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Toxicological evaluation of locally sourced Jordanian zeolite as a safe feed additive in broiler diets: Histopathological and hematological perspectives

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Abstract

Zeolite is a naturally occurring, highly absorbent mineral primarily composed of aluminosilicate. It is commonly used in animal feed due to its beneficial properties. Jordan possesses abundant natural deposits of zeolite, particularly rich in phillipsite and chabazite. However, no previous studies have been conducted in Jordan to assess the pathological effects of the local zeolite as a feed additive in broilers. This study aimed to assess the safety and evaluate the health impacts of locally sourced Jordanian zeolite inclusion in poultry diets. A total of 200 broiler chicks were randomly assigned to five dietary treatment groups containing 0%, 2%, 5%, 10%, and 15% Jordanian zeolite. The feeding trial lasted for 32 days. At the end of the experiment, postmortem examinations, histopathological analyses, and different hematological parameters were performed. Neither the postmortem nor the histopathological examinations revealed any significant abnormalities in the organs examined across all dietary groups. Biochemical parameters showed no statistically significant differences ($P > 0.05$). Similarly, all measured hematological parameters remained within normal physiological ranges. The findings demonstrate that Jordanian zeolite, rich in phillipsite and chabazite, is non-toxic under tested conditions and can be safely incorporated into broiler diets at levels up to 15%. Its use as feed additive shows promises as a functional and sustainable component in poultry nutrition without adverse effects on organ health or blood parameters.

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Introduction

Zeolite is a natural, powerful absorbent primarily consisting of alumino-silicate. Natural zeolites used in animal feeding are often metastable aluminosilicate minerals with a microstructure formed by a 3D interconnected network of SiO_4 and AlO_4 tetrahedra. This crystalline structure is characterized by geometrically regular, porous, cage-like bodies filled with interstitial cations (freely moving, exchangeable metallic cations, alkali and alkaline-earth metals) and polarizable or exchangeable (water- or "hydrated") ions. It completely absorbs far more water than its weight without losing performance (1). Zeolites can lose their adsorbed water or other substances by heating, dehydrating to lower-silica-content aluminosilicates, or forming allophanes from natural aluminosilicates to detoxify non-adsorptive or toxic compounds (2). Natural zeolites effectively detoxify economic pollutants, carcinogenic contaminants, and heavy metals, and encourage animal welfare and performance at higher growth rates (3). Activity-led structure differences in gaseous absorption capacities and chemical hydration. No other materials have structural properties that act on the body surface to draw in toxins and expel excess salts and hormones (4). There are 50 types of natural zeolites, but nine are used in animal feeding (5). Clinoptilolite is the most used type and the primary zeolite tested worldwide (6). Other tested or commonly used zeolite types include mordenite and phillipsite (7). The local zeolites are characterized by their mineral composition—primarily phillipsite and augite, with minor amounts of albite,

hematite, calcite, chabazite, and faujasite (8). Zeolites have been used in animal nutrition since the mid-1960s (9). Since then, many researchers have demonstrated that the dietary inclusion of zeolites improves average daily gain and/or feed conversion in pigs, calves, sheep, and broilers (10-13). EL-Nile (14) studied the effects of zeolite supplementation, in natural and Nano-Form, on ruminal fermentation characteristics, milk chemical composition, and milk production in dairy goats. The results revealed that the nanoform of zeolite had greater effects as a feed additive than the natural forms (14). A study was conducted to evaluate the use of natural zeolite as a feed additive in laying hens, and the results showed significant improvements in the eggshell ratio and eggshell density (15). Zeolites at concentrations ranging from 0 to 20 g/kg were used to evaluate the effects of zeolite as a feed additive on broiler growth performance and meat quality. There was a numerical increase in production performance with increasing the dose up to 10 g of zeolite, but it was not statistically significant (16). In addition, a study conducted in Jordan, using bentonite nanoparticles to assess the effect of zeolite on broiler performance and carcass characteristics, revealed that concentrations of 1%, 2%, and 3% enhanced broiler growth performance with no change in carcass characteristics (17).

No previous studies have been conducted in Jordan to assess potential toxicity and pathological changes associated with the use of local zeolite as a feed additive in broilers. The present study aimed to evaluate the potential use of locally sourced Jordanian zeolites as feed additives in broiler chickens. The study focused on assessing potential toxic effects on growth performance and biochemical responses in broilers to determine the safety and optimal inclusion level of these natural minerals in poultry diets.

Materials and Methods

Ethical approval and location

The study was conducted at the Animal House Facility / Poultry Research Unit, Faculty of Veterinary Medicine, Jordan University of Science and Technology (JUST). The study was read and approved by the Institutional Animal Care and Use Committee (IACUC) at JUST under approval number 16/4/12/738.

Zeolite source and characterization

Natural zeolite from the Alazraq area, Jordan, was milled (<250 µm) and stored airtight. Mineralogy was determined by powder XRD (Cu Kα, 40 kV, 30 mA, 5–70° 2θ; MEMR002-2020) at the Laboratories of Quality Directorate, Ministry of Energy and Mineral Resources, Amman.

X-Ray Diffraction profiles

Zeolite A: Phillipsite, Augite, Albite (major); Hematite, Calcite, Faujasite (minor). Zeolite B: Phillipsite, Augite (major); Hematite, Chabazite (minor); Faujasite, Calcite (trace). Zeolite C: Phillipsite, Augite, Albite, Officinite (major); Hematite (minor); Calcite (trace). Phillipsite predominated in all samples. ICP-MS showed that Pb, Cd, As, and Hg were below the feed additive limits. Moisture (at 105 °C for 4 h) and microbiological counts met hygiene standards.

Birds and housing

Two hundred broiler chicks (1 d old; mixed sex; 40 ± 2 g) were randomly allocated to 15 floor pens. Temperature was reduced from 32 ± 1 °C to 22 °C by d 21 (RH 55–65%); lighting 23L:1D (wk 1) then 20L:4D. Feed and water were provided ad libitum.

Experimental design and diets. Five treatments (0, 2, 5, 10, and 15% zeolite) in a completely randomized design with 3 replicates per treatment were tested. Diets were corn–soybean meal–based, iso-nitrogenous and iso-caloric, and met the local broiler requirements as shown in Table 1.

Table 1: Poultry feed formulation (percentage composition). All values represent the inclusion rate as a percentage of the total diet on an as-fed basis.

Item	Starter	Grower
Corn	48	52
Soybean meal	37	31
Wheat	10	12.5
Oil	1	1.3
Calcium Carbonate	1.3	1.2
Lysine	0.6	0.55
Methionine	0.37	.35
Threonine	.16	0.14
Vitamin Premix	0.1	0.1
Mineral Premix	0.1	0.1
Choline	0.075	0.075
Sodium Bicarbonate	0.15	0.14
Salt	0.26	0.28

Growth Performance and Production Efficiency

Birds were checked twice daily for clinical signs and mortality. Pen Body Weight (BW) and Feed Intake (FI) were recorded on days 0, 7, 14, 28, and 33. Based on that, Body Weight Gain (BWG), Average Daily BWG (ADBWG), Feed Conversion Ratio (FCR), and Average Daily Feed Intake (ADFI) were calculated. The parameters were determined for each feeding period and for the entire rearing period. Moreover, the European Production Efficiency Factor (EPEF) as an indicator of production efficiency was calculated as follows: EPEF = (BW (kg) * Livability (%) X 100) / (FCR X Age in days) (18).

Blood sampling and analysis

At the end of the experiment, on day 33, 3 birds were randomly selected from each pen with the closest mean BW. They were bled from the brachial vein (9 birds/treatment) and used for hematology and biochemistry determinations.

The hematology parameters include Red Blood Cell count (RBC), Hematocrit (Hct), White Blood Cell count (WBC), and Differential Cell Count (DCC). Biochemical parameters include: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Total bilirubin (TBIL), Total bile acid (TBA), creatinine (CRE), total protein (TP), albumin (Alb), globulin (Glo), glucose (GLU), cholesterol (CHOl), amylase (AMY), sodium (Na), potassium (K), calcium (Ca) and phosphorus (P).

Postmortem examinations

On the same day of blood collections, at day 33, the same birds that were bled were humanely killed. A complete postmortem examination was carried out. Representative tissue samples were cut from different body organs. These organs include: the liver, kidney, heart, spleen, bursa, proventriculus, gizzard, pancreas, and intestines. The tissue samples were fixed in 10% neutral buffered formalin for 48 hours.

Histopathological examinations

The formalin-fixed tissues were cut and processed routinely in an automatic tissue processor and embedded in paraffin blocks. Then the blocks were sectioned at 4–5 µm thickness and stained with hematoxylin and eosin stain (H&E). A certified anatomic veterinary pathologist examined the slides unthinkingly, and the results were recorded.

Statistical analysis

The data obtained were analyzed by one-way ANOVA (pen = experimental unit), blood and pathology data by bird. Tukey's test was used when $P < 0.05$ (19).

Results

Hematological parameters

Across the five groups, WBC counts and differential parameters remained within their respective reference intervals, with only a few outliers noted. In Groups 1–3, all measured values for WBCs, heterophils, lymphocytes, monocytes, eosinophils, and basophils were consistently within the reference intervals, indicating stable distributions and 100% compliance. Group 4 displayed higher WBC values (mean 17.0, range 10.5–29.0) with one outlier at 29.0, as well as single outliers for monocytes (1.8) and eosinophils (2.8), resulting in 89% compliance for these parameters. Group 5 demonstrated values well within expected limits, except for one eosinophil outlier (2.0). Overall, the results indicate that most hematological parameters were stable and aligned with reference intervals across all groups, with only minimal deviations due to isolated outliers in Groups 4 and 5 (Table 2).

Hematological Profiles

PCV and RBC counts for broilers across the five treatment groups (G1–G5) conformed to the established avian RI reported by (21), namely PCV: 24–42% and RBC: 1.6–3.1 $\times 10^6/\mu L$, as shown in Tables 3 and 4, respectively. Groups G2 (32.2%), G3 (28.7%), and G4 (29.9%) demonstrated complete compliance with the reference interval (100%). In contrast, G1 (29.0%) and G5 (27.9%) each had a single subclinical outlier at 22% and 23%, respectively, reducing compliance to 89% and 88%, respectively. These deviations were mild and not indicative of overt anemia. RBC concentrations were similarly robust, with all groups achieving 89–100% compliance with reference values. G2 presented one mildly polycythemic outlier ($3.28 \times 10^6/\mu L$), while G5 exhibited the lowest mean RBC count ($2.11 \times 10^6/\mu L$), consistent with its marginally reduced PCV. Despite these minor deviations, no hematological evidence of systemic pathology was observed.

Calculation of H-to-L Ratio (H:L Ratio)

Using absolute counts ($HX10^3$ and $LX10^3$ in $\times 10^9/L$), the H:L ratio is calculated as: H:L = (H count) / (L count). Across the five groups, the H:L ratio remained below the threshold of 1.0, indicating normal physiological status and low stress in most individuals (Table 5).

Biochemical parameters

The biochemical assessment of the five treatment groups (G1–G5) revealed consistent serum protein and bilirubin concentrations across all cohorts (Table 6). Total protein (TP) values ranged from 3.21 to 3.40 g/dL, with mean ± SD levels of 3.29 ± 0.31 g/dL in G1 and 3.21 ± 0.29 g/dL in G5. Albumin (Alb: 1.61–1.73 g/dL) and globulin (Glo: 1.51–1.70 g/dL) demonstrated minimal intergroup variability, while the albumin-to-globulin ratio (A/G) remained within a narrow range (1.00–1.14). Total bilirubin (TBIL) concentrations were uniformly low (0.19–0.24 mg/dL). Importantly, no group exhibited clinically abnormal extremes (e.g., G4 TP maximum: 4.4 g/dL; G2 Alb minimum: 1.3 g/dL), indicating preservation of protein metabolism and hepatic function under the experimental conditions used.

One-way ANOVA confirmed the absence of statistically significant differences (all $P > 0.05$) across the 17 biochemical parameters examined (Table 7). Representative biomarkers included TP ($F = 0.41$, $P = 0.80$), Alb ($F = 1.20$, $P = 0.324$), Glo ($F = 0.85$, $P = 0.503$), A/G ratio ($F = 2.10$, $P = 0.098$), and TBIL ($F = 1.65$, $P = 0.178$), all of which demonstrated intergroup homogeneity. Enzyme activities (ALT, ALP, AMY), metabolic markers (CHOL, GLU, CRE), electrolyte concentrations (Ca, P, K, Na), and derived indices (Ca×P, Na/K, TBA) similarly exhibited no group-wise divergence, with all values remaining above the significance threshold ($P > 0.05$).

Table 2: Leukocyte parameters across treatment groups

Group	Parameter	Mean	Median	Range	% Samples within RI
G1	WBC	12.3	11.5	10.0–16.5	100%
	H	4.8	4.7	3.5–6.4	100%
	L	5.2	4.8	3.0–8.0	100%
	M	0.7	0.6	0.5–1.6	100%
	E	0.5	0.4	0.3–1.6	100%
	B	0.5	0.5	0.2–1.1	100%
G2	WBC	13.2	12.0	10.0–15.5	100%
	H	5.0	4.8	3.6–8.2	100%
	L	5.4	5.1	3.5–6.3	100%
	M	0.8	0.7	0.4–1.6	100%
	E	0.9	0.9	0.7–1.7	100%
	B	0.6	0.6	0.3–1.1	100%
G3	WBC	14.1	13.5	12.0–18.5	100%
	H	5.2	4.9	3.6–10.5	100%
	L	6.1	5.8	4.8–8.1	100%
	M	0.8	0.7	0.4–1.4	100%
	E	0.6	0.5	0.3–2.2	100%
	B	0.6	0.5	0.3–0.9	100%
G4	WBC	17.0	15.5	10.5–29.0	89% (1 outlier: 29.0)
	H	5.8	5.3	3.0–15.0	100%
	L	7.8	7.2	5.2–9.6	100%
	M	0.9	0.8	0.6–2.8	89% (1 outlier: 2.8)
	E	1.1	0.9	0.3–2.8	89% (1 outlier: 2.8)
	B	0.8	0.7	0.3–1.2	100%
G5	WBC	13.9	13.0	12.0–16.5	100%
	H	4.5	4.2	2.6–5.9	100%
	L	6.5	6.1	5.4–9.7	100%
	M	0.9	0.8	0.4–2.0	100%
	E	1.0	0.9	0.7–2.6	89% (1 outlier: 2.6)
	B	0.5	0.5	0.1–1.2	100%

Values expressed as mean, median, and range); RI = Reference Interval (20): WBC (10.0–38.4), Heterophils (H) (1.5–16.2), Lymphocytes (L) (5.1–18.9), Monocytes (M) (0.0–2.1), Eosinophils (E) (0.0–2.2), and Basophils (B) (0.0–3.1) $\times 10^9$/L.

Table 3: Summary of PCV data across experimental groups

Group	Mean PCV (%)	Range (%)	% Samples within RI	Reference Interval
G1	29.0±5.4	22–38	89% (8/9)	24–42
G2	32.2±5.3	25–41	100% (9/9)	24–42
G3	29.7±2.5	26–33	100% (9/9)	24–42
G4	27.7±3.7	24–36	100% (9/9)	24–42
G5	27.9*±4.3	23–38	88% (7/8)	24–42

*Excludes G5-7 (missing data).

Table 4: Summary of RBC count data across experimental groups

Group	Mean RBC ($\times 10^6$/µL)	Range ($\times 10^6$/µL)	% Samples within RI	Reference Interval
G1	2.32±0.4	1.76–3.04	100% (9/9)	1.6–3.1
G2	2.6±0.4	2.00–3.28	89% (8/9)	1.6–3.1
G3	2.4±0.2	2.08–2.64	100% (9/9)	1.6–3.1
G4	2.2±0.3	1.92–2.88	100% (9/9)	1.6–3.1
G5	2.2±0.3	1.84–3.04	100% (8/8)	1.6–3.1

*Excludes G5-7 (missing data).

Table 5: H and L counts with derived H:L ratios

Group	Sample	HX10³ (×10⁹/L)	LX10³ (×10⁹/L)	H:L Ratio	Interpretation (vs. Normal <1)
	1	3.4	4.5	0.76	Normal (low stress)
	2	4.2	5.76	0.73	Normal
	3	6.375	4.75	1.34	Elevated (stress/immune)
	4	4.5	7.95	0.57	Normal
G1	5	4.6	3	1.53	Elevated
	6	4.83	5.29	0.91	Normal
	7	6.25	3.625	1.72	Elevated
	8	4.16	7.15	0.58	Normal
	9	5.445	7.755	0.70	Normal
Mean				0.88	
	1	3.96	5.06	0.78	Normal
	2	4	3.5	1.14	Elevated
	3	4.025	5.635	0.71	Normal
	4	4.83	4.14	1.17	Elevated
G2	5	3.6	4.32	0.83	Normal
	6	8.215	4.185	1.96	Elevated
	7	4.6	4.14	1.11	Elevated
	8	4.03	6.37	0.63	Normal
	9	5.365	5.945	0.90	Normal
Mean				1.0	
	1	4.05	6.345	0.64	Normal
	2	10.545	4.81	2.19	Elevated
	3	4.32	5.88	0.73	Normal
	4	5.74	6.72	0.85	Normal
G3	5	4.42	7.41	0.60	Normal
	6	5.4	5.805	0.93	Normal
	7	4.5	5.125	0.88	Normal
	8	4.48	7.84	0.57	Normal
	9	3.625	8.12	0.45	Normal
Mean				0.92	
	1	3.36	5.25	0.64	Normal
	2	3.08	7.7	0.4	Normal
	3	5.58	6.975	0.8	Normal
	4	6.84	7.2	0.95	Normal
G4	5	15.08	9.57	1.6	Elevated
	6	4.995	5.94	0.84	Normal
	7	3.72	8.99	0.41	Normal
	8	6.665	7.44	0.90	Normal
	9	5.95	6.65	0.89	Normal
Mean				0.88	
	1	4.785	9.735	0.49	Normal
	2	5.4	5.55	0.97	Normal
	3	3.5	8.12	0.43	Normal
	4	4.44	5.4	0.82	Normal
G5	5	3.5	6.625	0.53	Normal
	6	5.89	6.045	0.97	Normal
	7	4.16	5.46	0.76	Normal
	8	2.64	6.36	0.41	Normal
	9	4.75	5.875	0.81	Normal
Mean				0.66	

Comparison of PCV and RBC Values with Bounous et al. (20) Reference Intervals.

Table 6: Descriptive statistics for biochemical parameters in five groups of chickens

	G1					G2					G3				
Paramet	Mean	SD	Min	Median	Max	Mean	SD	Min	Median	Max	Mean	SD	Min	Median	Max
TP	3.3	0.31	2.8	3.3	3.7	3.3	0.28	3.1	3.2	4.0	3.2	0.25	2.9	3.2	3.8
Alb	1.6	0.1	1.5	1.6	1.8	1.7	0.09	1.5	1.7	1.8	1.7	0.16	1.5	1.7	2.0
Glo	1.7	0.26	1.2	1.6	2.0	1.7	0.23	1.4	1.6	2.2	1.5	0.15	1.3	1.5	1.8
A/G	1.0	0.16	0.8	1.0	1.3	1.0	0.12	0.8	1.0	1.2	1.2	0.14	1.0	1.1	1.4
TBIL	0.19	0.06	0.12	0.17	0.3	0.2	0.05	0.15	0.23	0.29	0.23	0.04	0.18	0.23	0.28
ALT	4.4	2.35	1	4	7	4.89	0.93	3	5	6	4	1.66	2	3	7
ALP	1202.89	424.84	705	1141	1936	1043.56	268.42	484	1029	1322	1522.56	451.50	819	1524	2000
AMY	587.11	148	408	567	805	629.44	140.31	461	573	869	578.67	106.96	437	576	742
CHOl	159.11	22.36	130	162	198	150	5.88	137	151	156	166.44	20.70	145	160	211
GLU	298	32.48	253	298	370	290	24.43	251	294	337	270.11	25.88	235	267	301
CRE	0.53	0.22	0.31	0.44	0.99	0.73	0.15	0.44	0.69	0.93	0.59	0.18	0.41	0.55	0.94
Ca	10.8	1.13	9.4	10.8	13.5	9.92	0.63	9.3	9.7	10.9	9.92	0.75	8.7	10.1	11
P	7.34	0.82	5.86	7.27	8.33	8.18	0.89	6.6	8.02	9.5	7.20	1.41	5.33	7.44	9.65
Ca*P	78.86	6.56	70	79	90	81.11	9.52	66	80	99	71.0	11.88	54.37	71	97
K	6.96	0.80	5.67	6.96	8	7.03	1.02	5.3	6.87	8	6.99	0.83	5.72	6.96	8
Na	148.89	3.02	144	149	154	149.33	2.65	146	149	154	147.89	4.11	144	147	155
Na/K	21.56	3.09	17	22	27	20.56	4.59	14	22	28	21	2.92	16	21	25
TBA	56.87	15.31	37.9	52.7	79.4	55.62	12.12	37.2	61.2	73.2	68.02	13.23	47.1	65.7	86
BUN	1.22	0.65	0.31	0.99	2.22	1.83	0.75	0.44	1.98	2.8	1.15	0.40	0.32	1.29	1.64
BUN/C	2.22	0.67	1	2	3	2.56	1.13	1	3	4	2.17	1.06	0.49	2	4
	G4					G5									
TP	3.2	0.48	2.3	3.4	4.0	3.4	0.56	2.8	3.3	4.4					
Alb	1.6	0.19	1.3	1.6	1.8	1.7	0.2	1.4	1.7	2.0					
Glo	1.6	0.34	1.0	1.6	2.3	1.7	0.34	1.4	1.6	2.4					
A/G	1.0	0.18	0.7	1.1	1.3	1.0	0.14	0.8	1.1	1.3					
TBIL	0.2	0.06	0.12	0.21	0.3	0.2	0.05	0.13	0.18	0.26					
ALT	4.11	1.83	2	4	8	3.67	2	1	4	6					
ALP	1403.13	290.46	868	1456	1768	1356.78	301.43	1009	1249	2000					
AMY	491.33	205.58	62	513	801	623.67	124.16	424	643	740					
CHOl	147.67	18.28	124	146	179	165.78	26.67	138	153	209					
GLU	279.89	25.93	234	290	308	273.89	21.49	230	275	299					
CRE	0.55	0.25	0.31	0.43	0.96	0.72	0.22	0.33	0.79	0.97					
Ca	10.34	1.02	8.9	10.3	12	9.92	0.80	8.3	10	10.9					
P	8.29	1.50	6.55	8.19	11.07	7.23	0.95	5.8	6.92	9.03					
Ca*P	86.61	22.65	60	88	133	71.70	11.56	57	69	98					
K	7.01	1.09	5.51	7.47	8	6.65	1.01	5.33	6.24	8					
Na	148.78	5.67	136	151	155	146.33	2.96	140	148	149					
Na/K	19.89	5.11	11	20	28	21.89	4.26	14	24	27					
TBA	64.87	16.05	31.8	72.1	77.9	63.56	16.94	41.4	58.1	87.5					
BUN	1.29	0.64	0.31	1.02	2.22	1.63	0.705	0.71	1.58	2.61					
BUN/C	2.78	1.48	1	2	6	2.56	1.42	1	3	5					

Body Weight (BW) and Weight Gain

Initial body weights (Day 1) did not differ significantly among the experimental groups (40.15–40.25 g; $P > 0.05$), confirming uniformity at the start of the trial. By day 33, however, significant differences were evident. The control group (G1) achieved the greatest final BW (2343.33 g), followed closely by G2 (2300.33 g) and G3 (2280.00 g). In contrast, G4 (2220.67 g) and G5 (2146.67 g) exhibited significantly lower final BW compared with G1 ($P < 0.05$) (Table 8). A similar trend was observed for cumulative weight gain (Day 1–33). G1 recorded the highest gain (2303.13 g), while G4 (2180.42 g) and G5 (2106.44 g) demonstrated significantly reduced gains relative to the control ($P < 0.05$) (Table 8).

Feed Utilization

Cumulative feed intake was identical across all groups (3690 g; $P > 0.05$), suggesting that treatment effects were not attributable to differences in voluntary feed consumption. Feed conversion ratio (FCR) values, however, revealed differences in feed efficiency. G1 (1.60) demonstrated the most efficient feed utilization, while G4 (1.69) was

moderately efficient. Conversely, G3 and G5 exhibited the poorest FCR (1.75), indicating less efficient conversion of feed into body mass (Table 8).

Production Efficiency

The European Production Efficiency Factor (EPEF) provided a composite measure of productivity. G1 achieved the highest score (448.98), confirming its superior overall performance. Efficiency declined progressively across the treatment groups, with G2 (432.37), G3 (421.72), G4 (402.72), and G5 (391.61). The reductions in G4 and G5 were statistically significant compared with G1 ($P < 0.05$) (Table 8).

Postmortem and histopathological findings

Representative birds from each replicate were subjected to thorough postmortem examinations. The birds were in good nutritional body conditions with no evidence of postmortem autolysis. The examined broiler chicken across all experimental groups exhibited no significant gross pathological abnormalities. All organs were within normal limits. Histopathological examination of the tissue sections from representative organs of all examined postmortem broiler chickens revealed no significant histopathological lesions. The tissues were histologically normal (Figures 1-11).

Table 7: One-way ANOVA Results for Biochemical Parameters across Five Groups of Chickens

Parameter	F-value	P-value	Significant (P<0.05)	Conclusion
TP	0.41	0.80	No	NS
Alb	1.20	0.32	No	NS
Glo	0.85	0.50	No	NS
A/G ratio	2.10	0.09	No	NS
TBIL	1.65	0.17	No	NS
ALT	0.59	0.67	No	NS
ALP	2.42	0.06	No	NS
AMY	1.24	0.31	No	NS
CHOl	1.70	0.17	No	NS
GLU	1.73	0.16	No	NS
CRE	1.86	0.14	No	NS
Ca	1.76	0.16	No	NS
P	2.0	0.11	No	NS
Ca*P	2.11	0.10	No	NS
K	0.25	0.91	No	NS
Na	0.86	0.49	No	NS
Na/K	0.34	0.85	No	NS
TBA	0.99	0.42	No	NS
BUN	1.86	0.14	No	NS
BUN/CRE	0.42	0.80	No	NS

NS: Not Significant.

Table 8: Indices of growth performance and production efficiency in broiler chickens under different treatments (G1–G5)

Treatment	BW Day 1	BW Day 33	BW gain	FI	FCR	EPEF
G1	40.20	2343.33	2303.13	3690	1.60	448.98
G2	40.21	2300.33	2260.08	3690	1.63	432.37
G3	40.15	2280.00	2239.75	3690	1.75	424.64
G4	40.25	2220.67*	2180.42*	3690	1.69	402.72
G5	40.25	2146.67*	2106.44*	3690	1.75	391.61

*P value <0.05.

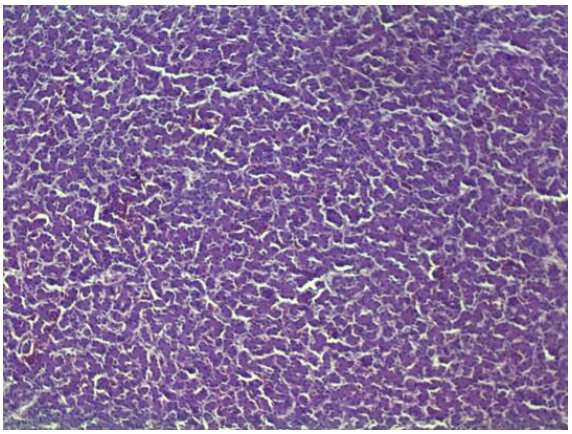

Figure 1: Normal hepatic histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 10X.

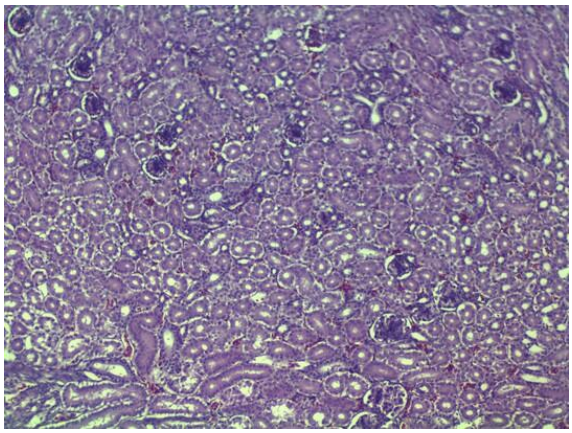

Figure 2: Normal renal histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 4X.

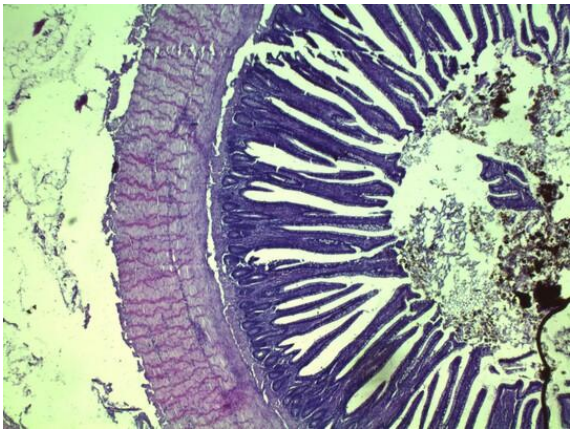

Figure 3: Normal intestinal histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 4X.

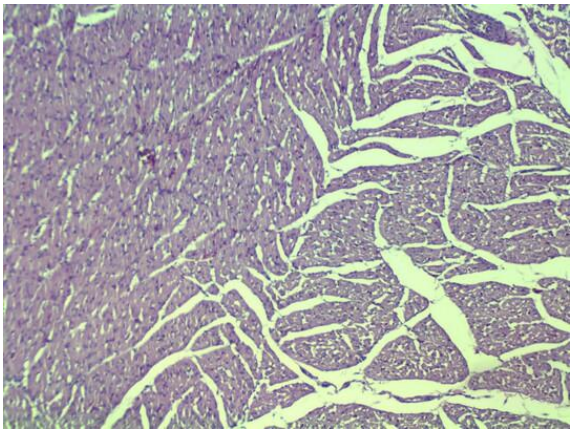

Figure 4: Normal cardiac histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 10X.

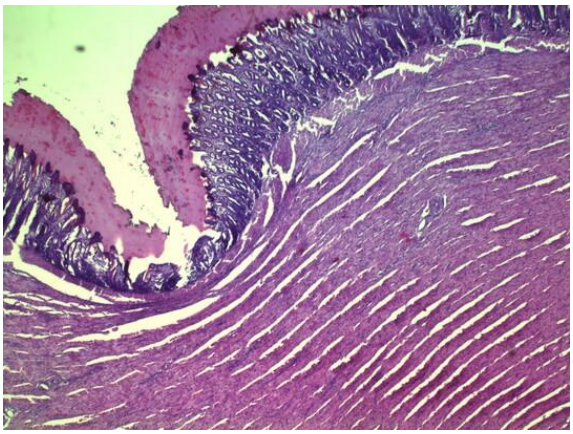

Figure 5: Normal gizzard histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 4X.

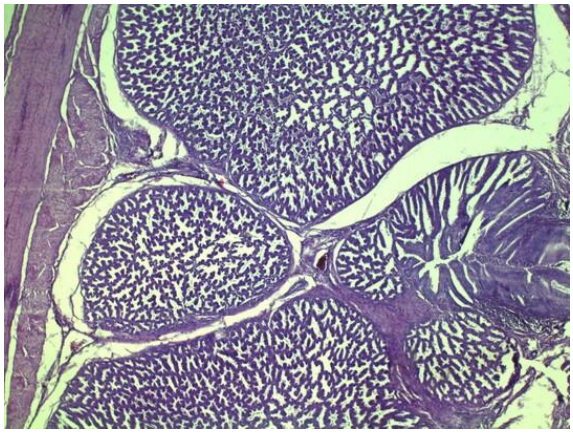

Figure 6: Normal proventriculus histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 4X.

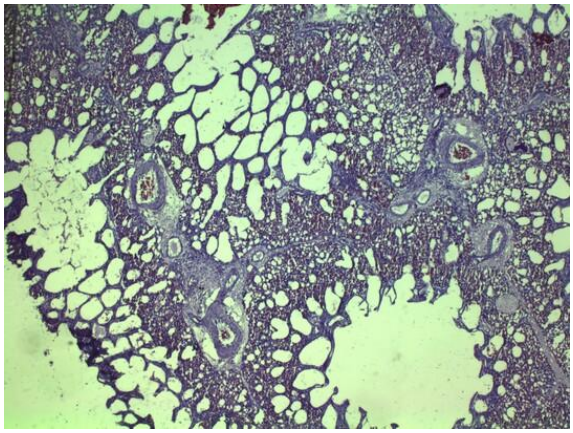

Figure 7: Normal lung histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 4X.

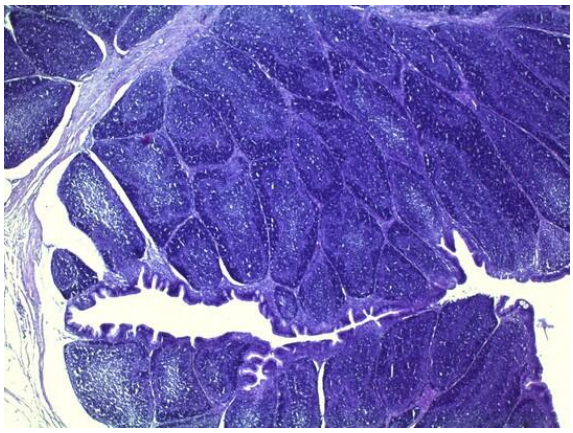

Figure 8: Normal bursa of Fabricius histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 4X.

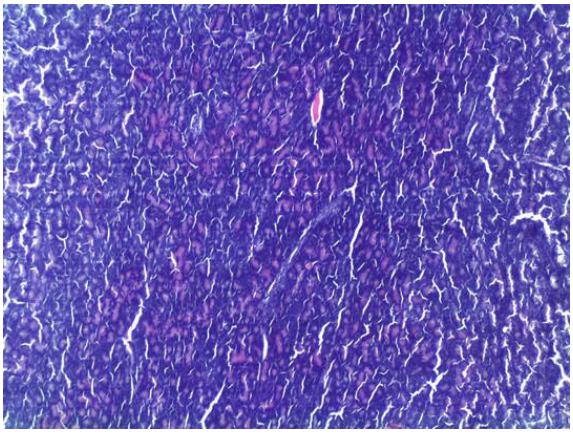

Figure 9: Normal pancreas histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 4X.

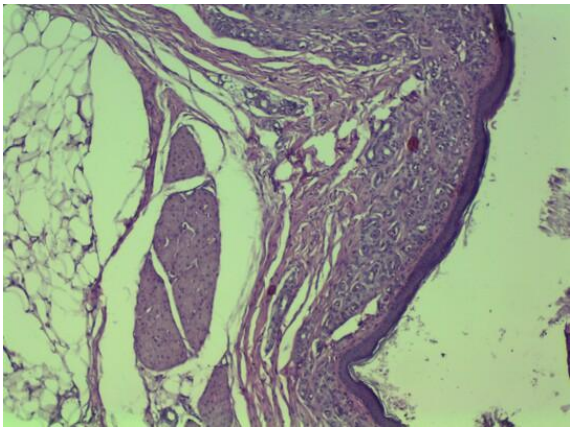

Figure 10: Normal Skin histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 4X.

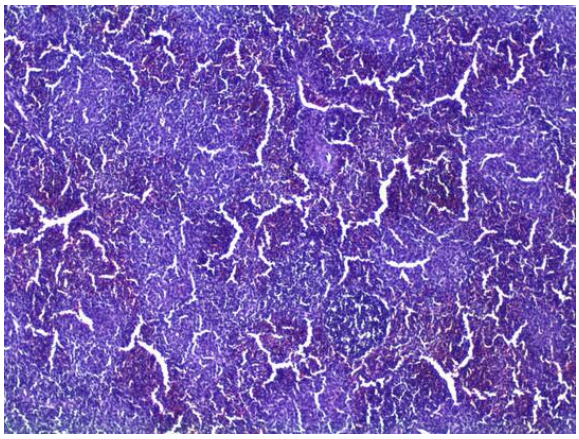

Figure 11: Normal spleen histology in broiler chickens (*Gallus gallus domesticus*) fed 15% zeolite containing diet (Group 5). H&E. 4X.

Discussion

The present study evaluated the effects of zeolite supplementation as a feed additive at different concentrations (0, 2, 5, 10, and 15%) on the growth performance, biochemical parameters, hematological parameters, and pathological effects in broiler chickens. Overall, the findings demonstrated that while growth efficiency was influenced by treatment, systemic biochemical and hematological parameters, as well as tissue stability, were maintained across groups. The hematological assessment of the examined broilers showed that all groups had leukocyte values within the established RIs for healthy birds. Total WBC counts (10.0–29.0 $\times 10^9$/L) and differential subsets (H: 2.6–15.1; L: 3.0–9.7 $\times 10^9$/L) were consistently in line with published RIs, suggesting a normal immune status across the flock. The average H:L ratio was 0.8 (range: 0.4–2.2), indicating low-stress conditions, as ratios below 1 are typically associated with unstressed poultry (21). Nevertheless, a few exceptions were observed, including one elevated H:L ratio in G3 (2.2) and sporadic increases in monocytes and eosinophils (three samples exceeding RIs). These findings could reflect short-term inflammatory responses or individual stress reactions (22).

Overall, 89% of samples fell within expected RIs for all measured parameters. Outliers, such as higher WBC values in G4 and elevated eosinophils in G5, highlight the need for careful interpretation in context. Slight deviations in avian leukograms can often reflect normal physiological variation rather than underlying disease (22). The role of the H:L ratio as a stress indicator was further supported, with 84% of samples showing values below 1 consistent with well-managed conditions (23). In summary, hematological values confirmed a healthy status across the groups.

The biochemical findings indicate that the experimental treatments had no measurable effects on the systemic biochemical status of the broiler chickens examined. The maintenance of biochemical stability across groups supports the conclusion that protein synthesis, hepatic function, renal clearance, and electrolyte balance remained physiologically uncompromised. The lack of treatment-related perturbations in these biomarkers, despite observed differences in growth performance, suggests that the dietary modifications influenced growth efficiency without inducing overt systemic or metabolic stress under a well-managed rearing system. Similar results have been reported by (24), who noted that dietary interventions may reduce growth efficiency without perturbing baseline biochemical markers.

The present study demonstrated that dietary treatments significantly influenced the growth performance and production efficiency of examined broilers. Although the initial body weights were uniform across groups, substantial differences emerged by day 33, indicating that the observed effects were attributable to the dietary interventions rather than baseline variation.

The superior growth performance of the control group (G1) highlights the adequacy of the standard diet in supporting optimal broiler development. In contrast, the reduced final BW and weight gain observed in G4 and G5 suggest that these dietary treatments may have limited nutrient availability or utilization. Previous studies have shown that suboptimal nutrient profiles, particularly with respect to amino acid balance and energy density, can impair growth performance and carcass yield in broilers (23,25).

Feed intake did not differ significantly among treatments, implying that variations in voluntary consumption did not drive the performance differences. Instead, efficiency of nutrient conversion was the critical factor. The lowest FCR in G1 supports this interpretation, as efficient feed conversion is a key determinant of profitability in commercial poultry production (26). Although G4 showed a favorable FCR compared with G3 and G5, its reduced BW and EPEF suggest that efficiency gains did not translate into proportional improvements in overall productivity.

The EPEF results provide further confirmation of the negative impact of dietary interventions in G4 and G5. These groups exhibited significantly lower production efficiency than the control, reflecting a combination of reduced growth rate, suboptimal feed efficiency, and lower final BW. Such declines in EPEF have direct economic implications, as they indicate higher per-unit production costs. It has been reported that zeolite supplementation at higher inclusion levels (≥3%) impairs production efficiency by reducing nutrient bioavailability and suppressing feed intake, thereby leading to decreases in body weight gain and EPEF (27). These findings are consistent with the reduced performance observed in the present study for groups G4 and G5.

The current study demonstrated that dietary inclusion of locally sourced Jordanian zeolite at concentrations ranging from 2% to 15% did not induce any significant gross or histopathological changes in the major visceral organs of broiler chickens. Both postmortem findings and histological assessments confirmed normal tissue architecture across all experimental groups, underscoring the safety of this zeolite variant in poultry nutrition.

The zeolite utilized was derived from natural deposits in Jordan. It was mineralogically characterized by the predominance of phillipsite and chabazite, with minor constituents including clinoptilolite, augite, albite, calcite, and hematite (28,29). While the majority of existing poultry studies have focused on clinoptilolite, phillipsite-rich zeolites exhibit comparable physicochemical properties, most notably a high cation-exchange capacity and a porous crystalline matrix, thereby facilitating adsorption and detoxification (30). The absence of tissue alterations in this trial suggests that Jordanian zeolite, despite its unique mineral composition, poses no health risk when incorporated into broiler feed.

These findings align with global literature supporting the safety of natural zeolites as feed additives (1). Moreover, it was reported that using zeolite as a feed additive enhanced intestinal morphology of the broiler chickens without adverse effects (31,32). Comparable outcomes have been documented in other livestock species (33). In addition to their safety, zeolites are widely recognized for functional benefits, including binding mycotoxins, improving nutrient absorption, and supporting gastrointestinal health (34,35). Recently, it was reported that zeolite supplementation positively affected cadmium accumulation and detoxification (36). Although performance indicators were not measured in this study, the absence of histopathological abnormalities strongly supports the safe inclusion of Jordanian zeolite in broiler diets. It's worth mentioning that the current results are consistent with Kraljević Pavelić et al. (37), who reported no adverse clinical, biochemical, or hematological effects following oral administration of purified clinoptilolite in human subjects. Despite species differences, the systemic tolerability observed in both studies supports the cross-species safety of zeolite, provided the material is appropriately purified and characterized. In contrast, Pavlovich-Cristopulos et al. (38) highlighted potential health risks associated with commercially available zeolite supplements, citing elevated concentrations of toxic metals such as arsenic and lead with high oral bio accessibility. This disparity underscores the critical role of source origin and mineral composition in determining the safety profile of zeolite-based products. The phillipsite- and chabazite-rich Jordanian zeolite used in our study did not elicit any pathological changes, suggesting its suitability as a safe, functional feed additive in poultry production when sourced and processed under controlled conditions.

Conclusion

The findings of this investigation affirm that dietary supplementation with up to 15% Jordanian zeolite is safe for broiler chickens, with no macroscopic or histopathological lesions observed in the organs and tissues examined. Across all treatment groups, growth performance, hematological profiles, and systemic health markers remained within established physiological norms, underscoring the local zeolite's non-toxic and biocompatible nature under the tested conditions. These results endorse its application as a functional feed additive with potential benefits for gastrointestinal integrity and overall poultry health. Future research should aim to elucidate its influence on nutrient bioavailability, immune modulation, and long-term production metrics under varying environmental and management conditions.

Conflict of interest

There is no conflict of interest.

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تقييم سمية الزيوليت الأردني كمضاف علفي للدجاج اللاحم: من منظوري التغييرات النسيجية والدموية

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الخلاصة

الزيوليت هو معدن طبيعي عالي الامتصاص يتكون أساسًا من الألومينوسيليكات. ويُستخدم عادةً في علف الحيوانات نظرًا لخصائصه المفيدة. تمتلك الأردن رواسب طبيعية وفيرة من الزيوليت، وغنية بشكل خاص بالفيليبسيت والشابازيت. ومع ذلك، لا توجد دراسات سابقة حول تأثيره. هدفت هذه الدراسة إلى تقييم السلامة والتأثيرات المرضية لإدراج الزيوليت الأردني المحلي في علف الدواجن. تم توزيع ٢٠٠ صوص لاحم عشوائيًا على خمس مجموعات معالجة غذائية تحتوي على ٠٪ و٢٪ و٥٪ و١٠٪ و١٥٪ من الزيوليت الأردني. واستمرت تجربة التغذية لمدة ٣٢ يومًا. في نهاية التجربة، تم إجراء الفحوصات بعد الذبح والتحليلات النسيجية المرضية وقياس معايير دموية مختلفة. لم تكشف الفحوصات بعد التشريح أو الفحوصات النسيجية المرضية عن أي شذوذات أو تغيّرات ذات دلالة في الأعضاء التي تم فحصها عبر جميع المجموعات الغذائية. وبالمثل، بقيت جميع المعايير الدموية التي تم قياسها ضمن النطاقات الفسيولوجية الطبيعية. تثبت النتائج أن الزيوليت الأردني، الغني بالفيليبسيت والشابازيت، غير سام ويمكن إدراجه بأمان في علف دجاج اللحم بمستويات تصل إلى ١٥٪. يُظهر استخدامه كمضاف علفي إمكانيات كونه مكونًا وظيفيًا ومستدامًا في تغذية الدواجن دون آثار ضارة على صحة الأعضاء أو معايير الدم.