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Effect of Natural Zeolite (NZ) of Growth Performance, Immunity Parameters and Gut Histology in Broiler Chicken

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ABSTRACT

The spread of food-borne illnesses through poultry is a major issue in many countries, including Iraq. Bacteria can be present in poultry products if proper hygiene and sanitation protocols are not followed during production and processing. This research aimed to examine the impact of zeolite on performance, immunity parameters, and gut histology. A total 162 one-day old broiler chicks were randomly assigned to a control group and two natural zeolite-supplemented groups, each containing 54 birds (with three replicates/group and 18 chicks/replicate). The group (control) was without any additives, while the other two groups were fed diets containing 1% or 0.5% natural zeolite (NZ). The findings indicated that the groups receiving 1% and 0.5% natural zeolite (NZ) showed significantly ($p<0.05$) higher BW and BWG compared to the group (control). Feed conversion ratio was affected by 0.5% natural zeolite (NZ) supplement compared to the control. There were no significant differences in production index (PI), spleen (%), bursa of Fabricius (%), total WBC, lymphocytes, heterophils and H/L ratio between the natural zeolite (NZ) groups and control. The research revealed that zeolite may have a beneficial effect on gut histology in broiler. Therefore, using zeolite as a feed supplement for poultry could improve performance.

تأثير الزيوليت الطبيعي على أداء النمو ومؤشرات المناعة ونسيج الأمعاء في فروج اللحم

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الخلاصة

انتشار الأمراض المنقولة عن طريق الغذاء من خلال منتجات الدواجن هو قضية كبيرة في العديد من البلدان، بما في ذلك العراق. يمكن أن تكون البكتيريا موجودة في منتجات الدواجن إذا لم يتم اتباع بروتوكولات النظافة والتطهير الصحيحة أثناء الإنتاج والتصنيع. هدفت هذه الدراسة إلى دراسة تأثير الزيوليت على الأداء ومؤشرات المناعة ونسيج الأمعاء لفروج اللحم ROSS. تم توزيع إجمالي 162 فرخ فروج لحم في يومها الأول عشوائيًا على مجموعة سيطرة ومجموعتين مكملتين بالزيوليت الطبيعي، كل منهما يحتوي على 54 طائرًا (مع ثلاث تكرارات (18 طير / تكرار). كانت المجموعة (السيطرة) بدون أي مضافات، في حين تم تغذية المجموعتين الأخريين بأعلاف تحتوي على 1٪ أو 0.5٪ زيوليت طبيعي .(NZ) أظهرت النتائج أن المجموعتين التي تلقتا 1٪ و 0.5٪ زيوليت طبيعي (NZ) أظهرت معدلات نمو والزيادة الوزنية افضل معنوياً ($p < 0.05$) مقارنة بالمجموعة (السيطرة). تأثر معدل كفاءة التحويل الغذائي بوجود مكمل زيوليت طبيعي بنسبة 0.5٪ (NZ)مقارنة بالسيطرة. لم تظهر اختلافات معنوية في مؤشر الإنتاج(PI) ، والطحال (%)، وغدة فابريشيا (%)، والعدد الكلي لخلايا البيضاء، والخلايا اللمفاوية ونسبة H/L بين مجموعتي الزيوليت الطبيعي (NZ) والسيطرة. كشفت الدراسة أن الزيوليت قد يكون له تأثير مفيد على نسيج الأمعاء في فروج اللحم ويمكن أن يحسن استخدام الزيوليت كمكمل غذائي للدواجن الاداء الانتاجي.

كلمات مفتاحية: فروج اللحم، النمو، مؤشرات المناعة، نسيج الامعاء.

INTRODUCTION

The quality of food has become a growing concern, leading to a shift towards producing higher quality food (Hanusova *et al*., 2021). However, the quality of these products is influenced by nutrition, and commercially produced feed may contain harmful substances, to improve feed quality, additives that can absorb these substances are being explored (Morsy, 2018; Anwar, 2023). Additionally, the ban on antibiotic growth promoters in broiler nutrition has led to the search for different products that can enhance growth, feed utilization, and gut health (Ameen and Shaman, 2003). On other side, the broiler sector encounters various hurdles in maintaining well-being, reducing environmental contamination, and enhancing feed efficiency as the demand for premium meat (Barbut and Leishman, 2022; Mohammed and Ameen 2023). Cohuo-Colli *et al.*, (2018) and Pavlak *et al.*, (2022) showed that zeolites offer a solution to some of these challenges by improving nutrient availability and absorption in poultry, reducing ammonia emissions, and improving litter quality. Zeolites are crystalline aluminosilicates with a three dimensional structure (SiO_4 and AlO_4 tetrahedra) by the sharing of oxygen atoms (Bakhtyari *et al*., 2020; Shareef *et al.*, 2023; Samer and Saeid , 2023). The size, shape, and connectivity of the channels and cages in zeolites

are determined by the arrangement of the tetrahedra and the types of cations present, resulting in a wide variety of zeolite structures with different pore sizes and shapes (Li *et al*., 2015).

Zeolites are useful in the development of broiler, according to a study by according to Fathi *et al*. (2018), zeolite can decreased ammonia emissions, and improve broiler production. On other hands, Zha *et al*., (2020) showed that zeolites may possess antibacterial activity that lower the incidence of bacterial infections in poultry, same results funded by Bilal *et al*., (2021) zeolite can reduced Salmonella infection in broilers. Abudabos *et al*., (2018) found that feeding broilers with a diet supplemented with zeolite to increase in villus height and crypt depth in the jejunum, which are indicative of a healthier intestinal mucosa. Another study also reported broilers fed with zeolite improved the gut histology in the ileum of (Zamani Moghaddam *et al*., 2014).

Zhou *et al*. (2014) found that a supplementation diet with zeolites improved broiler performance and the zeolite treatments showed improved growth performance. On other hands, Alharthi *et al*., (2022) showed use of zeolites as mycotoxin binders in poultry feed effectively adsorbed mycotoxins such as aflatoxins, preventing their absorption in the digestive system of poultry, this reduced the negative impact of mycotoxins on broiler chickens' performance. Morsy (2018), and Hanusova *et al*., (2021) using the zeolite in poultry diet and the results showed evidences to improve the performance. The finding of this study would provide the impact of natural zeolite (NZ) on performance, immunity parameters, antioxidant, growth hormone and gut histology in broiler chicken.

MATERIALS AND METHODS

Birds Housing: The project was implemented by department of agriculture branch, North Tikrit, poultry farms, Iraq. A total of 162 one-day old broiler (from the local hatchery). Chicks were divided into three groups, each split into 3 replicates (*n*=18 birds/replicate). Chickens were reared up to five weeks (35 days) of age, the temperature was 30 C^{o} and 75% relative humidity, the lighting program was provided for 23 hours L and 1 hour D per day.

Birds Feed: All feed formulation were evaluated by NRC to supply the nutrient requirements of the broiler Ross 308.

Starter diet = 23% Crude Protein with 3000 kcal ME/kg.

Finisher diet = 20% Crude Protein with 3250 kcal ME/kg

All the birds were allowed to access to the diets and water *ad libitum.*

Experimental groups: groups was Control, diet with 1% natural zeolite (NZ), and diet with 0.5% natural zeolite (NZ).

Performance parameters: The broiler chickens were weighed weekly, to estimate mean body weight (g), body weight gain (g), feed intake (g), and feed conversion ratio (Mohammed and Ameen, 2023).

Biochemical parameters: On day 35, three blood samples were collected from the jugular vein with EDTA tube, and the sample with EDTA was used to determine total white blood cells, heterophils, and lymphocytes. The H/L ratio was determine divided heterophils to lymphocytes.

Sample selected and Histology study: On day 35, 6 chickens randomly selected after 6 hours of feed starvation, Spleen and Fabricius were collected from each sample according to (Wu *et al*., 2013). The duodenal and Ileum samples were collected from six birds per groups, and flushed with formalin 10%, each tissue sample was cut into five sections and placed into a tissue cassette, the processing.

Statistical analysis

The data were analyzed using SAS software (9.0) version nine through one way ANOVA test with a completely randomized design between the groups by Duncan tests ($p<0.05$).

RESULTS AND DISCUSSION

Table 1 results showed that dietary supplementation with zeolite (NZ) at 1% and 0.5% levels had a significant positive effect on body weight and weight gain, compared to the control group which did not receive any zeolite. Furthermore, the broilers fed with 0.5% (NZ) had a significant on body weight and weight gain than those fed with 1% (NZ). According to results in Table 1, the birds group fed with 1% zeolite (NZ) had a significantly higher in feed intake compared to the birds control group and the birds group fed with 0.5% (NZ). Additionally, the feed conversion ratio was significantly better in birds group fed with 0.5% (NZ) compared to the bird's group control and the group fed with 1% NZ Table 1. On another hands, broilers fed with 0.5% zeolite (NZ) had a significantly higher production efficiency index compared to the birds group control and the birds group fed with 1% (NZ) figure 1.

Table-1. Effect of Natural Zeolite (NZ) on broiler performance at 35 days of age.

Performance	Control	Natural Zeolite (NZ)		*p* value
		1 %	0.5 %	
Body weight (g)	2230.67[b]	2301.33[a]	2359.00[a]	<.0001
Body weight gain (g)	1806.66[b]	1838.33[a]	2051.00[a]	0.0002
Feed intake (g)	3218.33[b]	3291.67[b]	3441.33[a]	0.0002
Feed conversion ratio (g/g)	1.78[a]	1.79[a]	1.67[b]	0.0069

[a,b] value with the different superscript in the same column are different ($p<0.05$).

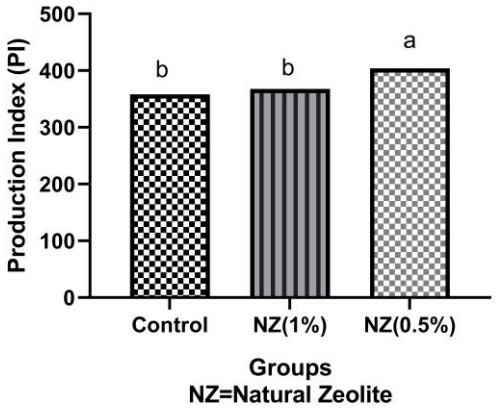


Fig.-1 Effect of Natural Zeolite (NZ) on Production Index (PI) broiler at 35 days of age

The positive impact of dietary zeolite supplementation on broiler performance experiential in this study is reliable with the findings of several previous studies. Abd El-Wahab *et al*., (2015) reported that the inclusion of zeolite in broiler diets improved bird's performance. Youssef *et al*. (2018) observed dietary supplementation with zeolite improved body weight gain and feed conversion ratio in broilers. However, Abd El-Wahab *et al*., (2015) has been suggested that zeolite improve nutrient absorption by improve intestinal morphology and function, and reducing the negative effects of mycotoxins on broiler (Kurtoglu *et al*., 2014). The observation that 0.5% zeolite supplementation was more effective than 1% zeolite supplementation in improving broiler performance is interesting. This findings agrees with the other investigations. According to Kurtoglu *et al*. (2014), 0.5% zeolite was superior to 1% zeolite in minimizing the detrimental effects of aflatoxin on broiler performance. The cause of this phenomena is unknown, however it could be because zeolite at high concentrations prevents other nutrients from being absorbed (Youssef *et al*., 2018; Haglan *et al.*, 2023). The findings are shown in Table (2), which details how (NZ) affected several parameters including the spleen (%), bursa of Fabricius (%), white blood cells, heterophils (%), lymphocytes (%), and H/L ratio (%). The (NZ) treatments did not have a significant effect on spleen (%) and bursa of Fabricius (%) table 2. Similarly, no significant impact was observed on total white blood cells, heterophils (%), lymphocytes (%), and H/L ratio (%) at 35 days of age.

The non-significant effects on immune parameters in broilers supplemented with zeolite (NZ) at 1% and 0.5% levels is consistent with Al-Sagan *et al*. (2018) found that dietary supplementation with zeolite had no significant effect on the total white blood cells, heterophils, and lymphocytes count in broilers, another study El-Katcha *et al*. (2018) found no significant effects of zeolite supplementation on immune organs' relative weight or total leukocyte count in broilers. It is important to note that the short term feeding time used in this study may be responsible for the absence of substantial effects on immunological results. Table 3 and figure 2 shows the intestinal villus height and crypt depth for broilers fed supplementations of 1 and 0.5% NZ. Jejunum VH and the ratio of VH to CD were substantially higher in the 0.5% NZ supplementation

group compared to the control and 1% NZ. The findings revealed no statistically significant differences in the ileum's VH, CD, and VH/CD ratio across all croups. According to a study, adding zeolite to the food of broilers increased the VH and CD in the jejunum, two markers of a healthier intestinal mucosa (Abudabos *et al*., 2018). Another study found that broilers fed zeolite had deeper crypts and has more length villus in the ileum (Zamani Moghaddam *et al*., 2014). Our results indicate that zeolite may have a favorable impact on the gut histology of chicken, which may enhance nutrient absorption and general health. However, broilers given zeolite by Torki *et al*. (2014) did not show any appreciable changes in VH or CD in the jejunum.

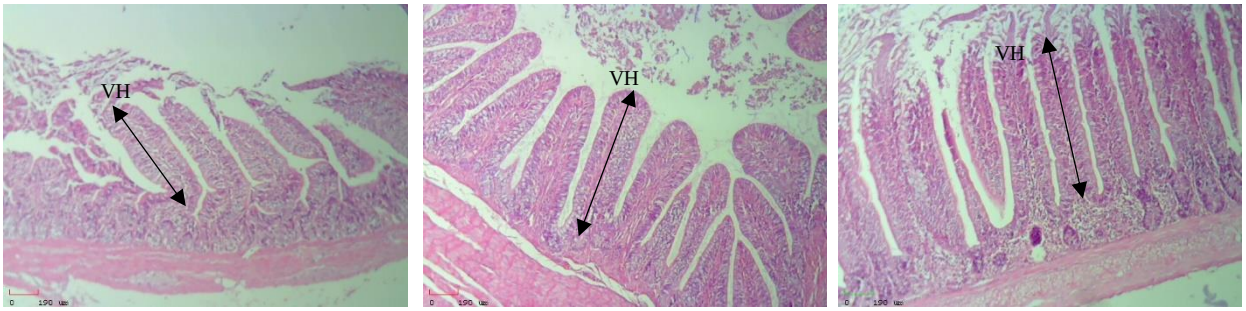
Table-2. Effect of Natural Zeolite (NZ) on spleen (%), bursa of Fabricius (%) and white blood cells, Heterophils, lymphocytes, and H/L ratio at 35 days of age.

Performance	**Control**	**Natural Zeolite (NZ)**		***p* value**
		1 %	**0.5 %**	
Spleen (%)	0.215	0.218	0.209	0.385
Bursa of Fabricius (%)	0.151	0.149	0.152	0.726
WBC x^{10}/L	24.39	23.79	23.82	0.924
Heterophils x^{10}/L	63.98	58.77	62.16	0.630
Lymphocytes x^{10}/L	21.15	21.83	25.31	0.108
H/L Ratio	0.33	0.37	0.40	0.126

Table-3. Villi height and crypt depth of broiler fed different levels of Natural Zeolite (NZ) supplementation.

Performance	**Control**	**Natural Zeolite (NZ)**		***p* value**
		1 %	**0.5 %**	
Jejunum				
Villi height (µm)	670.72^{b}	707.98^{b}	839.3^{a}	0.0010
Crypt depth (µm)	125.82	120.11	123.02	0.4095
Villi height to Crypt depth ratio	5.33^{b}	5.96^{b}	6.82^{a}	0.0068
Ileum				
Villi height (µm)	334.43	332.63	330.06	0.6405
Crypt depth (µm)	79.05	75.26	81.05	0.6922
Villi height to Crypt depth ratio	4.32	4.42	4.08	0.8826

^{a,b} value with the different superscript in the same column are different ($p<0.05$).



(A) Histological Section of broiler intestine of control group (HandE stain X40)

(B) Histological Section of broiler intestine with (1%) of Natural Zeolite (NZ) (HandE stain X40)

(C) Histological Section of broiler intestine with (0.5%) of Natural Zeolite (NZ) (HandE stain X40)

Fig.-2. Effect of Natural Zeolite (NZ) on intestine histology at 35 day s of age.

CONCLUSION

Dietary supplementation with zeolite at 0.5% and 1% levels had a significant positive. However, 0.5% zeolite was more effective than 1% zeolite in improving broiler performance. These findings suggest that zeolite can be used as a dietary supplement to enhance the growth performance of broilers, and may have a beneficial effect on gut histology in broiler, which could potentially improve nutrient absorption and health.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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