Annals of Agric. Sci., Moshtohor
Vol. 60(4) (2022), 1051 – 1062
ISSN 1110-0419
https://assjm.journals.ekb.eg

Effect of Zeolite Addition to Diets and Litter on Carcass Traits and Blood Biochemistry Parameters of Laying Hens (Silver Montazah)

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Abstract

The present study aimed to investigate the effect of zeolite addition to diet and litter of Egyptian local strain hens (Silver Montazah) on carcass traits and blood biochemical parameters. A total number of 720 laying hens and 96 cocks of Silver Montazah at age of 40 weeks, nearly equals in average body weight, randomly chosen, distributed into 12 groups (60 hens and 8 cocks / each group) in a factorial experimental design (4×3). Each group sub-divided into four replicates (each of 15 hens and 2 cocks) for evaluating dietary supplementation of zeolite with levels of (0, 10, 15 and 20 g/kg diet) and its addition to litter with levels of (0, 1.5 and 2 kg/ m^2) and the interaction effects between them. The obtained results revealed that significant (P<0.05) improve were found in carcass traits and internal organs and the most of blood biochemical parameters compared control groups of silver Montazah laying hens due to feeding on diet supplemented with different zeolite and reared on litter provided with zeolite at different levels. Furthermore, it is clearly observed that laying hens fed diet supplemented with zeolite at a level of 20 g/kg diet and raised on litter provided with zeolite at a level of 2 kg/m^2 showed the best improvement in carcass traits and blood biochemical parameters, compared with the other treatments applied and control group. Thus, it could be concluded that using of zeolite as a feed additive and mixing it with the litter in laying farms achieved the most favorable results.

Key words: laying hen, zeolite, carcass and blood parameters

Introduction

The construction diets of laying-hen mainly formulated using proteinaceous ingredients such as soybean meal and meat-and-bone meal to meet the recommended levels of essential amino acids contain relatively high levels of crude protein and excessive amounts of amino acids other than the first- and second-limiting amino acids (usually methionine and lysine, respectively). Because of, the poultry doesn't have any storage mechanisms for amino acids consumed beyond the requirement for protein synthesis, the amino acids consumed in excess are deaminated and the amino acid derived nitrogen is excreted in the urine mainly as uric acid (80%), ammonia (10%), and urea (5%) **(Goldstein and Skadhauge, 2000).** Once excreted, uric acid is readily converted to ammonia (NH_3) by a series of microbial enzymes present in the manure. Ammonia gas emitted from chicken litter and negatively effects on layers productive performance **(Beker *et al*., 2004).** Many studies were carried out as attempts to reduce the ammonia emission from animal production activities. One of an important trial in this studies area is using zeolite supplementation to diets of laying hens and its addition to litter of laying hen farms that reared in farm for a long time. Zeolite are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, having three-dimensional structures, characterized by the ability to lose and gain water reversibly and to exchange constituent cations without major change of structure (**Mumpton, 1984**). Moreover, molecules have a proper size which can selectively absorb and release specific molecules by ion exchange (**Shurson *et al.*, 1984**). In addition, zeolite used as a feed additive, it does not interact with other diet nutrients (vitamins, minerals, etc.) therefore it could be used as feed additives and mixed with litter safely to improve productive performance of layers and decrease of ammonia emission in poultry farms (**Kavan *et al.*, 2013**), reduce toxic effects of afaltoxins (**Gilani *et al.*, 2016**), help in litter management (**Eleroglu and Yalcin, 2005**) and controlling air quality in poultry house (**Li *et al.*, 2008**). Moreover, the mixing of zeolite to litters of poultry farms lead to decrease the moister litter content, whereas the chemical structure of zeolite enable it to absorb the excessive humidity from litter (**Dagtekin and Ozturk, 2015**). However, the action of it has been attributed to its high affinity for ammonium ions, resulting to the reduction in the uptake of ammonia produced from deamination of proteins during the digestive processes via the intestinal wall (**Pond *et al.*, 1988**). Ammonia is considered as a cell toxicant in animals thus, the reduction of the amount which the intestinal epithelial cells are exposed to, could lead to the reduction of epithelial turnover, a sparing of energy and improve nutrient utilization **(Safaeikatouli *et al.*, 2010)**. Using the zeolite in diets of laying hens and its addition to litter of laying farm promote the humoral and cellular immune response which characterize as a high concentdiet of pro-inflammatory and anti-inflammatory cytokines in serum of chicken **(Jarosz *et al.*, 2017)**. According to the previously mentioned facts, the present study aimed to evaluate the effect of zeolite supplementation to diets and its addition to litter on carcass traits and blood biochemical parameters of laying hens of Silver Montazah local strain.

Material and Methods

The present study was carried out at the Poultry Breeding Research Station at Inshas, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt, during the period from December 2015 to March 2016. This study was aimed to evaluate the effect of using different levels of zeolite in laying diets and litter on carcass traits and some blood biochemistry parameters of Silver Montazah hens.

A total number of 720 laying hens and 96 cocks of Silver Montazah local strain at 40 weeks of age were randomly chosen and distributed into 12 groups (60 hens and 8 cocks / each group) hens of each group were nearly equals in averages body weight and daily egg production. Each group sub-divided into four replicates, each of 15 hens and 2 cocks. A factorial experimental design (4×3) was used in 12 experiment groups as shown in (Table 1)

Table 1: Experimental design

Zeolite (g/kg diet)	Zeolite (kg/m2 litter)		
0	0	1.5	2
10	0	1.5	2
15 20	0	1.5	2

Each experimental group has 60 hens 8 cocks.

The photoperiod during the experimental period was fixed at 16 hours daily. All birds were housed on floor laying houses in pens each of 2 x 1.5 meter size for each replicate with semi closed windows, and tested for 16 weeks in this experiment. Birds were kept under similar, standard hygienic and environmental conditions and fed on basal layer diet shown in (Table 2) which formulated according to the recommended requirements of NCR, (1994).

Table 2. Composition and calculated analysis of the basal experimental diet

Ingredient	**%**
Yellow corn (8.5 %)	61.17
Soybean meal (44%)	16.70
Wheat bran (15.7 %)	6.70
Corn gluten (60%)	4.70
DL-Methionine (99%)	0.51
Limestone ($CaCo_3$)	8.13
Di. Calcium. Phosphate.	1.42
Salt (NaCl)	0.37
Vitamin and Minerals premix*	0.30
Total	**100**
Calculated analysis**	
ME Kcal/Kg	2699
Crude protein, %	16.02
Crude fiber,%	3.47
Calcium, %	3.45
Available phosphorus, %	0.40
Lysine %	0.73
Methionine (%)	0.34
Methionine + Cysteine (%)	0.62
Na (%)	0.19

***Supplied per Kg of diet**: Vit. A, 12000 IU; Vit.D_3, 2200 ICU; Vit.E, 10 mg; Vit K_3, 2 mg; Vit.B_1, 1 mg; Vit. B_2 5 mg; B_6 1.5 mg; B_{12} 10 mcg; Nicotinic acid 30 mg; Folic acid 1 mg, Pantothenic acid 10 mg; Biotein 1.5 mcg; Choline 250 mg; Copper 10 mg; Iron 30 mg; Selenium 0.1 mg; Cobalt 0.1 mg Manganse 60 mg; Zinc 50 mg; Iodine 1 mg.
** Calculated according to **NRC (1994).**

Parameters estimation and data collection:

At the end of the experimental period four birds were randomly taken from each treatment. Birds chosen were fasted for 12 hours prior slaughtering, individually weighed alive to the nearest gram and slaughtered according to Islamic traditional method by cutting the jugular vein and the throat near the first vertebra with a sharp knife. After complete bleeding, the birds were weighed and dressed by dry plucking, shank and heads were separated. The birds were then eviscerated and the intestinal viscera, intestine, gizzard, lungs, spleen, thymus gland, abdominal fat, liver, heart and reproductive organs were removed. The carcass, abdominal fat and giblets (empty gizzard, liver and heart) were separately weighed. The proportional weight of giblets, edible parts and abdominal fat to live weight were calculated as follows:

$$\text{Carcass weight } \% = \frac{EW}{LW} X\,100$$

$$\text{Giblets weight } \% = \frac{GW}{LW} X\,100$$

$$\text{Total edible parts } \% = \frac{EW + GW}{LW} X\,100$$

Where:
LW= live weight,
GW= giblets weight,
EW= eviscerated weight.

Blood samples were individually obtained at the end of the experimental period during slaughtering from four hens randomly chosen from each experimental group. Samples treated with anticoagulant EDTA then each sample was divided into two tubes, the first tube was used to determination of red blood cells (RBC's) count, white blood cells (WBC's) and lymphocytes. The second tube was used to collect plasma by centrifugation for 20 minutes at 2500 rpm and it stored at –20 °C until determination of plasma total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, low density liboprotein (LDL), high density liboprotein (HDL) and triglyceride using commercial chemical kits (**Bio meriex, Laboratorus reagent and products France)** similarly, to the method by **El-Gendi *et al*., (2019) and Okasha, (2021) .** Globulin was calculated by the difference between total protein and albumin.

Statistical analysis:

Data were statistically analyzed using the software **SAS, version 9.3 (SAS, 2004)**.Two-way ANOVA was used for analyzing the individual effects of zeolite diet and zeolite litter. One-way analysis of variance (one way ANOVA) was used to determine wet here significant variation existed between the treatments. When overall differences were found, differences between means were tested by **Duncan, (1955)** new multiple range test**.**The following model was used:

$$Y_{ijk} = \mu + R_i + L_j + (RL)_{ij} + e_{ijk}$$

Where:

Yijк= the ith observation.
μ=the overall mean of the respective variable.
Ri=Zeolite diet levels effect (i =1, 2, 3, and 4).
Lj=Zeolite litter levels effect (j =1, 2 and 3).
(RL)ij=Interaction effect between zeolite diet levels and Zeolite litter levels effects.
e_{ijk}=Random experimental error assumed to be normally distributed with zero mean and variance equals σ_e^2.

Results and discussion

Carcass traits

Data presented in Tables 3 and 4 revealed that the average of absolute and relative weights of carcass, giblets and total edible meat were significantly (*P*<0.001) increased for hens that fed diets supplemented with zeolite at a level of 20, 15 and 10 g /kg diet and those reared on litter mixed with 2 and 1.5kg zeolite/m^2 litter compared to those of control group. While, the averages of absolute and relative weights of abdominal fat, thymus, and spleen were recorded lower values in hens that fed diets supplemented with 20g zeolite/kg and those reared on litter mixed with 2 kg zeolite/m^2 litter compared with other treatments applied and control group, respectively. The improving in carcass characteristic due to dietary supplementation of different levels of zeolite may attributed to improve in health status, body weight gain as well as feed efficiency of birds (**Papaioannou *et al.*, 2004).** Moreover, the positive influence of zeolite supplementation on the spleen and liver weights may associated to the slower passage of ingesta through the digestive tract, that based on the levels of zeolites in chicken's diets (**Prvulovic *et al.*, 2008).** In addition, incorpodiet of zeolite into litter has reduced ammonia levels, through its ability to adsorb ions, litter chemical amendments are substances that improve the physical, chemical and microbiological integrity of litter and enhance the hygiene of poultry farm and productive performance consequently **(Oliveira *et al.*, 2004).** These results agree with those reported by **Amad, (2018), Emam *et al.*, (2019)** and **Rawia *et al.*, (2020)** who are stated that the absolute and relative carcass weights were significantly increased due feeding on diets supplemented with zeolite at different levels, compared to control group. On the other hand, these results disagree with those obtained by **Heba *et al.*, (2016) and Ergün *et al.*, (2017)** who are found no significant differences in both absolute and relative carcass weights and internal organs of laying hens due to treating with zeolite levels compared with control group. Highly significant effects (*P*<0.001) were found on carcass characteristics (carcass, abdominal fat, giblets, edible parts, and spleen) due to the interaction effects between dietary supplementation of zeolite levels and its addition to litter, except absolute and relative weight of thymus, at all periods of estimation. The interaction effects of (Z.R20×Z.L2), (Z.R20×Z.L1.5) and (Z.R20×Z.L0) showed significantly improved in absolute and relative weights of carcass, abdominal fat, giblets and edible parts at the end of the experiment.

Table 3. Least-squares means ($\overline{X}$ ± SE) for absolute and relative weights of carcass and abdominal fat of different experimental groups as affected by studied factors.

Treatment	Carcass weight		Abdominal fat weight	
	(g)	(%)	(g)	(%)
Z.R. (g/kg)				
0	1251.67±8.94^{d}	64.33±0.37^{d}	173.66±3.59^{a}	8.94±0.19^{a}
10	1329.17±8.27^{c}	68.33±0.44^{c}	141.00±2.05^{b}	7.24±0.10^{b}
15	1419.17±6.24^{b}	72.00±0.30^{b}	123.16±1.03^{c}	6.25±0.05^{c}
20	1577.67±25.15^{a}	75.66±0.41^{a}	114.00±1.46^{d}	5.48±0.11^{d}
Significant	***	***	***	***
Z.L. (kg/m^{2})				
0	1355.00±28.37^{c}	68.75±1.13^{c}	145.00±6.71^{a}	7.38±0.36^{a}
1.5	1385.13±27.02^{b}	70.00±1.01^{b}	138.62±6.22^{b}	7.05±0.34^{b}
2	1443.13±39.87^{a}	71.50±1.14^{a}	130.25±4.89^{c}	6.50±0.29^{c}
Significant	***	***	***	***
Interaction (R×L)				
Z.R 0× Z.L 0	1220.00±14.43^{i}	63.00±0.57^{k}	186.00±5.19^{a}	9.61±0.28^{a}
Z.R 0× Z.L 1.5	1252.50±4.33^{h}	64.50±0.28^{j}	175.00±0.57^{b}	9.04±0.04^{b}
Z.R 0× Z.L 2	1282.50±1.44^{g}	65.50±0.28^{i}	160.00±1.15^{c}	8.17±0.06^{c}
Z.R 10× Z.L 0	1295.00±5.77^{g}	66.50±0.28^{h}	146.50±0.86^{d}	7.51±0.04^{d}
Z.R 10× Z.L 1.5	1332.50±1.44^{f}	68.50±0.28^{g}	145.00±0.23^{d}	7.48±0.01^{d}
Z.R 10× Z.L 2	1360.00±2.88^{e}	70.00±0.20^{f}	131.50±0.28^{e}	6.74±0.02^{e}
Z.R 15× Z.L 0	1395.00±5.77^{d}	71.00±0.15^{e}	127.50±1.44^{e}	6.49±0.09^{e}
Z.R 15× Z.L 1.5	1422.50±4.33^{c}	72.00±0.11^{d}	121.50±0.28^{f}	6.15±0.02^{f}
Z.R 15× Z.L 2	1440.00±5.77^{c}	73.00±0.57^{c}	120.50±0.28^{f}	6.11±0.01^{f}
Z.R 20× Z.L 0	1510.00±2.88^{b}	74.50±0.28^{b}	120.00±0.32^{f}	5.92±0.06^{f}
Z.R 20× Z.L 1.5	1533.00±4.61^{b}	75.00±0.22^{b}	113.00±1.73^{g}	5.54±0.09^{g}
Z.R 20× Z.L 2	1690.00±23.09^{a}	77.50±0.28^{a}	109.00±0.34^{g}	4.99±0.04^{h}
Significant	***	***	***	***

$^{a\text{-}b\text{-}c\ etc.}$ Means, within column, with different superscripts are significantly different (Ns=Non-significant and ***=p<.001); Z.R. zeolite in diet, Z.L. zeolite in litter.

Table 4. Least-squares mean ($\overline{X}$ ± SE) for absolute and relative weights of giblet weight, total edible meat weight, thymus weight and spleen weight of different experimental groups as affected by studied factors.

Treatment	Giblet weight		Total edible meat weight		Thymus weight		Spleen weight	
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Z.R. (g/kg)								
0	76.66±3.06^{d}	3.95±0.15^{d}	1328.33±11.51^{d}	68.37±0.49^{d}	2.48±0.13	0.19±0.01a	3.71±0.11a	0.29±0.01a
10	86.75±1.20^{c}	4.47±0.06^{c}	1415.92±9.37^{c}	72.76±0.49^{c}	2.61±0.16	0.19±0.01b	3.43±0.05b	0.25±0.01b
15	94.08±1.02^{b}	4.84±0.04^{b}	1513.25±7.18^{b}	76.78±0.31^{b}	2.36±0.14	0.16±0.01c	3.23±0.12bc	0.22±0.01c
20	106.33±1.89^{a}	5.47±0.05^{a}	1684.00±26.84^{a}	80.77±0.43^{a}	2.34±0.17	0.15±0.01d	3.09±0.09c	0.19±0.01d
Significant	***	***	***	***	n.s	**	***	***
Z.L. (kg/m^{2})								
0	83.68±3.48^{c}	4.31±0.16^{c}	1438.69±31.65^{c}	72.97±1.28^{c}	2.58±0.12	0.19±0.01	3.55±0.11a	0.26±0.01a
1.5	91.81±2.39^{b}	4.73±0.09^{b}	1476.94±29.36^{b}	74.80±1.09^{b}	2.33±0.12	0.17±0.01	3.29±0.09b	0.24±0.01b
2	97.37±2.78^{a}	5.01±0.08^{a}	1540.50±42.60^{a}	76.24±1.21^{a}	2.43±0.14	0.17±0.01	3.26±0.08b	0.22±0.01c
Significant	***	***	***	***	n.s	n.s	**	***
Interaction (R×L)								
Z.R 0× Z.L 0	62.75±0.7	3.24±0.	1282.75±13	66.28±0.	2.52±0.	0.20±0.0	4.12±0.0	0.33±0.0

	5^{h}	04^{k}	.71^{j}	61^{k}	21	1	2a	1a
Z.R 0× Z.L 1.5	81.50±1.19^{g}	4.21±0.05^{j}	1334.00±5.49^{i}	68.92±0.19^{j}	2.47±0.25	0.19±0.02	3.49±0.16b	0.28±0.01b
Z.R 0× Z.L 2	85.75±1.25^{f}	4.41±0.06^{h}	1368.25±2.56^{h}	69.89±0.08^{i}	2.44±0.30	0.19±0.02	3.53±0.19b	0.27±0.01b
Z.R 10× Z.L 0	82.75±1.10^{g}	4.26±0.05^{i}	1377.75±6.79^{h}	70.65±0.34^{i}	2.92±0.07	0.22±0.01	3.40±0.06c	0.26±0.01b
Z.R 10× Z.L 1.5	86.00±0.81^{f}	4.43±0.03^{h}	1418.50±1.04^{g}	73.21±0.21^{h}	2.37±0.31	0.17±0.02	3.54±0.12b	0.26±0.01b
Z.R 10× Z.L 2	91.50±1.04^{e}	4.71±0.05^{f}	1451.50±3.06^{f}	74.43±0.06^{g}	2.56±0.37	0.18±0.02	3.37±0.05c	0.24±0.01c
Z.R 15× Z.L 0	90.00±0.57^{e}	4.65±0.01^{g}	1485.00±6.35^{e}	75.57±0.11^{f}	2.06±0.24	0.14±0.01	3.75±0.12b	0.27±0.01b
Z.R 15× Z.L 1.5	94.25±0.62^{d}	4.87±0.03^{e}	1516.75±4.51^{d}	76.79±0.11^{e}	2.28±0.23	0.16±0.01	2.97±0.17e	0.21±0.01d
Z.R 15× Z.L 2	98.00±0.40^{c}	5.04±0.02^{d}	1538.00±5.78^{d}	77.97±0.34^{d}	2.74±0.22	0.19±0.01	2.97±0.06e	0.20±0.01d
Z.R 20× Z.L 0	99.25±0.25^{c}	5.12±0.01^{c}	1609.25±3.03^{c}	79.37±0.09^{c}	2.84±0.25	0.18±0.01	2.94±0.08e	0.19±0.01e
Z.R 20× Z.L 1.5	105.50±1.19^{b}	5.43±0.06^{b}	1638.50±4.17^{b}	80.31±0.08^{b}	2.21±0.21	0.14±0.01	3.16±0.14d	0.20±0.01d
Z.R 20× Z.L 2	114.25±0.47^{a}	5.88±0.05^{a}	1804.25±22.95^{a}	82.66±0.34^{a}	1.98±0.19	0.12±0.01	3.17±0.23d	0.18±0.01f
Significant	***	***	***	**	n.s	n.s	**	**

$^{a-b-c\ etc..}$ Means, within column, with different superscripts are significantly different (Ns=Non-significant and ***=p<.001);
Z.R. zeolite in diet, Z.L. zeolite in litter

Blood parameters:

Plasma total proteins, albumin (A), globulin (G) and A/G ratio:

The obtained results presented in Table 5 revealed that the averages of plasma total protein, albumin and globulin significantly increased in hens fed diets supplemented diet with different levels of zeolite, compared with untreated groups, the rate of increase differed according to the levels of treatments applied. Laying hens fed diet supplemented with 20 g zeolite/kg diet showed the highest averages of plasma total protein, albumin and globulin (10.00, 3.42 and 4.03 mg/dl, respectively) followed by those fed diet with 10 g zeolite /kg diet (8.14, 3.27 and 3.86 mg /dl, respectively), compared with control group and the other treatments. However, the highest averages of A/G ratio were recorded by birds of control group (96.67) and those fed diet containing 15 g zeolite /kg diet (85.68) followed by birds fed diet with 20 and 10 g zeolite /kg diet, respectively. concerning to the effect of zeolite mixing with the litter, it is clearly observed that the addition of zeolite to litter at a level of 1.5 kg zeolite/m^2 showed the highest averages of plasma total protein, albumin and globulin (7.82, 3.64 and 3.79 mg/dl, respectively) and A/G ratio (96.04) compared with those reared on litter mixed with 2 kg zeolite/m^2 litter and those of control group which showed the highest average of total protein (9.90). These results agreed with those reported by **Rawia *et al.*, (2020)** who are found a significant increase in total serum protein concentdiets due to addition of zeolite to laying hen's diets compared with control. The interaction effects between (Z.R20×Z.L1.5) and (Z.R20×Z.L0), respectively showed the significant higher averages of plasma total protein, albumin and globulin. While, the interaction effects between (Z.R0×Z.L0) and (Z.R20×Z.L2), respectively showed higher averages of A/G ratio. However, the interaction effects between (Z.R0×Z.L1.5) showed the lower averages of plasma total protein, albumin and globulin.

Table 5. Least-squares means ($\overline{X}$ ± S.E) for plasma total protein, albumin, globulin and A/G ratio of different experimental groups as affected by studied factors.

Treatment	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	A/G ratio
Z.R. (g/kg)				
0	5.94±0.85^c	2.90±0.48	3.00±0.44^c	96.67±0.46^b
10	8.14±1.01^b	3.27±0.84	3.86±0.25^b	84.71±0.54^a
15	8.01±0.67^b	3.23±0.57	3.77±0.42^b	85.68±0.49^a
20	10.00±1.27^a	3.42±0.71	4.03±0.66^a	84.86±0.68^b
Significant	**	**n.s**	**	*
Z.L. (kg/m^2)				
0	9.90±0.79^a	3.34±0.58^a	4.06±0.32^a	82.26±0.45^a
1.5	7.82±0.99^b	3.64±0.57^b	3.79±0.57^b	96.04±0.57^b
2	6.34±0.69^b	2.89±0.42^c	3.39±0.33^b	85.25±0.37^b
Significant	**	**	*	**
Interaction (R×L)				
Z.R 0× Z.L 0	6.88±0.31^c	3.39±0.58	3.49±0.26^c	97.13±0.42^b
Z.R 0× Z.L 1.5	4.13±0.05^e	2.03±0.40	2.58±0.42^e	78.68±0.41^b
Z.R 0× Z.L 2	6.82±2.51^c	3.30±1.31	3.52±1.20^c	93.75±1.25^b
Z.R 10× Z.L 0	10.78±2.39^{ab}	3.31±1.90	3.99±0.48^b	82.95±1.19^a
Z.R 10× Z.L 1.5	6.61±0.42^c	3.12±0.02	3.48±0.44^c	89.6±0.23^b
Z.R 10× Z.L 2	6.82±1.16^c	2.72±0.73	4.10±0.43^b	66.3±0.58^c
Z.R 15× Z.L 0	10.64±0.38^b	4.65±0.09	4.98±0.48^{ab}	91.56±0.28^b
Z.R 15× Z.L 1.5	6.91±0.28^c	4.02±1.24	4.89±0.95^d	82.20±1.09^a
Z.R 15× Z.L 2	6.48±1.10^c	3.03±0.92	3.44±0.18^c	88.0±0.55^b
Z.R 20× Z.L 0	10.82±1.55^{ab}	5.34±0.83	5.78±0.72^{ab}	92.3±0.77^b
Z.R 20× Z.L 1.5	10.94±1.72^a	4.42±1.66	4.81±0.87^a	91.89±1.26^b
Z.R 20× Z.L 2	5.24±0.41^d	3.82±0.62	4.02±0.43^d	95.02±0.52^b
Significant	**	**n.s**	**	**

[a-b-c etc..] Means, within column, with different superscripts are significantly different (Ns=Non-significant and ***=p<.001); Z.R. zeolite in diet, Z.L. zeolite in litter

Plasma cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL):

The results obtained in Table 6 showed that averages of total plasma cholesterol, triglycerides and low-density lipoprotein (LDL) of laying hens have significant increased due to feeding diets supplemented with different levels of zeolite compared to control group, which decreased all averages of lipids parameters. It is clearly observed that, in most cases hens fed diet with zeolite at a level of 20g/kg diet showed the highest averages of plasma cholesterol, triglycerides and low-density lipoprotein (LDL) (118.88 , 90.96 and 36.11 mg/dl, respectively), when compared with hens of the control group and those fed diet supplemented with 10g and 15 zeolite/kg diet. However, litter supplementation with zeolite at a level of 2 kg/m^2 significantly decreased averages of plasma cholesterol, triglycerides, (HDL) and (LDL), compared with hens of the control group. While, plasma LDL was significantly increased (28.68 mg/dl) by adding zeolite at a level of 1.5 kg/m^2 litter of laying hens. The effects of zeolite supplementation to diets on the blood parameters generally depend on the balance of element content in the diets **(Eleroglu *et al*., 2011)**. These results disagree with those reported by **Ergün *et al*.,(2017)** and **Rawia *et al*.,(2020)** who are found that plasma cholesterol, triglycerides, HDL and LDL levels in blood of laying hens were significantly (P<0.05) decreased due to feeding on diet supplemented with different levels of zeolite compared to control group. The highest averages of plasma cholesterol, plasma triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were showed by hens due to the interaction among the different levels of natural zeolite in diet and/or litter, as affected by Z.R20×Z.L1.5, Z.R20×Z.L1.5, Z.R0×Z.L0 and Z.R20×Z.L1.5, respectively. However, the lowest levels of plasma triglycerides, plasma cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were showed by the interactions between Z.R0×Z.L2, Z.R10×Z.L2, Z.R20×Z.L2 and Z.R0×Z.L2, respectively.

Table 6. Least-squares means ($\bar{X}$ ± S.E) for plasma total cholesterol, triglycerides, high density lipoprotein (HDL) and low-density lipoprotein (LDL) of different experimental groups as affected by studied factors

Treatments	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Z.R. (g/kg)				
0	93.20±5.33^{c}	80.06±5.01^{b}	0.11±0.03	22.64±1.09^{c}
10	112.35±8.67^{b}	53.70±6.57^{c}	0.06±0.01	24.48±1.80^{b}
15	115.43±7.59^{a}	55.72±3.83^{c}	0.06±0.01	24.31±1.92^{b}
20	118.88±8.23^{a}	90.96±11.09^{a}	0.06±0.01	36.11±2.81^{a}
Significant	*	***	n.s	***
Z.L. (kg/m^2)				
0	122.74±6.01^{a}	80.05±4.30^{a}	0.09±0.02	27.03±1.73
1.5	107.85±6.32^{b}	70.80±9.34^{b}	0.06±0.01	28.68±2.91
2	99.31±7.14^{c}	59.48±6.61^{c}	0.06±0.01	24.93±1.61
Significant	*	*	n.s	n.s
Interaction (R×L)				
Z.R 0× Z.L 0	103.62±0.24^{c}	64.47±7.45^{f}	0.21±0.09^{a}	24.76±0.05^{c}
Z.R 0× Z.L 1.5	105.28±7.42^{b}	78.22±2.16^{d}	0.05±0.01^{b}	24.97±1.80^{c}
Z.R 0× Z.L 2	70.72±2.25^{e}	97.50±5.71^{c}	0.07±0.01^{b}	18.20±0.32^{d}
Z.R 10× Z.L 0	120.92±21.39^{ab}	81.62±3.12^{d}	0.06±0.01^{b}	28.63±5.06^{bc}
Z.R 10× Z.L 1.5	102.91±9.64^{c}	43.06±8.15^{h}	0.06±0.01^{b}	22.39±1.16^{cd}
Z.R 10× Z.L 2	113.24±14.91^{b}	36.43±1.61^{i}	0.06±0.01^{b}	22.41±0.27^{cd}
Z.R 15× Z.L 0	134.36±0.72^{a}	71.23±4.61^{e}	0.04±0.01^{b}	22.13±0.10^{cd}
Z.R 15× Z.L 1.5	89.05±4.92^{d}	53.02±1.75^{j}	0.07±0.01^{b}	20.10±0.56^{d}
Z.R 15× Z.L 2	122.88±15.49^{ab}	42.92±0.66^{h}	0.06±0.01^{b}	30.69±4.38^{b}
Z.R 20× Z.L 0	132.09±8.19^{a}	102.90±1.55^{b}	0.08±0.01^{b}	32.60±3.73^{b}
Z.R 20× Z.L 1.5	134.17±16.37^{a}	108.90±28.46^{a}	0.05±0.01^{b}	47.28±2.82^{a}
Z.R 20× Z.L 2	90.40±1.93^{d}	61.07±9.45^{f}	0.04±0.01^{b}	28.45±0.32^{bc}
Significant	*	**	*	***

$^{a,b\text{-}c\text{-}d\text{-}e}$ Means, within column, with different superscripts are significantly different (P≤0.05).

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT):

Data presented in Table 7 show that averages of plasma AST and ALT significantly (P<0.001) increased with increasing the dietary zeolite level in hen's diet, the rate of increase differed according to the treatments applied. It is quite clear that, hens fed diet supplemented with zeolite at a level of 10 g/kg diet and those of control group showed the lowest averages of plasma AST and ALT (0.31mg/dl) and (0.39 mg/dl) respectively, compared with the other treatments applied. However, the higher averages of plasma AST and ALT were showed by hens fed diet supplemented with zeolite at a levels of 20 and 15g /kg, respectively, compared with the other treatments applied and control group.

The improving takes place in hebetic enzymatic functions in laying hens due to dietary supplementation of zeolite may be attributed its support of the antioxidant system **(Wang *et al*., 2012)**, reduce of lipid peroxidation and normalized the liver functions in birds **(Morsy ,2018)**.These results disagreed with those reported by **(Straková *et al*., 2008**) who found that AST were significantly (P<0.05) increased due to feeding of layer hens on diet supplemented with zeolite. The obtained results showed that, hens reared on litter provided with zeolite at levels of 1.5 and 2 kg/m^2 litter showed the highest averages of plasma AST (0.59 mg/dl) and ALT (0.70 mg/dl), compared with the other treatments applied and control group. Concerning to the effect of interaction between dietary and litter supplementation of zeolite, the results showed significant effect (P<0.05) on plasma AST and ALT due to the interactions among studied factors. The highest averages of plasma AST and ALT were showed by the interactions between Z.R15×Z.L2 and Z.R15×Z.L1.5, respectively. However, the interaction between Z.R10× Z.L 2 and Z.R10×Z.L0 showed the lowest averages of plasma AST and ALT, respectively.

Table 7. Least-squares means ($\overline{X}$ ± S.E) for plasma transaminases (AST and ALT) of different experimental groups as affected by studied factors

Treatments	**AST(mg/dl)**	**ALT(mg/dl)**
Z.R. (g/kg)		
0	$0.39±0.09^c$	$0.38±0.04^d$
10	$0.31±0.06^d$	$0.51±0.10^c$
15	$0.70±0.14^b$	$0.92±0.04^a$
20	$0.83±0.07^a$	$0.73±0.05^b$
Significant	*****	*****
Z.L. (kg/m^2)		
0	0.54±0.09	0.62±0.08
1.5	0.59±0.07	0.59±0.08
2	0.53±0.12	0.70±0.06
Significant	**n.s**	**n.s**
Interaction (R×L)		
Z.R 0× Z.L 0	$0.12±0.01^g$	$0.39±0.15^e$
Z.R 0× Z.L 1.5	$0.84±0.08^{ab}$	$0.38±0.01^e$
Z.R 0× Z.L 2	$0.22±0.02^f$	$0.37±0.01^e$
Z.R 10× Z.L 0	$0.57±0.03^c$	$0.26±0.02^g$
Z.R 10× Z.L 1.5	$0.27±0.09^e$	$0.29±0.07^f$
Z.R 10× Z.L 2	$0.09±0.01^h$	$0.99±0.02^a$
Z.R 15× Z.L 0	$0.40±0.02^d$	$0.92±0.09^a$
Z.R 15× Z.L 1.5	$0.61±0.18^c$	$1.02±0.09^a$
Z.R 15× Z.L 2	$1.10±0.31^a$	$0.84±0.02^b$
Z.R 20× Z.L 0	$1.09±0.08^a$	$0.92±0.04^a$
Z.R 20× Z.L 1.5	$0.67±0.05^b$	$0.68±0.08^c$
Z.R 20× Z.L 2	$0.72±0.10^b$	$0.60±0.02^d$
Significant	*****	*****

$^{a-b-c-d-e}$ Means, within column, with different superscripts are significantly different (P≤0.05).

Red blood cells (RBC's), White blood cells (WBC's) and Lymphocytes:

The obtained results listed in Table 8 showed that significant increase in averages of RBC's and lymphocytes (3.88106/ml) and (43.0 %) due to dietary supplementation of zeolite at a levels of 20 and 10 g/kg diet, respectively. While, the higher average of WBC's was hens of by laying hens of control group. It is clearly evidence that laying hens reared on litter supplemented with zeolite at a level of 2 kg/m^2 showed higher averages of RBC's, WBC's and lymphocytes mounted 3.45, 11.68 and 39.37, respectively. However, the lowest averages of WBC's and lymphocytes were recorded by layers reared on litter provided with zeolite at a level of 1.5 kg/m^2. The biochemical enhancement was found in this study due to treating with zeolites, could be attributed to its role for ion-exchangers, participate in certain biochemical transformations, normalize the homeostasis of animals and increase the nutrient conversion, thus, it may be reversible in increase the values of RBC's **(Shadrin, 1998 and Tsitsishvili *et al*., 1977)**. Moreover, zeolite have a positive influence on the immunity and the inflammatory processes by diminishing the synthesis of nitric oxide and superoxide anions **Visnja *et al*.,(2004)**. They enhance the immune activity in chickens **Jung *et al.*, (2010)**. These results agreed with those founding by **Jarosz *et al.*, (2017)** and **Emam *et al*.,(2019)** who are indicated that RBC's and lymphocytes of chicken were significantly (P<0.05) increased due to feeding on diets supplemented with different levels of zeolite compared to control group (untreated one).While, these results disagreed with those reported by **Rawia *et al*.,(2020)** who found that hematological parameters of RBC's and lymphocyte were not affected by adding zeolite to broiler's diets. The interaction effects of (Z.R20×Z.L2), (Z.R0×Z.2) and (Z.R15×Z.L0) showed the highest averages of RBC's, WBC's and lymphocytes (4.03, 12.20 and 57.0), respectively, compared to the other interaction effects.

Table 8. Least-squares means ($\overline{X}$ ± S.E) for RBC's, WBC's and Lymphocytes of different experimental groups as affected by studied factors.

Treatments	RBCs (10^6/ml)	WBCs (10^6/ml)	Lymphocytes (%)
Z.R. (g/kg)			
0	2.63±0.08^{d}	11.95±0.17	41.50±1.88^{b}
10	3.14±0.04^{c}	11.58±0.11	43.00±4.44^{a}
15	3.51±0.02^{b}	11.48±0.14	41.66±3.75^{b}
20	3.88±0.04^{a}	11.49±0.16	31.16±1.91^{c}
Significant	***	n.s	*
Z.L. (kg/m^2)			
0	3.10±0.14^{c}	11.63±0.10	39.37±3.50
1.5	3.31±0.11^{b}	11.57±0.17	39.25±2.44
2	3.45±0.11^{a}	11.68±0.12	39.37±2.98
Significant	***	n.s	n.s
Interaction (R×L)			
Z.R 0× Z.L 0	2.31±0.15^{j}	11.65±0.17	35.50±1.44^{f}
Z.R 0× Z.L 1.5	2.73±0.02^{i}	11.99±0.41	46.00±1.15^{c}
Z.R 0× Z.L 2	2.86±0.01^{ih}	12.20±0.31	43.00±4.04^{d}
Z.R 10× Z.L 0	2.95±0.02^{h}	11.71±0.25	39.50±7.21^{e}
Z.R 10× Z.L 1.5	3.14±0.02^{g}	11.50±0.25	39.00±9.23^{e}
Z.R 10× Z.L 2	3.32±0.03^{f}	11.53±0.14	50.50±7.21^{b}
Z.R 15× Z.L 0	3.43±0.01^{e}	11.51±0.34	57.00±4.04^{a}
Z.R 15× Z.L 1.5	3.52±0.02^{d}	11.40±0.26	39.50±0.28^{e}
Z.R 15× Z.L 2	3.60±0.01^{c}	11.54±0.18	28.50±0.86^{h}
Z.R 20× Z.L 0	3.73±0.02^{bc}	11.64±0.14	25.50±1.44^{i}
Z.R 20× Z.L 1.5	3.87±0.02^{b}	11.38±0.46	32.50±1.44^{g}
Z.R 20× Z.L 2	4.03±0.06^{a}	11.47±0.21	35.50±4.33^{f}
Significant	*	n.s	***

[a-b-c-d-e] Means, within column, with different superscripts are significantly different (P≤0.05).

Conclusion

From the present study it could be concluded that laying hens of silver Montazah fed diet supplemented with zeolite at a level of 20 g/kg diet and raised on litter provided with zeolite at a level of 2 kg/m^2 showed the best improvement in carcass traits and blood biochemical parameters, compared with the other treatments applied and control group. Thus, it could be recommended to use zeolite as a feed additive and mixing with the litter in laying farms to obtain favorable results.

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تأثير إضافة الزيولايت للعليقة والفرشة على صفات الذبيحة وصفات الدم البيوكميائية للدجاج البياض (المنتزه الفضى)

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تهدف هذه الدراسة إلى تقييم تأثير استخدام مستويات مختلفة من الزيولايت في العليقة و/ أو الفرشة على صفات الذبيحة وخصائص الدم في دجاج المنتزه الفضي البياض. استخدم في هذه الدراسة 720 دجاجة بياضة و96 ديك من سلاله المنتزه الفضي عمر 40 أسبوع تم اختيارها وتقسيمها عشوائيا الى 12 مجموعه (كل مجموعه بها 60 دجاجه و8 ديوك)، قسمت كل مجموعه الى 4 مكررات (بكل منها 15 أنثى و2 ديك). صممت تجربة عامليه 4 X 3 لتقييم اضافة أربع مستويات من الزيولايت للعليقة (صفر، 10، 15 و20 جرام/ كجم عليقه) وثلاثة مستويات من الزيولايت للفرشة (صفر، 1.5 و2 كجم/ م^2 فرشه) والتداخلات بينهما. استمرت التجربة لمده 16 أسبوع. أظهرت النتائج أن إضافة الزيولايت بمستويات مختلفة لكلاً من العليقة والفرشة أدى لتحسن في صفات الذبيحة والأعضاء الداخلية ومعظم خصائص الدم مقارنة بمجموعة الكنترول. علاوة على ذلك لوحظ أن تغذية الدجاج البياض على مستوى 20 جرام زيولايت/ كجم عليقة والتربية على فرشة مضاف إليها زيولايت بمستوى 2 كجم/ م^2 أعطت أفضل النتائج الخاصة بصفات الذبيحة وخصائص الدم مقارنة بالمعاملات الأخرى ومجموعة الكنترول. وعليه يمكن أن نوصى بإضافة الزيولايت بمعدل 20 جرام زيولايت/ كجم عليقة وكذا للفرشة بمعدل 2 كجم/ م^2 والتداخل بين كلا من Z.R20× Z.L2 في مزارع الدجاج البياض وذلك للحصول على أعلي كفاءة إنتاجية واقتصاديه.

الكلمات المفتاحية: الدجاج البياض، الزيولايت، صفات الذبيحة، خصائص الدم.