterial Counts of Local Developed Laying Hens

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Abstract

This study was conducted to investigate the effects of dietary zeolite and synbiotic supplementation on egg production traits, Intestinal bacteriology and blood constituents of Mandarah (Egyptian local developed strain) laying hens. A total number of 270 laying hens + 27 cock, 24 weeks old were randomly taken to be similar in body weights (1381.30±1.27), which were randomly divided into nine experimental groups, (30 hens + 3 cocks in each). Each group was contained three replicates (10 hens+1 cock in each). The experimental groups designed a factorial arrangement (3x3), 3 zeolite level groups (0, 1, 2 %); 3 levels symbiotic (0, 1, 2 %), respectively, during the experimental periods lasted six months from 24 to 48 weeks of age**.** The obtained results showed that supplementing with 2 % zeolite improved significantly (P<0.01) in final body weight (FBW); body weight change (BWC, %); total egg number (TEN); egg production rate (EP, %); daily egg mass (DEM, g) and feed conversion ratio (FCR) as kg feed /eggs when compared to hens in receiving 1% zeolite and control group, during period 24 at 48 weeks of age. Addition of zeolite to laying hens' diets at levels 2% improved of IgG, IgM, T-AOC and neutrophil (Nut) values as compared to control group. Concerning effect of 2% synbiotic supplementation in layer diets caused to increase significantly (P<0.01) in FBW, BWC, TEN, EP, DEM and FFCR (kg feed/ eggs) as compared to 1% supplementation with synbiotic and control group. Hens received synbiotic at level 2% could be improved (P<0.01) significantly of IgG, IgM, T-AOC and bacterial count. The interaction effects between zeolite and synbiotic supplementation were significant (P<0.01) in TFI trait, while the other traits at productive performanc e were not significant. Supplementation of 2 % zeolite and 2 % synbiotic caused to improve significantly (P<0.01) IgM and lactic acid bacterial counts when compared to other treatments groups. Conclusively, it can be concluded that, supplemental layer diets with zeolite or synbiotic at level 2% were more effective for improving productive performance traits, biochemical, immunological blood parameters and bacterial count of Mandarah laying hens.

Keywords: Zeolite, Synbiotic, Productive performance, Biochemical & Hematological Parameters, Immunology

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1. Introduction

Poultry production plays a major role in providing a large and cheap source of animal protein in Egypt, beside pure Egyptian breeds there were some local developed strains that established for both meat and egg production. In 1966 cross breeding was produced between Fayoumi x Barred Plymouth Rock to give Dokki-4 local strain [1]**,** while Mandarah as local strain was produced from a crossing between Alexandria x Dokki-4 strains [2]. In recent years, there are evidences in the literature that using of zeolite (Clinoptilolite) has encouraging effects on the poultry performance traits such as BW, BWC, EW, EM, TFI and FC [3,4,5]. [6] reported that zeolite-natural and modified, because of their specific structure, are excellent adsorbed and thus can diminish the harmful effect of heavy metals. The same authors found that clinoptilolites, due to its structural stability under high temperatures and acidity, are the most widely used zeolite in animal studies. The important research data indicated the positive influence of the feed inclusion of clinoptilolite on poultry health. Zeolite is an excellent "trapper" of waste products and heavy metals because of its chemical composition and specific lattice structure**.** [7] reported that, these minerals are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations (Na, K and / or Ca caution). Zeolite is porous material, able to adsorb molecules of appropriate cross-sectional diameter and to exchange their constituent captions without major change of their structure. Thus, zeolite appears to posse two important properties: adsorption and ion-exchange. The exploitation of these properties underlies the use of zeolite in a wide range of agricultural applications and particularly in poultry nutrition**.** Synbiotics (prebiotic and probiotic) are defined as beneficially affects the host by activating the metabolism and survival of one or a limited number of health promoting bacteria and/or by selectively stimulating their growth in ways that can improve the host's welfare [8, 9, 10, 11 and 12]. The same authors added the dietary supplementation with synbiotic had a significant (P<0.05) increase on live body weight, weight gain and improve feed intake, feed conversion ratio, egg production, egg weight and egg mass of laying hens as compared to those of control group. Moreover, [13] demonstrated that chickens fed with synbiotic had an ability to improve intestinal colonization via decrease *E. coli* and total aerobic bacteria count in the ileum than in the control group. Therefore, the aim of this study to evaluate supplementation of zeolite and synbiotic in the diet on productive performance traits and biochemical, immunological blood parameters and bacterial counts of Egyptian local of developed laying hens (Mandarah strain).

2. Materials and Methods

2.1. Birds, management and experimental design

The present study was carried out at Inshas Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. A total number of 270 Mandarah laying hens+ 27 cocks, 24 weeks old was randomly taken to be similar in body weights (1381.30±1.27). Birds were randomly divided into nine treatment groups (30 hens + 3 cocks in each) and then each treatment group was divided into three replicates (10 hens+ 1 cock / replicate). The experimental groups designed a factorial arrangement (3 x 3), 3 zeolite levels (0.0, 1.0 and 2.0 %) and 3 levels synbiotic 0.0, 1.0, 2.0 %). respectively, during the experimental period from 24 to 48 weeks of age. Birds were fed a balanced basal diet, during the experimental period lasted six months from 24 to 48 weeks of age. All birds were housed individually in layer's pens and maintaining in similar managerial and conditions environment with a photoperiod length of 17 h daily. Feed and water were provided ad libitum throughout, the experimental period (24-48 weeks of age). Experimental diets were formulated to be iso-nitrogenous and iso- caloric to cover the nutrients requirements as recommended by [14] and Agriculture Ministry Decrees, [15] as shown in Table 1. Chemical analyses of basal diet and zeolite as show in an Tables 1 and 2, respectively was determined in the Central Laboratory For Soil, Foods and Feedstuffs (International Accredited Lab, has ISO 17025 since 2012), Faculty of Technology and Development, Zagazig University, Zagazig, Egypt.

2.2. Zeolite and Synbiotic products

Zeolite was product (Manufactured by Mec Enerji, Turkey) importer from Al –Zahraa Vetmedical for Veterinary products and feed additives. Synbiotic was used in this experiment (Poultry Star® me) is an International Product of Biomin Singapore Pte, Ltd, Biomin GmbH, Austria. It was purchased from an Egyptian Veterinary Medicinal dealer Company. According to the Biomin Company, each one gram of the used symbiotic contains 0.9 g Fructo- oligosaccharides (pure soluble inulin, chicory) and 0.1 g blend of probiotic bacteria (Enterococcus faecium (3 x 10^9 CFU/g), Bifidobacterium animalis (5 x 10^8 colonies forming unit per gram, CFU/g), Pediococcus acidilactici (1.3 x 10^9 CFU/g), Lactobacillus reuteri (1 x 10^8 CFU/g) and Lactobacillus salivarius (1 x 10^8 CFU/g)].

2.3. Measurements studied

Body weight (BW), change body weight (BWC) (%), daily and total egg number, egg weight (g) were recorded individually of each group, while daily and total feed intake were recorded weekly in each replicate, during the experimental periods (24 - 48 weeks of age). Egg production rate (%) was calculated at four weeks intervals, during the production periods as egg number/hen/period x100 for each replicate and calculated the average of the whole experimental period (24-48 weeks of age). Egg mass was calculated by multiplying egg number X average egg weight. Feed conversion (Kg feed/ eggs) was calculated as Kg feed consumption produced number of eggs at four weeks intervals and the whole experimental period.

2.4. Blood samples assay

At the end of experiment (48 weeks of age), blood samples were collected from wing vein from three hens in each treatment into two tubes anticoagulant of Ethylene diamine tetra acetic acid (EDTA) treated and non-EDTA tubes). Samples treated with anticoagulant EDTA used for determination of hematology parameter such as red blood cells (RBC's), white blood cells (WBC), hemoglobin (Hb), platelet count (PLT), lymphoid (LYM), neutrophils (NUT) and Hematocrit values (HCT) by the coulter (HA-VET, Clinging, Belgium). Plasma was collected by centrifugation for 15 minutes at 3000 rpm and it stored at – 20 °C until determination of blood metabolites in each at (total protein (TP), albumin (Alb.), glucose, creatinine. Total antioxidant capacity (TAOC), neutrophil (NUTI) were determined according to method described by [17]. Concentrations of immunoglobulin's (IgG and IgM) were determined according to [18]. Globulin (Glob) was calculated by the difference between TP and Alb value. Other samples (non-EDTA tubes) used to collected serum by centrifugation for 15 minutes at 3000 rpm and it stored at –20°C until determination blood minerals in each of calcium(Ca.) and phosphorus(Ph.) by calorimetrically using commercial kits were determined in the Accredited Medical Lab.

2.5. Intestinal bacterial counts studies

At the end of experiment, the same three birds slaughtered were chosen for intestinal bacterial count studies. All viscera were carefully removed by hand from the carcass under sterile conditions, and one gram of the intestinal content from the ileo-cecal junction portion was transferred to a sterile test tube containing nine ml of 1% sterile peptone water (first dilution 10-1) and vortexed for 1 min to homogenize. The homogenate was diluted serially from 10-1 dilution to 10-8. For each dilution 0.1 ml was plated onto sterile selective medium agar for enumeration of the tested bacteria groups. MRS (de man, rogosa and sharpe) agar (Oxoid, Uk) for enumerating total aerobic count and lactic acid bacteria, brilliant green agar (Fischer scientific, USA) for enumerating Salmonella ssp., Violet red bile glucose agar (Sigma-Aldrich, UK) for enumerating Escherichia coli. After preparing the media according to manual descriptions, it was poured in Petri dishes previously sterilized at 180 °C for 3 hours, and left to hardening at room temperature (28 ± 2°C). Then 0.1 ml of each dilution was planted (duplicate) for each microbial group and left to dry. The dishes were then incubated at 37 °C for 24 hours for Salmonella (Pink or colorless colonies with a red halo), 72 hours for E. coli (purple – pink م) and 48 hours for Lab in anaerobic jar with GAS Pack (Oxoid, UK), The number of colonies were then counted to determine the colony forming units (CFU). CFU per gram of fresh caecal content were then expressed on logarithms [19].

2.6. Statistical analysis

The experiment data were statistically examined by analysis of variance according to [20] using ANOVA procedures of SAS [21]. The statistical model was used as follows:

$$\mathbf{Y_{ijk} = \mu + Z_i + S_j + (ZS)i_j + e_{ijk}}$$

Where, Y_{ij}: An Observation; µ: Overall mean, Zi: Effect of the feed additives Zeolites groups (i=1,2, and 3); S_j: Synbiotic supplementation (j=1,2and 3); (ZS)ij: Interaction effect (ij=1,2…+9), e_{ijk}: Random error. The differences among means were tested by using Duncan᾿s multiple range test procedures [22]. The percentage values were subjected to be arcsine transformation before performing the analysis of variance. Means were presented after recalculated from the transformed value to percentages.

3. Results and Discussion

3.1. Productive performance traits

The effect of dietary zeolite or synbiotic supplementation and their interaction on productive performance traits of laying hens for the whole experimental period (24-48 weeks of age) are shown in Table 3. Supplementing with 2% zeolite was significantly (P<0.01) caused to improve in final body weight (FBW); body weight change (BWC, %); total egg number (TEN); egg production (EP, %); daily egg mass (DEM, g) and feed conversion ratio (FCR); kg feed /eggs and Egg production rate as compared to hens in receiving 1% zeolite and control groups. Similar results were obtained by [23, 24 and 25] who found that positive significant effects of dietary zeolite were noticed on the number of eggs laid per hen, egg weight, and efficiency of feed utilization. Addition of natural zeolite to broiler diet led to promote of chicken performance [24] and improve body weight gain and feed conversion ratio [27]. At present, use of natural zeolite develops by utilizing features of ion-exchange, water and gas absorption [28]. The exploitation of these properties underlies the use of zeolite in a wide range of industrial and agricultural applications and particularly in animal nutrition [29]. Concerning effect of 2% synbiotic supplementation in layer diets improved significantly (P<0.01) in FBW, BWC, TEN, EP, DEM and FCR (kg feed/eggs) as compared to 1% Synbiotic supplementation and control group during period 24-48 weeks of age (Table 3). The same results were obtained by [30] showed that synbiotic of the starter diets and 0.5 kg/ton of the grower diets) increased significantly (P<0.05) the FBW, DWC and FCR of broiler chicks as compared with the control group. Regarding the interaction, it could be shown that total feed intake was significantly (P<0.01) influenced by supplementation with zeolite and synbiotic, while the other traits of egg production with not significant, during 24-48 weeks of age as shown in Table 3.

3.2. Hematological parameters of blood

Data in Table 4 show zeolite or synbiotic and their interaction effect on blood hematology parameters. Insignificantly effects on most blood hematology parameters, except of white blood cells (WBC) and platelet counts (PLT), with in the normal physiological range for healthy hens were significantly (P≤0.01) affected by zeolite supplementation (Table 4). Concerning effect of synbiotic, at level 2% supplementation in layer diets increased significantly (P≤0.01) in red blood cells (RBC) and platelet counts (PLT) and decreased WBC as compared with the other groups. [31] Indicated that synbiotics supplementation did not effect on hemoglobin, except the packed cell volume, which was increased in the additive treatments with restriction at the end of the experiment. Regarding the interaction effects between zeolite and synbiotic supplementation were not significant on all hematological blood parameters (Table 4).

3.3. Biochemical blood parameters

Results of zeolite or synbiotic and their interaction on fraction and function liver, kidney function and blood minerals, it could be seen in Table (5). There were insignificant differences in blood liver of fraction and function, kidney function and blood minerals, except GPT and Uric acid (with in the normal range), which were significantly (P≤0.01) decreased affected by zeolite diet at levels 1 or 2% as compared with control group. Zeolite (Clinoptiolite) diet might be reduced lipid peroxidation and normalized the liver functions in hens drinking saline water and/or may be safe supplements even though more Biolological histological studies were needed to prove it [32, 33, 34 and 5]. Concerning effect of synbiotic supplementation at level 2% in layer diets decreased significantly (P≤0.01) in GOT and GPT values when compared with control group (Table 5). It could be noticed that Creatinine and Uric acid values as kidney function and blood minerals were insignificantly affected by synbiotic supplementation of laying hens. Synbiotic supplementation at different levels was positive effect on the plasma total protein and globulin may be belonged to the immune stimulant effect of these feed additives in poultry [35]. These results were in concord with, [36] who observed that feeding broiler chickens on a prebiotic supplemented diet, increased serum total protein and globulin. Similarly, [37] revealed that the prebiotic inclusion in the quail's diet caused to increase significant (P<0.05) in the concentration of total plasma protein and total globulin. On the other hand, these results were in contrast to those of [38, 39], where they revealed that the synbiotic had no significant effect on blood total protein, albumin, globulin and albumin / globulin ratio in chickens.

Table 1. Composition and chemical analysis of the basal diet

Ingredients	**(%)**
Yellow corn	63.15
Soybean meal (44%)	23.29
Corn gluten meal (60%)	3.02
Mono calcium phosphate	1.39
Lime stone	8.40
NaCl	0.40
Vitamins and minerals mixture*	0.30
DL-methionine	0.05
Total	**100.00**
Chemical analysis calculated **	
Crude protein (%)	17.00
Crude fiber (CF)	3.09
Available phosphorus (%)	0.42
Calcium (%)	3.41
Lysine (%)	0.868
Methionine (%)	0.377
Methionine + Cystine (%)	0.666
Metabolizable energy (Kcal ME/kg diet)	2748
Chemical analysis determined***	
Dry matter, %	90.73
Crude protein, %	16.97
Ether extract, %	2.45
Crude fiber, %	3.96
Ash, %	6.37
Nitrogen free extract, %	60.98

*Each 3 kg of Vitamins and Minerals mixture * contains: Vit. A 10000,000 IU; Vit.D3 2000,000 IU; Vit. E 10,000 mg; Vit.K3 1000 mg; Vit.B1 1000 mg; Vit.B2 5000 mg; Vit.B6 1500 mg; Vit. B12 10 mg; Pantothenic acid 10,000 mg; Niacin 30,000 mg; Folic acid 1000 mg; Biotin 50 mg; Choline 250,000 mg; Manganese 60,000 mg; Copper 4,000 mg; Iron 30,000mg; Iodine 300 mg; Cobalt 100 mg; $CaCO_3$ to 3,000gm.
**According to [14] and [15].
*** According to [15].

Table 2. Composition and chemical analysis of zeolite products.

Chemical analyses of zeolite*		**Composition: Each 1 kg zeolite contains (%) ****	
P	0.002 %	**Sio2**	69.60
K	0.29 %	**Al2o3**	12.70
Na	0.44 %	**Fe2o3**	1.40
Ca	1.71 %	**Cao**	2.40
Fe	1523.48 mg/kg	**Mgo**	1.00
Mn	81.82 mg/kg	**Na2o**	0.30
-	-	**K2o**	4.00
-	-	**Tio2**	0.10
-	-	**P2o5**	0.10
-	-	**Mno**	0.10

* Chemical composition of zeolite according to central lab for soil, food and feedstuff (CLSFF), Faculty of Technology and Development, Zagazig University, Zagazig, Egypt.
** Country of Origin, Turkey.

Table 3. Effect of zeolite and synbiotic *levels* as feed additives on productive performance parameters of laying hens from 24 to 48 weeks of age

Items		Productive performance parameters								
		IBW g	FBW g	BWC %	TEN	EW g	DEM g / day	TFI kg	FC (kg feed/ eggs)	EP %
Effect of zeolite (ZY), %										
0.0		1383.9	1704.4^{c}	23.16^{c}	104.5^{b}	47.49	29.6^{b}	18.97^{a}	5.51^{c}	62.2^{b}
1.0		1379.4	1737.8^{b}	25.98^{b}	107.9^{a}	47.54	30.6^{a}	18.96^{a}	5.69^{b}	64.2^{a}
2.0		1380.6	1766.7^{a}	27.97^{a}	109.5^{a}	47.56	31.1^{a}	18.87^{b}	6.16^{a}	65.2^{a}
SEM		2.13	6.38	0.46	0.51	0.09	0.16	0.07	0.07	0.30
Sig.		NS	**	**	**	NS	**	*	**	**
Effect of synbiotic (SB), %										
0.0		1383.33	1718.89^{b}	24.26^{b}	106.0^{b}	47.4	30.05^{b}	18.96	5.59^{b}	63.10^{b}
1.0		1381.11	1743.89^{a}	26.27^{a}	107.4^{ab}	47.7	30.59^{a}	18.90	5.68^{a}	63.94^{ab}
2.0		1379.44	1746.11^{a}	26.58^{a}	108.5^{a}	47.5	30.77^{a}	18.95	5.72^{a}	64.57^{a}
SEM		2.19	10.17	0.75	0.82	0.09	0.25	0.17	0.07	0.49
Sig. test		NS	**	**	**	NS	**	NS	**	**
Effect of interaction (ZY x SB), %										
0.0	0.0	1385.0	1680.0	21.30	109.2	47.4	29.3	19.22^{a}	5.38	61.6
	1.0	1381.7	1710.0	23.76	108.2	47.8	30.0	18.96^{bc}	5.59	62.5
	2.0	1385.0	1723.3	24.43	108.4	47.3	29.7	18.91^{bc}	5.56	62.6
1.0	0.0	1383.3	1730.0	25.06	108.5	47.5	30.1	18.83^{bc}	5.64	63.2
	1.0	1381.7	1746.7	26.42	108.9	47.6	30.6	19.10^{ab}	5.64	64.2
	2.0	1373.3	1736.7	26.46	109.4	47.5	31.2	18.93^{abc}	5.79	65.3
2.0	0.0	1381.7	1746.7	26.42	109.8	47.5	30.8	18.83^{bc}	5.76	64.5
	1.0	1380.0	1775.0	28.62	110.8	47.6	31.2	18.79^{bc}	5.81	65.1
	2.0	1380.0	1778.3	28.87	110.6	47.5	31.4	18.86^{bc}	5.82	65.9
SEM		3.62	7.47	0.46	0.67	0.16	0.40	0.04	0.07	0.08
Sig. test		NS	NS	NS	NS	NS	NS	*	NS	NS

a,b,c: Means in each classification in the same column with different superscripts, differ significantly (P<0.05). N.S: Not Significant, * P < 0.05, ** P< 0.01. SEM: Mean at standard error. IBW,g=Initial body weight, FBW,g : Final body weight, BWC,% : Body weight change , TEN: Total egg number, EW, g: Egg weight, EP, % : Egg production, DEM / day : Daily egg mass, TFI, kg: Total feed intake, FC: Feed conversion (Kg feed/ eggs).

Table 4. Effect of zeolite and Synbiotic *levels* as feed additives on hematology parameters of laying hens at 48weeks of age

Items		Blood hematology parameters					
		RBC (10^{12}/L)	WBC (10^9/L)	Hb (g/dl)	PLT (10^9/L)	LYM %	HCT %
Effect of zeolite, (ZY), %:							
0.0		2.84	91.11^{a}	10.76	51.21^{b}	78.92	31.20
1.0		2.75	85.87^{b}	10.94	65.14^{a}	79.98	32.10
2.0		2.76	84.76^{b}	11.08	67.71^{a}	80.14	32.54
SEM		**0.05**	**1.10**	**0.15**	**2.16**	**1.12**	**0.51**
Sig. test		**NS**	**	**NS**	**	**NS**	**NS**
Effect of Synbiotic (SB),%:							
0.0		2.69^{b}	89.97^{a}	10.63	55.79^{c}	78.08	31.02
1.0		2.76^{b}	86.99^{b}	11.01	60.82^{b}	80.12	31.98
2.0		2.91^{a}	84.78^{b}	11.13	67.46^{a}	80.84	32.84
SEM		**0.05**	**1.29**	**0.14**	**2.89**	**1.04**	**0.51**
Sig. test		**	**	**NS**	**	**NS**	**NS**
Effect of interaction (ZY x SB), %:							
00.0	**0.0**	2.76	92.27	10.23	47.47	76.63	30.47
	1.0	2.83	91.57	10.97	49.70	79.50	31.23
	2.0	2.94	89.50	11.07	56.47	80.63	31.90
1.0	**0.0**	2.63	89.83	10.70	58.43	78.53	31.33
	1.0	2.70	86.00	11.00	65.77	80.33	32.20
	2.0	2.91	81.77	11.13	71.23	81.07	32.77
2.0	**0.0**	2.68	87.80	10.97	61.47	79.07	31.27
	1.0	2.74	83.40	11.07	67.00	80.53	32.50
	2.0	2.87	83.07	11.20	74.67	80.83	33.87
SEM		**0.08**	**1.40**	**0.23**	**0.35**	**2.51**	**0.81**
Sig. test		**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

a,b,c: Means in each classification in the same column with different superscripts, differ significantly (P<0.05)
N.S: Not Significant, ** P< 0.01, RBC: Red blood cells, WBC: White blood cells, HGB: Hemoglobin, PLT: Platelets count, LYM: Lymphoid, HCT: Hematocrit.

Table 5. Effect of zeolite and synbiotic *levels* as feed additives on biochemical blood parameters (With in normal range) of laying hens at 48 weeks of age

Items		Liver fractions			Liver function		Kidney function		Blood minerals	
		T. Protein (g/dl)	Globulin (g/dl)	Albumin (g/dl)	GOT (IU/L)	GPT (IU/L)	Creatinine (mg/dl)	Uric acid (mg/dl)	Phos. (mg/dl)	Ca (mg/dl)
Effect of zeolite, (ZY), %:										
0.0		6.97	4.20	2.77	5.66	7.98^{a}	0.70	7.03^{a}	7.24	9.97
1.0		6.85	4.25	2.60	5.55	6.79^{ab}	0.64	4.57^{b}	7.26	10.89
2.0		6.84	4.26	2.57	4.54	5.87^{b}	0.59	4.02^{b}	7.27	11.63
SEM		**0.19**	**0.18**	**0.16**	**0.47**	**0.56**	**0.03**	**0.38**	**0.18**	**0.46**
Sig. test		**NS**	**NS**	**NS**	**NS**	**	**NS**	**	**NS**	**NS**
Effect of synbiotic (SB),%:										
0.0		6.86	4.09	2.77	6.35^{a}	8.16^{a}	0.69	5.90	7.10	10.46
1.0		6.89	4.28	2.61	5.04^{b}	6.81^{ab}	0.65	5.05	7.23	11.07
2.0		6.92	4.35	2.57	4.35^{b}	5.67^{b}	0.60	4.67	7.44	10.96
SEM		**0.21**	**0.18**	**0.16**	**0.40**	**0.50**	**0.03**	**0.57**	**0.17**	**0.52**
Sig. test		**NS**	**NS**	**NS**	**	**	**NS**	**NS**	**NS**	**NS**
Effect of interaction (ZY x SB), %:										
0.0	0.0	6.97	4.00	2.97	6.56	10.17	0.80	8.21	7.02	10.19
	1.0	6.96	4.26	2.70	5.48	7.57	0.68	6.64	7.27	9.51
	2.0	6.99	4.34	2.65	4.93	6.20	0.62	6.24	7.44	10.20
1.0	0.0	6.57	3.85	2.71	6.78	7.77	0.65	5.28	7.12	9.87
	1.0	7.01	4.42	2.59	5.47	7.03	0.66	4.52	7.23	10.91
	2.0	6.98	4.48	2.50	4.40	5.57	0.62	3.92	7.42	11.90
2.0	0.0	7.04	4.42	2.62	5.71	6.53	0.62	4.21	7.17	11.31
	1.0	6.69	4.15	2.54	4.17	5.83	0.60	3.99	7.20	12.78
	2.0	6.77	4.21	2.56	3.73	5.23	0.56	3.84	7.45	10.79
SEM		**0.08**	**0.35**	**0.30**	**0.05**	**0.69**	**0.31**	**0.77**	**0.57**	**0.30**
Sig. test		**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

a, b,c: Means in each classification in the same column with different superscripts, differ significantly (P<0.05).
N.S: Not Significant; ** P< 0.01, GOT: Glutamic-Oxaloacetic transaminase; GPT: alanine aminotransfera; Phos: Phosphorus, Ca: Calcuim,

Table 6. Effect of zeolite and synbiotic *levels* as feed additives on Immunological response and antioxidants parameters of laying hens at 48 weeks of age

Items		Immunological response		Antioxidants		MR, %
		IgG (mg/mL)	IgM (mg/mL)	T-AOC (m mol/L)	NUT %	
Effect of zeolite, (ZY), %:						
0.0		117.56^{b}	209.56^{c}	0.70^{b}	9.57	1.11
1.0		126.11^{a}	226.44^{b}	0.86^{a}	10.28	1.11
2.0		131.22^{a}	245.11^{a}	0.90^{a}	10.83	0.00
SEM		**3.46**	**5.05**	**0.03**	**0.45**	**0.74**
Sig. test		**	**	**	NS	NS
Effect of synbiotic (SB),%:						
0.0		115.11^{c}	212.44^{c}	0.73^{b}	9.01^{b}	2.22
1.0		126.11^{b}	228.00^{b}	0.84^{a}	10.52^{a}	0.00
2.0		133.67^{a}	240.67^{a}	0.90^{a}	11.14^{a}	0.00
SEM		**2.92**	**6.00**	**0.04**	**0.38**	**0.49**
Sig. test		**	**	**	**	NS
Effect of interaction (ZY x SB), %:						
0.0	**0.0**	101.33	184.33^{e}	0.53	8.60	3.33
	1.0	120.33	214.67^{d}	0.76	9.83	0.00
	2.0	131.00	229.67^{dbc}	0.82	10.27	0.00
1.0	**0.0**	119.00	221.67^{dc}	0.81	9.37	3.33
	1.0	125.33	228.00^{dc}	0.85	10.40	0.00
	2.0	134.00	229.67^{dbc}	0.93	11.07	0.00
2.0	**0.0**	125.00	231.33^{bc}	0.84	9.07	0.00
	1.0	132.67	241.33^{b}	0.92	11.33	0.00
	2.0	136.00	262.67^{a}	0.95	12.10	0.00
SEM		3.75	4.64	0.04	0.61	**0.40**
Sig. test		NS	**	NS	NS	NS

a,b,c: Means in each classification in the same column with different superscripts, differ significantly (P<0.05)
N.S: Not Significant, ** P< 0.01
IgG= Immunoglobulin G, IgM =Immunoglobulin M, T-AOC = Total antioxidants capacity,
NUT =Neutrophils, MR, %: Mortality rate.

Table 7. Effect of zeolite and Synbiotic *levels* as feed additives on total aerobic, lactic acid, *E. coli* and salmonella of laying hens at 48 weeks of age

Items		**Total aerobic count X10^{6} (Log CFU/g)**	**Lactic Acid Bacteria X 10^{4} (Log CFU/g)**	***E. Coli* X 10^{2} (Log CFU/g)**	***Salmonella Spp*. X 10^{2} (Log CFU/g)**
Effect of zeolite(ZY), %:					
0.0		7.88^{b}	5.23^{b}	2.35^{a}	2.05^{a}
1.0		8.49^{a}	5.75^{a}	1.74^{b}	1.49^{b}
2.0		8.72^{a}	5.93^{a}	1.22^{c}	1.30^{b}
SEM		**0.17**	**0.11**	**0.13**	**0.16**
Sig. test		**	**	**	**
Effect of synbiotic (SB), %:					
0.0		7.92^{b}	5.37^{c}	2.14^{a}	2.09^{a}
1.0		8.43^{a}	5.63^{b}	1.67^{b}	1.48^{b}
2.0		8.75^{a}	5.90^{a}	1.50^{b}	1.27^{b}
SEM		**0.18**	**0.13**	**0.19**	**0.16**
Sig. test		**	**	**	**
Effect of interaction (ZY x SB), %					
0.0	**0.0**	7.03	4.62^{c}	2.96	2.74
	1.0	8.07	5.23^{b}	2.10	1.79
	2.0	8.55	5.83^{a}	1.98	1.62
1.0	**0.0**	8.34	5.68^{a}	1.93	1.93
	1.0	8.49	5.71^{a}	1.84	1.37
	2.0	8.65	5.84^{a}	1.45	1.15
2.0	**0.0**	8.38	5.79^{a}	1.52	1.58
	1.0	8.72	5.97^{a}	1.07	1.27
	2.0	9.07	6.03^{a}	1.06	1.05
SEM		**0.21**	**0.12**	**0.13**	**0.19**
Sig. test		**NS**	**	**NS**	**NS**

a,b,c: Means in each classification in the same column with different superscripts, differ significantly (P<0.05)
N.S: Not Significant, ** P< 0.01

[31] Indicated that synbiotics supplementation did not effect on serum total protein, albumin, globulin and glucose, except the packed cell volume, which was increased in the additive treatments with restriction at the end of the experiment. Moreover, [40-41] indicated that the supplementing broiler diet with probiotics or prebiotics did not any effect on each of total protein, albumin, globulin and albumin to globulin ratio.

3.4. Immunological response and antioxidants

Supplementations of zeolite or synbiotic and their interaction on immunological response, antioxidants and mortality rate are shown in Table (6). Significantly (P≤0.01) increased of IgG, IgM and T-AOC values by received zeolite supplementation as compared with control group. However, NUT value was increased insignificant affected by zeolite diet as compared with control. [6 and 42] reported that reactive oxygen species concentration decreased in the liver of mice fed zeolite supplementation as antioxidant. This reducing effect might be associated with adhesion-adsorption, ion-exchange and action binding properties of clinoptilolite. Effect of 1 or 2% synbiotic supplementation in layer diets increased significantly (P≤0.01) IgG, IgM, TAC and NUT values as compared with control group (Table 6). Mortality rate percentages were insignificantly when hen's diet contained zeolite or synbiotic supplementation. [43] reported that there were Positive effects of zeolite on decrease on mortality rate of laying hens and enhancing prevention of some diseases and improving the health status by reduced colony counts in the gut microflora of the proximal and distal gut and described reduced mortality in broilers and layers. Indeed, zeolites are used as effective adsorbents of toxic agents, particularly aflatoxins from the feeds [44, 45, 46, 47]. Concerning the interaction, it could be notice immunological response and antioxidants parameters were insignificantly affected by the interaction between zeolite and synbiotic supplementation, except IgM value was significant (P≤0.01). The highest values recorded that the interaction between treated with 2% zeolite and 2% synbiotic as compared with other treatment groups (Table 6).

3.5. Bacterial count

The effect of zeolite supplementation at different levels on total aerobic, lactic acid, *E. coli* and salmonella, was noted in Table (7). Significantly (P≤0.01) effect on total aerobic and lactic acid, while, *E. coli* and salmonella were decreased (P≤0.01) by received 1 or 2 % zeolite as compared with control group. Similar results were obtained by [48] who reported that zeolite caused to improve in the morphology of the intestinal mucosa may be explained by the lower numbers of E. coli and Salmonella. This ultimately decreases inflammatory processes at the intestinal mucosa, increasing, at the same time, villus height and secretory activity. However, supplementation with zeolite had no effect on Salmonella reduction [49]. Concerning synbiotic, at levels 1 or 2% supplementation in layer diets increased significantly (P≤0.01) in total aerobic and lactic acid, while it was decreased significantly (P≤0.01) of *E. coli* and salmonella as compared to control group. These results are in agreement with findings of [50] demonstrated that the addition of the synbiotic (Biomin Imbo) reduced *Escherichia coli* and total coliform populations in the intestines of broiler chickens. On the contrary they added that different levels of symbiotic increased the numbers of *Lactobacillus* in the intestine of broiler chickens. [51] showed that the addition of synbiotic to the diet resulted in a decrease of caecal coliform organism counts, which could be positive effects of synbiotic on gut microbial ecology. but differed from the results reported by [52]. Moreover, [53] reported that the challenges with nutritional interventions for Salmonella control were variable depending on the nutritional management and Salmonella status of the flock. Synbiotic supplementation had limited efficacy on decreasing SE colonization, although it was not certain that the microorganisms present in these products failed to colonize the enteric microenvironment. Furthermore, it is necessary to consider the composition of the commercial products, their dosage, the route of administration (feed or water) and the farm sanitary conditions. All these factors are able to influence the efficacy of the products [54]. It is possible that synbiotic could balance the intestinal microeco-system by controlling pathogenic bacteria via a competitive exclusion, which improve the count of beneficial bacteria. Previous studies have indicated that probiotics and prebiotics as synbiotic could regulate the intestinal micro ecological environment in different ways [55-56]. The use of synbiotic (prebiotic, probiotic) as feed additives for pathogen control and performance enhancement in poultry production has gained attention recently due to the increasing restriction of antibiotics as growth -promoting agents [57]. According to [58] the prophylactic and curative use of antibiotics to control Salmonella is not recommended for three reasons), which were antibiotic resistant Salmonella (and other) strains have emerged; there is a concern about the presence of antibiotic residues in meat and most antibiotics fail to eliminate Salmonella from animals, although some decreased contamination from this pathogen in animals has been observed. Results in Table 7, could be noticed that total aerobic, lactic acid, *E. coli* and salmonella values were insignificantly affected by interaction between zeolite and synbiotic supplementation at level 2% except, of lactic acid was significant (P≤0.01) as compared to other treatment groups.

4. Conclusion

It can be concluded that, supplemental layer diets with zeolite or synbiotic at level 2% were more effective for improving productive performance traits, biochemical, immunological blood parameters and bacterial count of Mandarah laying hens.

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