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Research Article

The Use of Glauconite as a Feed Additive in Broiler Nutrition and Its Effect on Growth Performance, Intestinal Histomorphology and Biomechanical Properties of Bones

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Abstract

The purpose of this study was to investigate the effects of using glauconite as a feed additive in broiler nutrition and its effect on fattening performance, intestinal histomorphology and biomechanical properties of bones in broilers. A total of 288 one-day-old male chicks were included in the study and they were randomly divided into 3 groups and these groups were divided into 8 subgroups. The trial continued for 35 days. The control group (C) was fed with basal ration while experimental groups were fed respectively with 1% glauconite (G1) and 2% glauconite (G2) added to the basal ration. The results showed that the use of different doses of glauconite in the rations did not statistically affect fattening performance. On the 21st and 35th day of the experiment, when histomorphology of ileum was examined, it was observed that villus height (VH), crypt depth (CD) and VH/CD ratio were not affected by glauconite addition. When histomorphology examination of jejeum was performed on the 35th day of the experiment, it was seen that the villus height was statistically affected. The effect of glauconite on biomechanical properties of femur and tibia bones was not statistically significant. As a result, the addition of glauconite in broiler rations did not affect the performance parameters, biomechanical properties of bones and histomorphology of the ileum, but adversely affected jejunum histomorphology.

Keywords: *Glauconite, Broiler, Intestinal histomorphology, Performance, Biomechanical properties of bones*

Broyler Beslemede Glokonitin Yem Katkı Maddesi Olarak Kullanımı ve Büyüme Performansı, Bağırsak Histomorfolojisi ve Kemiklerin Biyomekanik Özellikleri Üzerine Etkisi

Öz

Bu çalışmanın amacı, broyler beslenmede glokonitin yem katkı maddesi olarak kullanımı ve besi performansı, bağırsak histomorfolojisi ve kemiklerin biyomekanik özellikleri üzerine etkilerini araştırmaktır. Çalışmada bir günlük yaşta toplamda 288 adet civciv (Ross 308) rastgele üç gruba (Herbir grupta 96 civciv bulunmaktadır) ayrılmıştır ve bu gruplar 8 altgruba (Her alt grupta 12 civciv bulunmaktadır) ayrımıştır. Çalışma 35 gün sürmüştür. Deneme guplarına basal rasyona ek olarak %1 (G1) ve %2 (G2) glokonit ilavesi yapılırken kontrol grubu (K) bazal rasyonla beslenmiştir. Deneme sonunda rasyona farklı dozlarda glokonit ilavesinin performans parametrelerine istatistiki bir etkisi olmamıştır. Denemenin 21. ve 35. günlerinde ileum histomorfolojisi incelendiğinde, villus yüksekliği, kript derinliği ve villus yüksekliği/ kript derinliği oranının glokonit ilavesinden istatistiksel olarak etkilenmediği görülmüştür. Denemenin 35. gününde jejunum histomorfolojisi incelendiğinde, villus yüksekliği istatistiksel olarak anlamlı bulunmuştur. Denemenin sonunda tibia ve femura ait biyomekanik özellikler incelenmiştir. Glokonitin tibia ve femur ait biyomekanik özelliklere etkisi istatistiksel olarak anlamlı bulunamamıştır. Nitekim, broyler rasyonlarına glaukonit ilavesi performans, parametreleri, kemiklerin biyomekanik özellikleri ve ileum histomorfolojini etkilememiştir fakat jejunum histomorfolojisini olumsuz etkilemiştir.

Anahtar sözcükler: *Glokonit, Broiler, Bağırsak histomorfolojisi, Performans, Kemiklerin biyomekanik özellikleri*

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INTRODUCTION

Glauconite is a natural mineral with chemical formula (K, H_{20})) ($Fe^{3+,}$ Al, $Fe^{2+,}$ Mg)$_2$ [Si_3AlO_{10}] $(OH)_2$ x nH_2O) and it is a three-layer silicate mineral [1]. Glauconite is slightly acidic and capable of absorbing moisture 10 times more than ordinary sand. It consists of marine potash, silica, iron, magnesium, and lime, plus up to 30 other trace minerals. Glauconite has the property of bonding sandy soils and loosening clay soils. It is used in industrial areas such as magnetic separation and alkaline soil improvement with glauconite mineral enrichment process [2]. Element, granulometric, morphological composition of natural mineral glauconite (Belozersky field of Saratov region). Due to their sorption, physicochemical properties and environmental safety properties, they have been found to be suitable for water treatment, soil improvement and premix components for animals [3]. Minerals as ingredients in the premix are usually bentonite - montmorilloniet, glauconite, minerals of the zeolite group, limestone and others. Minerals are biostimulators of growth in poultry and livestock [4].

Glauconite is one of the clay mineral. Glauconite was historically applied as remedies in medicine. Clay-based antimicrobials consist essentially of a known antimicrobial drug or metal nanoparticles. Glauconite has the capacity to absorb various substances on the surface. Also, it can intercalate many inorganic and organic ions, replacing K^+, Ca^{2+}, etc in interlayers sites (ion-exchange capacity) [5]. Veing et al.[6] stated to be benefited from the ability to absorb many organic and inorganic ions on the surface of glauconite. Copper nanoparticles nested and adsorbed with glauconite-matrix (3-7 nm) mineral surface (30-50 nm). Superb antibacterial activity composites was reported for *Staphylococcus aureus* and *Escherichia coli*. Glauconite mineral is known to be used as dermatological and gastro-intestinal agents [6]. The clays supplemented to the ratio have the ability to bind, immobilize toxic substances in the gastrointestinal tract of animals and reduce their toxicity [7]. Clays are inherently non-toxic to environment [8]. The presence of glauconite in the gastrointestinal tract positively affects the metabolism. That is, glauconite regulates the content of intestinal fluid, electrolyte and acid-base balance and mineral metabolism. The use of glauconite in combination with probiotics in pig rations increased immune and natural organism resistance [9].

Prohibition of the use of antibiotics and other growth factor chemicals for residual release and resistance to bacteria has led to the search for alternative feed additives [10,11]. The use of glauconite clay mineral as a non-toxic and historically therapeutic use has aroused interest in its usability as a feed additive. There is no study in the literature that the glauconite mineral is used as a feed additive in any animal experiment. Due to these properties of glauconite mineral, this study was carried out to determine the effect of glauconite usage on the performance, intestinal morphology and biomechanical properties of bones in broiler feeding.

MATERIAL and METHODS

Animals, Experimental Design and Feed

This study was carried out with the permission of the Ankara University Animal Experiments Local Ethics Committee (Decision No: AU-HAYDEK /2014-23-157) report.

The glauconite mineral used in the experiment was supplied by Russia Saratov University. The particle size of the glauconite mineral used in the experiment is 60 microns. Chickens used in this study (Ross 308) was provided from a commercial hatchery (Beypiliç, Bol, Turkey). A total of 288 one-day-old male chicks (Ross 308) were included in the study and they were randomly divided into 3 groups (96 chicks in each group) and these groups were divided into 8 subgroups (12 chicks in each group). The animals were fed with corn, soy bean meal basal ration and trial continued for 35 days. The house temperature was monitored thermostatically throughout the study. The temperature, which was 32-35°C on the first day, was gradually lowered and maintained at 22°C in the last two weeks. The starter, grower, and finisher rations were given to the animals for 0-14, 15-28, and 29-35 day intervals, respectively *(Table 1)*. All rations were formulated to NRC [12] nutrient recommendations. Each subgroups were equipped with manual feeders and automatic nipple drinkers. Water and feed were given *ad libitum*. Ration treatments were as follows: C, basal diet (Control; without addition); G1, glauconite 1% and G2, glauconite 2%. Ration nutrient analyzes were performed according to AOAC [13]. The chemical composition of glauconite mineral is given in *Table 2*.

Growth Performance

In the study, live weights (LW) were recorded for each subgroup weekly. Live weight gain (LWG) was determined with the difference between these measurements. For each subgroups feed intake (FI) of animals was recorded weekly and used for the calculation of the feed conversion ratio. Performance parameters were calculated by considering the subgroup averages.

Histomorphologic Measurements

On 21th and 35th days of the study one animal from each subgroup was randomly selected for histomorphological analysis. Animals were cut off with a suitable method for the intestinal histomorphological and the biomechanical properties of bones.

The tissue samples for histomorphological examination were taken from the jejunum and ileum. In order to provide uniformity for each animal, specimens were obtained from 8 cm proximal of the Meckel's diverticula for jejunum and from 8 cm proximal of the ileocecal junction for ileum.

Table 1. *The composition of the rations used in the study (%)*[1]

Ingredient	Broiler Starter 0-14. days	Broiler Grower 15-28. days	Broiler Finisher 29-35. days
Corn	51.00	52.25	56.45
Soybean (Full fat), 38%	19.62	18.00	14.00
Vegetable oil	1.00	2.00	3.00
Soybean meal, 48%	24.00	24.00	23.00
DCP	2.40	2.00	2.00
Limestone	0.8	0.85	0.85
Bicarbonate	0.10	0.10	0.10
Salt	0.25	0.25	0.25
DL-Metiyonin	0.37	0.25	0.15
L-lizin	0.2	0.10	0
Vitamin premix[2]	0.10	0.10	0.10
Mineral premix[3]	0.10	0.10	0.10
Anticoccidial	0.06	-	-
Total	100.00	100.00	100.00
Chemical composition, calculated			
Crude protein, %	22.01	21.56	20.03
ME, kcal/kg	3099	3158	3219
Ca, %	1.01	0.92	0.91
P, %	00.5	0.44	0.44
Methionine + Cysteine, %	1.09	0.96	0.82
Lysine, %	1.44	1.33	1.14
Analysis values			
ME, kcal/kg	3131	3153	3200
Crude protein, %	23.45	21.70	19.60
Ca, %	1.04	1.00	0.93
Total P, %	0.53	0.50	0.48

[1] As-fed basis; [2] Provided per kilogram of complete diet: Vit. A, 12.000 IU; Vit. D_3, 2.500 IU; Vit. E, 40 IU; Vit. K_3, 5 mg; thiamin, 2.5 mg; riboflavin, 6 mg; pyridoxine, 5 mg; pantothenic acid, 15 mg; niacin, 25 mg; folic acid, 1 mg; biotin, 50 µg; Vit.B_{12}, 20 µg; [3] Provided per kilogram of complete diet: Cu, 5 mg; I, 1 mg, Co, 200 µg; Se, 150 µg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg. Folic Acid 1.000 mg/kg, Biotin 50 mg/kg, Copper 5.000 IU/kg, Iodine 1.000 IU/kg, Cobalt 200 mg/kg, Selenium 150 mg/kg, Iron 60.000 mg/kg, Zinc 60.000 mg/kg, Mangan 80.000 mg/kg

Table 2. *The chemical composition of glauconite used in this study (%)*

Glauconite	
Moisture	10.30
SiO_2	61.82
Al_2O_3	21.08
Fe_2O_3	3.25
CaO	2.44
MgO	2.67
CaO + MgO	5.11
K_2O	0.95
Na_2O	2.44

Each intestinal specimen (jejunum and ileum) has a size of 1 cm. Tissue samples were fixed in 10% neutral buffered formaline for 24 h and then dehydrated in graded ethanol solutions, washed with tap water, purged with xylol and embedded in paraffin, respectively. Intestinal segments were cut off the thickness of 5 µm with a microtome. Cross sections were prepared and stained with Mallory's triple stain modified by Crossman in order to determine the intestinal morphometry [14].

Villus height (VH) was determined as the area from the top of the villus to the crypt mouth. Crypt depth (CD) was measured as the area between adjacent crypt terminals [15]. Histological sections were examined using a light microscope and photographed with Leica DFC450 digital microscope camera. The images were then evaluated with ImageJ software.

Femur and Tibia Biomechanical Properties

Left femur and tibia samples were thawed at 4ºC and cleaned of all tissue. Length and width of femur and tibia samples were measured using digital calliper. Afterwards bone samples were stored at -20°C for further analyses. Femurs and tibias were subjected to the three-point bending tests until problem occurred, with Instron 5944 testing frame (Instron, Norwood, MA, USA). The loading rate was 5 mm/min. Spon length was 70 mm for bones. The load was applied to the midpoint of the shaft. Load versus displacement data was collected for each sample. Stiffness values were determined from the slope of the linear region of the load displacement curves. Ultimate load (UL) and displacement at ultimate load (DUL) were calculated from the load displacement curves as well. The load at which the permanent deformation of the system begins is the yield load (YL). The displacement at which the permanent deformation begins is the displacement at the yield load (DYL) [16].

Statistical Analysis

The one-way variance analysis method (ANOVA) was used for the statistical calculations of the groups and the importance of the differences between the mean values in the groups and a suitable post hoc test (Duncan) was used for the importance control of the differences between the groups. The statistical analysis was done with the SPSS software package [17].

RESULTS

The results obtained in this study are presented with appropriate tables.

Performances

On the 7th and 28th days of the experiment, the live weight value between the groups was statistically significant ($P<0.05$). Live weight gain (LWG) values between the groups were

Table 3. *Effects of dietary supplementation of glauconite on live weight, live weight gain, feed intake and feed conversion ratio in the broiler (g)*

Performance Parameters	Days	Control		G1		G2		Significance
		X	Sx	X	Sx	X	Sx	P
Live Weight, g Days	0	41.68[a]	0.08	41.29[ab]	0.06	41.25[b]	0.07	0.001***
	7	149.29[a]	2.8	145.67[ab]	2.11	140.39[b]	1.56	0.032*
	14	386.84	4.46	395.34	4.45	383.31	4.84	0.187
	21	818.83	7.7	799.98	10.11	809.35	7.7	0.319
	28	1445.23[a]	13.46	1383.99[b]	19.82	1394.69[b]	16.67	0.040*
	35	2175.42	29.62	2089.75	36.43	2070.50	26.32	0.060
Live Weight Gain, g, Days	0-7	107.61[a]	2.73	104.37[ab]	2.11	99.14[b]	1.56	0.039*
	7-14	237.54	4.37	249.67	4.09	242.91	4.74	0.175
	14-21	431.99	8.18	404.64	9.72	426.03	6.93	0.074
	21-28	626.39	10.83	584.00	17.86	585.34	13.52	0.083
	28-35	730.19	27.13	705.75	21.83	675.80	25.27	0.320
	0-35	2133.73	29.61	2048.45	36.38	2029.24	26.30	0.061
Feed Intake, g Days	0-7	140.87	2.85	119.03	15.12	135.29	3.97	0.240
	7-14	393.63[ab]	6.96	379.93[b]	5.96	416.72[a]	14.44	0.047*
	14-21	587.87	27.51	628.84	15.33	656.48	7.72	0.053
	21-28	929.43	20.84	1024.66	37.03	1083.89	69.31	0.087
	28-35	1279.47	57.01	1248.80	60.96	1248.32	50.77	0.905
	0-35	3331.29	76.81	3401.29	70.97	3540.68	118.18	0.276
Feed Conversion Ratio Days	0-7	1.31	0.02	1.12	0.14	1.36	0.04	0.156
	7-14	1.66[a]	0.03	1.52[b]	0.02	1.71[a]	0.03	0.001***
	14-21	1.35[b]	0.05	1.55[a]	0.02	1.54[a]	0.03	0.005***
	21-28	1.48[b]	0.03	1.77[a]	0.10	1.84[a]	0.09	0.014*
	28-35	1.76	0.09	1.78	0.10	1.86	0.09	0.752
	0-35	1.56[b]	0.03	1.66[ab]	0.06	1.74[b]	0.05	0.055

*Statistically not significant (P>0.05). The mean (X) and standard error (Sx) values of 8 subgroups in each group. [a, b,c] Differences between the mean values of different letters in the same row are statistically significant. * P<0.05, *** P<0.01; Groups; C: Control, G1: 1% glauconite, G2: 2% glauconite*

statistically significant in the first week of the experiment (P<0.05). Feed intake (FI) values between the groups were statistically significant in the second week of the experiment (P<0.05). In the mentioned week, the lowest feed intake value belongs to the G1 group. Feed intake was affected positively by the use of low-dose glauconite in the second week of the trial. The feed conversion ratio (FCR) between the groups at the 2nd, 3rd and 4th weeks of the experiment was found to be statistically significant (P<0.01). In the mentioned weeks, the lowest feed conversion ratio value belongs to the control group. At the end of the experiment, the use of different doses of glauconite in rations did not statistically affect the LW, LWG, FI and FCR. The effect of glauconit use at 1% and 2% as feed additive in broiler rations during the experiment on the parameters of LW, LWG, FI and FCR is given in *Table 3*.

Morphological Measurement of the Jejunum and the Ileum

On the 21st and 35th day of the experiment, when histology of ileum was examined, it was observed that VH, CD and VH/CD ratio were not affected by glauconite addition. On the 21st day of the trial, when the jejenum histomorphology was examined, it was seen that VH and VH/CD ratio values were not affected by the addition of glauconite. However, CD value was found to be statistically significant. The crypt depth of the control group was higher than those of the other groups (P<0.05). When histological examination of jejeum was performed on the 35th day of the experiment, CD and VH/CD ratio values between groups were not statistically significant. However, VH value was found to be adversely significant (P<0.05). Histomorphological measurements of jejunum and ileum at 21st and 35th day of the experiment are given in *Table 4*.

Femur and Tibia Biomechanical Properties

On the 35th day of the experiment, biomechanical properties of the femur and tibia bones from each subgroup were investigated. The effect of glauconite addition on bio-mechanical properties of femur and tibia bones was not statistically significant. The femur and tibia biomechanical properties on the 35th day of the trial are given in *Table 5*.

Table 4. *Effect of glauconite supplementation on histomorphology of ileum and jejunum on the 21st and 35th days of the trial (µm)*

Histomorphology Parameters	**C**		**G1**		**G2**		**P**
	X	**Sx**	**X**	**Sx**	**X**	**Sx**	
Ileum 21							
Ileum villus height (µm)	777.87	98.78	690.85	38.99	754.75	66.67	0.708
Ileum crypt depth (µm)	166.62	13.62	142.00	8.38	162.29	10.59	0.297
Ileum villus height/crypt depth	4.89	0.80	4.94	0.33	4.75	0.46	0.974
Jejenum 21							
Jejenum villus height (µm)	1017.33	77.20	1011.33	94.40	994.66	22.30	0.973
Jejenum crypt depth (µm)	196.27^{a}	12.71	147.38^{b}	6.98	167.55^{ab}	11.15	0.017*
Jejenum villus height/crypt depth	5.27	0.45	6.84	0.47	6.09	0.48	0.094
Ileum 35							
Ileum villus height (µm)	938.83	64.94	92.33	69.22	913.33	21.28	0.949
Ileum crypt depth (µm)	157.66	15.77	126.77	7.14	155.83	11.81	0.168
Ileum villus height/crypt depth	6.11	0.40	7.42	0.72	6.04	0.48	0.172
Jejenum 35							
Jejenum villus height (µm)	1379.00^{a}	38.20	1031.00^{b}	71.22	1106.33^{b}	77.12	0.004*
Jejenum crypt depth (µm)	201.33	7.89	195.50	9.32	186.00	7.03	0.426
Jejenum villus height/crypt depth	6.90	0.31	5.35	0.49	5.98	0.43	0.062

*Statistically not significant (P>0.05). The mean (X) and standard error (Sx) values of 8 subgroups in each group. Groups; C: Control, G1: 1% glauconite, G2: 2% glauconite. ^{a,b} Differences between the mean values of different letters in the same row are statistically significant * (P<0.05), *** (P<0.001)*

Tablo 5. *Effects of dietary glauconit treatments on femur and tibia parameters on d 35th*

Bone	**Item**	**Dietary Treatment**						
		C		**G1**		**G2**		**P**
		X	**Sx**	**X**	**Sx**	**X**	**Sx**	
Femur	Length, mm	67.33	0.39	67.00	0.32	65.98	0.69	0.141
	Width, mm	9.62	0.10	9.61	0.17	9.68	0.18	0.940
	UL, N	237.55	13.83	242.54	8.00	250.65	12.60	0.732
	DUL, mm	4.06	0.23	4.39	0.29	4.52	0.39	0.582
	YL, N	165.66	16.54	162.55	16.91	174.22	19.46	0.891
	DYL, mm	2.03	0.12	2.07	0.23	2.40	0.32	0.497
	Stiffness, N/mm	80.54	4.85	79.48	3.61	76.49	6.54	0.848
Tibia	Length, mm	92.12	0.67	91.90	0.76	91.47	0.85	0.828
	Width, mm	9.05	0.22	9.41	0.24	9.24	0.23	0.563
	UL, N	210.15	14.50	225.75	11.31	235.74	16.50	0.455
	DUL, mm	3.39	0.14	3.62	0.11	3.52	0.19	0.577
	YL, N	194.00	12.57	202.66	11.48	213.81	15.92	0.589
	DYL, mm	2.75	0.07	2.77	0.05	2.70	0.12	0.831
	Stiffness, N/mm	70.67	4.77	72.93	3.62	78.90	3.47	0.342

Statistically insignificant (P<0.05). The values show the mean (X) and standard error (Sx) of the 8 subgroups in each group. Groups; C: Control, G1: 1% glauconite, G2: 2% glauconite
Data represent mean values of 8 replicates per treatment; K: corn-soybean meal basal diet containing 0% glauconit; G1: basal diet containing 1% glauconit, G2: basal diet containing 2% glauconit; UL: Ultimate Load, DUL: Displacement at Ultimate Load, YL: Yield Load, DYL: Displacement at Yield Load

DISCUSSION

The use of clays in animal nutrition affects digestibility of nutrients, live weight gain and feed conversion rate positively [18]. In the literature research, there are few studies in which glauconite mineral is used as animal feed additive and it has not been investigated in terms of certain parameters examined in this study. In our experiment, the use of different doses of glauconite did not statistically affect the LW and LWG on the 35th day. There are many current studies using clay group minerals that support the results of our study [19,20]. However, there are many recent studies suggesting that clay group minerals have a positive effect on the performance of broiler feeding [21,22]. There are no studies on the use of glauconite in poultry nutrition. Therefore, glauconite studies in different animals were used in the discussion section. The use of glauconite in bull-calves rations increased live weight [23]. In our experiment, the use of different doses of glauconite did not statistically affect feed intake on the 35th day. It was observed that the feed intake increased between 7-14 days and the highest value was in G2. In a different study using bentonite, feed intake was reported to be increased [24]. Research results are consistent with recent articles. The use of glauconite at the level of 4% in broiler diets did not affect FI and FCR values [25]. The researchers associated that increased feed intake increase the pellet quality of bentonite by acting as a pellet binder. Similarly, a study conducted with sepiolite showed an increase in pellet quality [26]. The effect of glauconite in this direction and the related feed intake changes were not observed since this study was carried out with powder feed. Although the positive effects of glauconite application on the feed conversion ratio were observed during the experiment period, no significant effect was observed at the end of the experiment. It was reported that kaolin and zeolite will have a positive effect on feed conversation ration whereas in the same study, it was reported that bentonite increases the ratio [27]. It was stated that the feed conversation ratio will be affected positively in studies performed with different silica minerals [22]. However, different studies indicate that silica minerals do not have a significant effect on feed conversation ratio [20,24]. The results obtained can be affected by different conditions such as the quality of the clay mineral used and the feed structure. It is suggested that the mineral structure and metal oxide content of clay mineral can differentiate the results of the study [26].

Morphological changes in the small intestine, villus height (VH), crypt depth (CD) and VH/CD ratio may improve poultry performance by improving nutrient digestion and absorption [28]. When used in animal feeding, the clays caused morphological changes in the intestinal mucosa, such as an increase in villus height and an increase in crypt depth. These morphological changes increase the surface area of the gastrointestinal tract and thereby increase nutrient digestion [7]. In our study, it was observed that the addition of ration glauconite did not affect the ileum histomorphology on the 21st and 35th days of the study. In our study, the addition of ration glauconite on the 21st day of the study affected the jejunum CD. The highest crypt depth was in the control group and the lowest in the G1 group. On the 35th day of the study, the addition of ration glauconite affected the VH of the jejunum. The highest VH in the control group and the lowest G1 group. In glauconite use, high doses were found to be more beneficial for intestinal health than low dose.It has been reported that clinopililoies in different structure increased VH but had no effect on CD [29]. In a different study, the use of zeolite has been reported to increase in the length of intestinal villus [30]. In the study performed with sepiolite, an increase in the length of the duodenum villus was reported [22]. In the studies carried out with Cu-montmorillonite, an increase in villus length was formed [31]. The results obtained can be affected by different conditions such as the quality of the clay mineral used and the feed structure.

Leg abnormalities are very important problems in rapidly growing broilers, leading to economic losses and reduced welfare [32]. A study of the effect of glauconite mineral on the biomechanical properties of bones in poultry has not been found in literature review. In our study, biomechanical properties of femur and tibia bones were not affected by the addition of glauconite to the ration and no adverse effect was observed. There are studies on clay minerals and other minerals in different animals. Safari et al.[25] reported that the effect of 4% glauconite on broiler diets on the weight, length and density of the tibia was statistically insignificant. It was reported that the addition of organic trace minerals and 25-hydroxycholecalciferol to turkey rations has a positive effect on the biomechanical properties of bones [33]. The effect of different levels of boron on the laying hen rations is not insignificant on the all of tibial biomechanical properties [34]. In a different study, use of calcium soaps of animal fats in broiler feeding did not affect on the biomechanical properties of femur [35]. Although this study is compatible with our study, many studies are needed in this area. The results obtained may be affected by different conditions such as particle size, structure and dosage of the glauconite mineral used. The conflicting results might depend on the type and dose of the used clay mineral, chemical composition, coop conditions and environmental factors.

In conclusion, the use of glauconite at 1% and 2% levels in broiler rations did not affect performance parameters, biomechanical properties of bones and histomorphology of ileum but adversely affected jejunum histomorphology. In the use of glauconite, high doses were found to be more beneficial for intestinal health than low doses. There is not enough study on the use of glauconite as a feed additive in broiler feed, and studies to be carried out in different doses with glauconites in different structures will shed light on the potential of using this substance as feed additive. In our study, it is seen that many studies are needed in order to explain the effect levels of the investigated parameters. Our study is a good literature for further studies.

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