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Research Article

Effect of Clinoptilolite and/or Phytase on Broiler Growth Performance, Carcass Characteristics, Intestinal Histomorphology and Tibia Calcium and Phosphorus Levels [1]

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Abstract

The effect of clinoptilolite alone or in combination with phytase on the performance, carcass characteristics, intestinal histomorphology, and tibia ash and Ca, P levels were determined in male broiler chickens. Total of 192 one-day-old male broiler chicks (Ross 308) were randomly assigned to 4 different treatments with 8 replicates containing 6 birds each. Treatment 1 (T1) was served as control fed a diet without clinoptilolite and/or phytase, Treatment 2 (T2) was supplemented with 2% clinoptilolite. Treatment 3 (T3) and Treatment 4 (T4) were (formulated Ca; 0.87, 0.80 and 0.75%, available P; 0.30, 0.28 and 0.26% for starter, grower, and finisher periods respectively) supplemented 0.01% phytase and 2% clinoptilolite + 0.01% phytase, respectively. The results showed that combined use of clinoptilolite and phytase (T4) in broiler diets decreased (P=0.044) body weight gain at 1-14 d. Dietary treatments had no effect on body weight gain, feed intake (FI) and feed conversion ratio (FCR) during the entire experimental period. Supplementation with phytase (T3) significantly (P=0.006) decreased crypt depth in duodenum on d 21. Tibia ash and phosphorus level were decreased due to combined use of clinoptilolite and phytase (T4) in comparison to those fed control (T1) and clinoptilolite (T2) diets on d 21. At 42 days of age, tibia ash, Ca, P levels and Ca/P ratio was not changed by the treatments. It is concluded that there might be a positive effect of 2% clinoptilolite on the performance of male broilers after starting period.

Keywords: *Broiler, Clinoptilolite, Carcass, Intestinal histomorphology, Performance, Tibia ash*

Klinoptilolit ve/veya Fitaz İlavesinin Broilerlerin Büyüme Performansı, Karkas Karakteristikleri, Bağırsak Histomorfolojisi ve Tibia Kalsiyum ve Fosfor Düzeylerine Olan Etkisi

Özet

Bu çalışmada erkek broiler rasyonlarında klinoptilolitin tek başına veya fitaz ile birlikte kullanılmasının performans, karkas karakteristikleri, bağırsak histomorfolojisi ve tibia külü ile Ca, P düzeyleri üzerine etkisi incelenmiştir. Toplamda 192 adet günlük yaşta erkek civciv (Ross 308) 4 deneme grubuna ve her biri 6 civcivden oluşan 8 tekerrür grubuna rasgele olacak şekilde ayrılmıştır. Birinci grup (T1) kontrol grubu olarak düzenlenerek bazal rasyon ile beslenmişlerdir. İkinci gruba (T2) %2 düzeyinde klinoptilolit ilave edilmiştir. Daha düşük düzeyde Ca ve yararlanılabilir P içeren (başlangıç, büyütme ve bitirme dönemleri için sırasıyla Ca; %0.87, 0.80 ve 0.75 yararlanılabilir P; %0.30, 0.28 ve 0.26) 3. ve 4. gruba ise sırasıyla %0.01 fitaz ve %2 klinoptilolit + %0.01 fitaz ilave edilmiştir. Araştırmanın 1-14. günlerde, klinoptilolit ve fitazın birlikte kullanıldığı grupta (T4) canlı ağırlık artışının daha düşük düşük olduğu (P=0.044) görülmüştür. Ancak tüm deneme boyunca gruplar arasında canlı ağırlık artışı, yem tüketimi ve yemden yararlanma oranı açısından farklılık tespit edilmemiştir. Araştırmanın 21. gününde yalnızca fitaz ilavesinin (T3) duodenum kript derinliğini önemli ölçüde azalttığı (P=0.006) sonucuna varılmıştır. Denemenin 21. gününde tibia külü ve fosfor düzeyinin klinoptilolit ve fitazın birlikte kullanılmasına bağlı olarak önemli ölçüde daha azaldığı görülmüştür. Ancak denemenin 42 gününde tibia parametreleri açısından gruplar arasında farklılık tespit edilememiştir. Sonuç olarak başlangıç döneminden sonra rasyonlarda kullanılacak %2 klinoptilolitin performans üzerine olumlu etkileri olabileceği düşünülmektedir.

Anahtar sözcükler: *Bağırsak Histomorfolojisi, Broiler, Karkas, Klinoptilolit, Performans, Tibia külü*

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INTRODUCTION

Zeolites are hydrated aluminosilicates of volcanic origin, which is derived from the same molecular frame containing alkali (Na, K, Li, Cs) and alkaline-earth metals (Ca, Mg, Ba, Sr), having ion exchange and adsorption characteristics and ability to lose and gain water reversibly [1]. Clinoptilolite is one of the natural zeolites used successfully as feed additive due to its specific physicochemical properties. Previous studies have revealed that the dietary clinoptilolite may increase growth performance [2], help to control ammonium in poultry houses [3], improve immune response [4], help to bind toxin [5], and improve the hygienic and sanitary conditions of litter [6]. More recently Nikolakakis et al.[7] revealed that dietary clinoptilolite supplementation improved broiler growth performance and litter quality.

Zeolites are used in animal nutrition in particular to their absorption/adsorption properties, antimicrobial effects and preventive nature against diarrheal infections [8,9]. In addition, dietary natural zeolites may improve the digestibility of the nutrients by increasing the mean retention time of the digesta in layers [10].

Zeolites can also influence Ca utilization and metabolism [11], by selectively retaining or releasing of Ca as it passes through the digestive tract [12]. Moreover zeolites may impact electrolyte balance of broilers [13]. It is well known that mineral metabolism and electrolyte balance are play a crucial role on bone formation [14,15]. Clinoptilolite has a beneficial effect on tibia bone mineralization and calcium and phosphorus utilization in broiler chicks [13]. On the other hand, Elliot and Edwards [16] and Leach et al.[17] reported that zeolites suppressed P utilization by forming an indigestible compound, and had an indirect effect on P absorption and metabolism.

Previous studies have suggested that dietary zeolite improves growth performance [17], and increase Ca utilization and bone ash deposition [13,18]. However contradictory results were reported on growth performance [16] and bone ash content [19]. Discrepancies between the studies might be partially attributable to the type of zeolite, purity and physicochemical properties of the product used in the experiments [20], supplementation level to the diets, and dietary calcium level [21].

Considerable amount of phosphorus (P) in poultry diets in the form of phyate-P, which is not utilized efficiently by monogastric animals [22,23]. In this sense inorganic phosphate sources, such as di-calcium phosphate, included to poultry diets to meet available P requirements [24]. Phytase is widely used in poultry feeds to improve P availability [25] and to reduce the cost of the ratio [24]. In addition, it may also enhance broiler performance by increasing the utilization of dietary energy and amino acids [26]. El-Sherbiny et al.[27] reported that dietary addition of 500 U/kg phytase improved body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of the birds. In contrast, Chung et al.[28] reported that both fungal and bacterial phytase supplementation had no consistent effect on broiler growth performance from d 0 to 21.

The improvement in the availability of P and other cations by dietary phytase supplementation has been generally well accepted. More recently Lalpanmawia et al.[29] concluded that laboratory phytase and commercial phytase supplementation resulted in 30% reduction in P excretion. However the effect of combined use of clinoptilolite and phytase, in diets that contain low level of P, on broiler performance and bone characteristics heretofore unreported.

The objective of the current study is to evaluate the effects of clinoptilolite addition to the broiler diets with or without phytase enzyme on the performance, carcass characteristics, intestinal histomorphology, and tibia ash, Ca and of P levels in male broiler chickens.

MATERIAL and METHODS

Experimental Design, Diets and Management

The experimental design, diet and management used in this trial were approved by the Animal Ethics Committee of the Ankara University (2012-8-60). A total of 192 one-day-old male broiler (Ross 308) chicks, with an average body weight (BW) of 42.64±0.22 g, were obtained from a commercial hatchery (Beypiliç AŞ, Turkey) and allocated to 4 treatments in a randomized complete block design with 8 replicates of 6 birds in each for a 42 day feeding trial. All diets were formulated to meet or exceed NRC [30] nutrient recommendations (except P and Ca in diets containing phytase) of broilers for starter (0-14 days), grower (14-35 days) and finisher (35-42 days) periods. Treatment 1 (T1) was served as control group fed diet without clinoptilolite and/or phytase supplementation, Treatment 2 (T2) was supplemented with 2% clinoptilolite (NAT-MIN 9000, Gördes Zeotile, Izmir, Turkey). Phytase containing diets (T3 and T4) were formulated at a low level of Ca and P (Ca; 0.87, 0.80 and 0.75%, available P; 0.30, 0.28 and 0.26% for starter, grower, and finisher periods, respectively). Treatment 3 and 4 were supplemented with 0.01% phytase (5000 FTU/g) (P500, Tempe Chemical Feed Additives, Istanbul, Turkey), and 2% clinoptilolite with 0.01% phytase, respectively. Chemical and physical characteristics of clinoptilolite (according to the manufacturer's data sheet) is shown in *Table 1*. Composition and nutrient levels of experimental diets are shown in *Table 2*.

All birds were housed in floor pens with fresh wood shavings served as litter material. Each pen (90x80x80 cm) contained one feeder and two-nipple waterer. Temperature was controlled with electrical heater, maintained at 34°C for the first three days and then gradually reduced by 2-3°C per week to final temperature of 22°C. Water and diets

Table 1. *Chemical and physical characteristics of clinoptilolite used in the experiment* [1]

Tablo 1. *Denemede kullanılan klinoptilolitin kimyasal ve fiziksel özellikleri*

Item	Composition
Chemical Formula	$[(Na_{0.5}K_{2.5})(Ca_{1.0}Mg_{0.5})(Al_6,Si_{30})O_{72}.24H_2O]$
Specific gravity	2 g/cm^3
Particle size	0-0.8 mm
Pore diameter	0.041µm
Moisture	Max 12%
Cation Exchange Capacity (NH_4^+)	1.6-2.0 meq/g

[1] *According to the manufacturer's data sheet*

(in mash form) were provided *ad libitum* throughout the experimental period.

During the experimental period, body weights were recorded for each replicate weekly to determine weight gain. Also feed intake was recorded for each pen, and feed/gain ratio (F/G) was calculated during the same periods to determine the growth performance of birds. Mortalities were recorded on as it occurred, and were used to adjust feed conversion.

Sample Collection and Procedures

At d 21 and d 42, 8 birds from each treatment (1

Table 2. *Composition of experimental diets*

Tablo 2. *Deneme yemlerinin kompozisyonu*

Item	Experimental Diets[1]											
	Starter 0 to 14 d				Grower 14 to 35 d				Finisher 35 to 42 d			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Ingredients, %												
Corn	39.98	39.50	40.78	40.65	47.05	43.70	48.03	44.39	45.00	44.88	49.46	47.04
Soybean meal (CP, 47%)	30.00	30.00	30.00	30.00	24.00	23.36	24.00	23.37	24.65	24.55	25.00	24.00
Full fat soy (CP, 35%)	13.00	14.00	13.00	14.00	13.00	14.00	13.00	14.00	8.50	9.00	8.00	9.50
Wheat	10.00	6.50	10.00	6.54	8.00	8.00	8.00	8.00	13.30	10.00	10.00	9.00
Vegetable oil	2.50	3.50	2.50	3.10	3.90	4.90	3.60	4.80	5.00	6.00	4.60	5.50
Limestone	0.86	0.85	1.00	1.00	0.80	0.79	0.95	1.00	0.77	0.78	0.85	0.85
DCP	2.25	2.25	1.30	1.30	2.00	2.01	1.20	1.20	1.85	1.85	1.15	1.15
DL-Methionine	0.40	0.40	0.40	0.40	0.31	0.32	0.30	0.31	0.18	0.19	0.19	0.20
L-Lysine	0.26	0.25	0.26	0.25	0.19	0.17	0.16	0.17	-	-	-	-
L-Threonine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
NaCl	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Anticoccidial	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Clinoptilolite	-	2.00	-	2.00	-	2.00	-	2.00	-	2.00	-	2.00
Phytase	-	-	0.01	0.01	-	-	0.01	0.01	-	-	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values												
Crude protein, %	23.00	22.90	23.00	23.00	20.60	20.30	20.60	20.40	19.40	19.20	19.40	19.20
ME kcal/kg	3012	3012	3037	3012	3176	3174	3177	3175	3227	3227	3226	3223
Ca, %	1.00	1.00	0.87	0.87	0.90	0.90	0.80	0.80	0.85	0.85	0.75	0.75
Available P, %	0.50	0.50	0.30	0.30	0.45	0.45	0.28	0.28	0.42	0.42	0.26	0.26
Meth+Sist, %	1.10	1.10	1.10	1.10	0.95	0.95	0.95	0.95	0.80	0.80	0.80	0.80
Lysine, %	1.44	1.44	1.44	1.44	1.24	1.22	1.22	1.22	1.01	1.01	1.01	1.01
Threonine, %	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90	0.86	0.86	0.86	0.86
Analyzed values												
Crude protein, %	23.02	23.06	23.10	23.00	20.50	20.22	20.60	20.45	19.25	19.15	19.25	19.10
Ca, %	1.02	1.03	0.88	0.88	0.95	0.93	0.81	0.72	0.88	0.89	0.74	0.75
Total P, %	0.86	0.85	0.66	0.66	0.78	0.77	0.62	0.61	0.74	0.73	0.60	0.59

[1] ***T1:*** *Control, a standard corn-soybean meal basal diet;* ***T2:*** *2% clinoptilolite;* ***T3:*** *0.01% phytase (5000 FTU/g);* ***T4:*** *2% clinoptilolite and 0.01% phytase;* [2] *Provides per kg of diet: vitamin A, 15000 IU; vitamin D_3, 5000 IU; vitamin E, 50 mg; vitamin K_3, 10 mg; vitamin B_1, 4 mg; vitamin B_2, 8 mg; vitamin B_6, 5mg; vitamin B_{12}, 0.025mg; niacin, 50 mg; pantothenic acid, 20 mg; folic acid, 20 mg; biotin, 0.25 mg; choline,175 mg;* [3] *Provides per kg of diet: manganese, 100 mg; zinc, 150 mg; iron, 100 mg; copper, 20 mg; iodine, 1.5 mg; cobalt, 0.5 mg; selenium, 0.2 mg; molybdenum, 1mg; magnesium, 50 mg*

bird per replicate) were randomly selected for sample collection. After slaughtering, intestinal tract was removed immediately. To ensure the uniformity of samples, approximately 2 cm lengths of the mucosal segments of duodenum, jejunum and ileum were excised as follows: duodenum (from gizzard outlet to the end of the pancreatic loop), jejunum (8 cm proximal to Meckel's diverticulum), and ileum (8 cm proximal to the ileo-cecal junction). Afterwards the tissue samples were flushed with saline solution to remove adherent intestinal contents and fixed in 10% neutral buffered formaline solution for 24 h. Tissue samples were taken from formaline and dehydrated in graded ethanol solutions, cleared with xylol and embedded in paraffin, respectively. Intestinal samples were sectioned at the thickness of 6 μm with microtome. Mounted sections were stained with Mallory's modified triple staining technique [31]. Villus height was measured from the top of the villus to crypt mouth and crypt depth was defined as distance between basements of the crypt-to-crypt mouth [32]. Histological sections were examined under the light microscope (Leica DM 2500, Leica Microsystems GmbH, Wetzlar, Germany) and photographed with Leica DFC450 (Leica Microsystems, Heerbrug, Germany) digital microscope camera. The images were evaluated using ImageJ software (Image J, US National Institutes of Health, Bethesda, MD, USA).

The left tibia was removed and cleaned of adhering tissue, defatted and crushed into small particles. Ash contents were determined for each replicate (one bone from each pen) as described by the AOAC [33]. The ash percentage of each bone was determined with regard to defatted individual dry matter in tibia. Calcium was determined by gloxal-bis method according to Farese et al.[34]. Phosphorus content was measured according to the ammonium vanadate/molybdate method as described by Gericke and Kurmies [35].

Statistical Analysis

Data were analyzed as a completely randomized block design with 4 dietary treatments and 8 replicates using the ANOVA procedure [36] of the SPSS version 14.01 (SPSS Inc., Chicago, IL, USA). Significant differences among treatment groups were tested by Duncan's multiple range tests. Mortality rates were compared using a chi-squared test. Statistical differences were considered significant at P<0.05.

RESULTS

Growth Performance and Carcass Characteristics

The effect of dietary addition of clinoptilolite and phytase on BWG, FI, FCR and mortality rate (%) are shown in *Table 3*. Weight gain was lower in birds fed with clinoptilolite and phytase (T4) supplemented diet in comparison to control diet at 0-21 days of the study (P=0.044). Feed intake was similar among the treatment groups. The relative weight of internal organs, carcass and carcass components are shown in *Table 4*.

Table 3. *Influence of dietary treatments on performance of broiler* [1]
Tablo 3. *Deneme rasyonlarının broiler performansı üzerine etkisi*

Age (day)	Dietary Treatment[2]				Statistics	
	T1	T2	T3	T4	SEM	*P-value*
Weight gain, g						
1-14	468.62[a]	455.75[ab]	457.48[ab]	429.85[b]	5.13	0.044
1-21	977.98	974.03	997.77	941.27	8.93	0.159
1-42	3149.43	3225.14	3206.76	3147.26	21.76	0.494
Feed intake, g						
1-14	574.87	572.27	588.69	554.04	7.67	0.479
1-21	1356.74	1309.27	1346.19	1328.04	10.54	0.416
1-42	5132.50	5067.87	5105.79	5068.53	30.00	0.858
Feed intake/weight gain (FCR)						
1-14	1.23	1.26	1.29	1.29	0.01	0.335
1-21	1.39	1.34	1.35	1.41	0.01	0.161
1-42	1.63	1.57	1.59	1.61	0.01	0.071
Mortality, %						
1-42	2.08	4.16	4.16	6.25	-	0.791

[a-b] *Means with different superscripts in the same row are significantly different (P<0.05);* [1] *Data represent mean values of 8 replicates per treatment;* [2] ***T1:*** *Control, a standard corn-soybean meal basal diet;* ***T2:*** *2% clinoptilolite;* ***T3:*** *0.01% phytase (5000 FTU/g);* ***T4:*** *2% clinoptilolite+0.01% phytase*

Table 4. *Effect of dietary treatments on carcass components and relative organ weights on d 42* [1]
Tablo 4. *Deneme rasyonlarının karkas bileşenleri ve relatif organ ağırlıkları üzerine etkisi (42. gün)*

Item	Dietary Treatment[2]				Statistics	
	T1	T2	T3	T4	SEM	*P-value*
Dressing percentage, %	74.04	74.21	74.41	74.33	0.41	0.992
Breast weight, g	624.63	666.37	618.62	624.50	18.20	0.793
Breast % of live weight	17.28	19.44	18.46	19.00	0.34	0.876
Wings, g	210.50	226.75	222.00	228.00	3.50	0.281
Wings % of live weight	6.78	7.00	7.36	7.29	0.11	0.267
Leg quarters, g	626.25	673.00	642.50	669.71	13.51	0.579
Leg quarters % of live weight	20.20	20.78	21.00	21.41	0.22	0.296
Gizzard, g	41.37	36.62	35.37	36.12	0.95	0.098
Gizzard % of live weight	1.35	1.13	1.17	1.15	0.03	0.078
Liver, g	41.50	44.00	38.12	44.37	0.97	0.080
Liver % of live weight	1.34	1.36	1.25	1.42	0.03	0.130
Heart, g	15.00	14.00	13.62	13.75	0.42	0.675
Heart % of live weight	0.49	0.43	0.45	0.44	0.01	0.477
Abdominal fat, g	46.14	44.00	38.00	49.50	2.34	0.387
Abdominal fat % of live weight	1.06	1.14	0.98	1.13	0.07	0.307

[1] *Data represent mean values of 8 replicates per treatment;* [2] ***T1:*** *Control, a standard corn-soybean meal basal diet;* ***T2:*** *2% clinoptilolite;* ***T3:*** *0.01% phytase (5000 FTU/g);* ***T4:*** *2% clinoptilolite+0.01% phytase*

There were no significant differences among treatment groups on internal organ weights and processing characteristics.

Intestinal Histomorphology

Morphological measurements of small intestine are shown in *Table 5*. At d 21, duodenal villus height was not affected by dietary treatments, however crypt depth was significantly (P=0.006) decreased by phytase (T3) addition compared to control group (T1). On the other hand, dietary treatments have more pronounced effect on duodenal villus height at d 42. In jejunum and ileum, villus height and crypt depth were not affected by clinoptilolite and/or phytase addition either 21-d or 42-d of ages. However jejunum villus height:crypt depth ratio (VH:CD) was significantly increased (P=0.037) by phytase supplementation on d 42.

Tibia Ash, Ca and P Levels

At 21 days of age, combined use of dietary clinoptilolite and phytase reduced (P=0.002) tibia ash content of the birds compared to those fed without phytase supplemented groups *(Table 6)*.

Moreover dietary clinoptilolite and phytase (T4) reduced (P<0.001) tibia P level on d 21. However dietary treatments did not affect tibia Ca level. At 42 days of age, tibia ash, Ca, P levels and Ca/P ratio were not affected by dietary treatments.

DISCUSSION

Growth Performance

Clinoptilolite and phytase supplementation to Ca and P deficient diets decreased the growth performance of broilers at starting period. However, with the advancing age, dietary treatments did not affect broiler performance during the entire experimental period. These effects might be attributed to the level of clinoptilolite in Ca and P deficient diets. According to these results it can be assumed that dietary 2% clinoptilolite may be high at starting period. Suchy et al.[2] reported that dietary 1% clinoptilolite supplementation improved weight gain of broiler chickens than those fed with 2% clinoptilolite on d 30. However body weight was increased with both supplementation levels (1 and 2%) on d 40. In agreement with our findings, they suggested that supplementation of 2% clinoptilolite in younger chickens may have a suppressive effect on broiler performance. Moreover they concluded that the level of clinoptilolite in the diets might be increased with chickens' age. In addition, Eser et al. [37] revealed that dietary 1% sepiolite supplementation increased body weight of broiler chickens on d 42. Contrary to our results, Karamanlis et al.[38] revealed that clinoptilolite addition to the broiler diet and litter were significantly improved live weight and such differences were more pronounced after 28-d of ages. Also, there was a significant interaction between feeding and bedding treatments on

Table 5. *Effects of clinoptilolite and/or phytase addition on intestinal histomorphology of broiler chicken at d 21 and d 42* [1]
Tablo 5. *Klinoptilolite ve/veya fitaz ilavesinin 21 ve 42 günlerdeki broiler bağırsak histomorfoloji üzerine etkisi*

Item	Dietary Treatment[2]				Statistics	
	T1	T2	T3	T4	SEM	*P-value*
Duodenum						
d 21						
Villus height (μm)	1831.81	1858.69	1828.25	1785.13	35.69	0.918
Crypt depth (μm)	281.69[a]	261.94[ab]	233.88[b]	260.50[ab]	5.14	0.006
VH:CD[3] ratio	6.57	7.20	7.94	6.89	0.22	0.160
d 42						
Villus height (μm)	1760.75[b]	2190.69[a]	2051.13[a]	2166.06[a]	46.36	0.001
Crypt depth (μm)	255.50[b]	271.19[ab]	283.94[ab]	305.75[a]	6.54	0.039
VH:CD ratio	6.96	8.10	7.37	7.12	0.17	0.089
Jejunum						
d 21						
Villus height (μm)	1382.00	1399.06	1192.69	1375.00	32.53	0.104
Crypt depth (μm)	246.75	222.31	230.25	233.25	5.55	0.491
VH:CD ratio	5.65	6.38	5.31	5.79	0.18	0.213
d 42						
Villus height (μm)	1734.44	1653.06	1839.81	1740.25	43.96	0.538
Crypt depth (μm)	262.44	258.44	242.00	271.81	6.00	0.373
VH:CD ratio	6.67[b]	6.47[b]	7.63[a]	6.42[b]	0.17	0.037
Ileum						
d 21						
Villus height (μm)	788.75	907.69	834.69	949.94	24.63	0.080
Crypt depth (μm)	205.56	207.69	190.56	216.00	4.85	0.320
VH:CD ratio	3.84	4.40	4.44	4.41	0.10	0.108
d 42						
Villus height (μm)	1492.31	1347.94	1443.50	1527.31	34.54	0.443
Crypt depth (μm)	257.81	237.69	227.44	265.25	5.58	0.051
VH:CD ratio	5.80	6.08	6.35	5.79	0.12	0.326

*[a-b] Means with different superscripts in the same row are significantly different (P<0.05); [1] Data represent mean values of 8 replicates per treatment; [2] **T1:** Control, a standard corn-soybean meal basal diet; **T2:** 2% clinoptilolite; **T3:** 0.01% phytase (5000 FTU/g); **T4:** 2% clinoptilolite+0.01% phytase; [3] VH:CD; villus height:crypt depth*

days 28 (P<0.05) and 42 (P<0.001). On the other hand, dietary and bedding treatments did not affect feed conversion ratio. Similar to our results, Horniakova and Busta [39] reported that the average live weight of broiler chickens tended to increase by a feed additive containing clinoptilolite compared with the control group. In addition to the natural clinoptilolites, modified clinoptilolite [40] and zinc-bearing clinoptilolite [41] had no consistent beneficial effects on broiler overall performance from 1 to 42 d. As mentioned by the numerous reports clinoptilolite is a harmless feed additive for broilers. However discrepancies between the studies may be related to the nature, purity, concentration and composition of the clinoptilolite [40]. In this study, dietary 2% clinoptilolite and phytase supplementation decreased broiler weight gain between 1 to 14 d of the study. However, clinoptilolite and phytase combination had no pronounced effect on broiler growth performance during 42 d experimental period. It is necessary to mention that the current study was conducted under good hygienic conditions and the day old male chickens were provided from a young breeders flock. As a result, the need for dietary feed additives to increase the production performance might be decreased to minimum. In addition it can be assumed that effects of clinoptilolite on performance might be more pronounced under suboptimal environmental conditions.

Intestinal Histomorphology

The morphological changes in the small intestine, such as increasing villus height and VH:CD ratio might

Table 6. *Effect of dietary clinoptilolite and/or phytase supplementation on tibia ash, Ca and P content* [1]
Tablo 6. *Klinoptilolite ve/veya fitaz ilavesinin broiler tibia külü ile Ca ve P düzeylerine etkisi*

Item	Dietary Treatment[2]				Statistics	
	T1	T2	T3	T4	SEM	*P-value*
d 21						
Tibia ash, %	56.72[a]	57.07[a]	55.89[ab]	55.14[b]	0.21	0.002
Ca, %	20.36	20.27	19.67	19.68	0.15	0.228
P, %	10.32[a]	10.24[a]	10.07[a]	9.68[b]	0.06	<0.001
Ca/P ratio	1.97	1.98	1.95	2.03	0.02	0.326
d 42						
Tibia ash, %	59.09	60.24	58.83	58.35	0.29	0.115
Ca, %	19.98	20.47	20.29	19.80	0.18	0.538
P, %	10.15	10.30	10.56	10.17	0.07	0.099
Ca/P ratio	1.97	1.99	1.92	1.95	0.02	0.537

*[a-b] Means with different superscripts in the same row are significantly different (P<0.05); [1] Data represent mean values of 8 replicates per treatment; [2] **T1:** Control, a standard corn-soybean meal basal diet; **T2:** 2% clinoptilolite; **T3:** 0.01% phytase (5000 FTU/g); **T4:** 2% clinoptilolite+0.01% phytase*

have beneficial effects on birds performance. So that, these changes enhance the absorptive surface area that is prominent when the alternative growth stimulators are applied. Previous studies have revealed that dietary clinoptilolite can influence intestinal mucosa and protect intestinal mucosa from damage [42-44]. In the current study, duodenal villus height and crypt depth were affected by dietary treatments on d 21 and d 42 *(Table 5)*. However no significant differences were observed on jejunum and ileal histomorphology during the experimental period. Xia et al.[45] reported that dietary Cu-montmorillonite increased villus height and villus height:crypt depth ratio in small intestine. They suggested that such improvements on intestinal integrity might be related to the decreased numbers of *E. coli* and *Clostridium* spp. and increased mucus resistance. Similarly Wu et al.[40] suggested that improvement in the intestinal integrity might be related to the lower numbers of *E. coli* and Salmonella. In addition Tang et al.[41] observed an increase in villus height and villus height:crypt depth ratio of the small intestinal mucosa of the chickens fed the diet supplemented with zinc-bearing clinoptilolite. As reported by previous researchers, montmorillonite acts as a mucus stabilizer and attaches to the mucus to support the intestinal mucosal barrier and help regeneration of the epithelium [46,47]. In addition, due to the porous structure and high cation exchange capacity, clinoptilolite can bind amines, ammonia and toxins. These suggested favorable effects of clinoptilolite may help to protect gut wall to the deleterious effects of such products.

More recently, Wu et al.[4] reported that dietary addition of natural and modified clinoptilolite, at the level of 2%, may have protective effects on intestinal mucosa against LPS-mediated damage. Previous studies indicated that natural and modified clinoptilolite supplementation has beneficial effect on intestinal integrity. In addition to previous studies, our results suggested that combined use of clinoptilolite and phytase in low Ca and P diets had no detrimental effect on male broiler intestinal histomorphology.

Tibia Ash and Mineral Content

According to the previous studies [17,21] dietary zeolite supplementation improves bone ash in birds fed low Ca diets. Moreover, Leach et al.[17] reported that dietary sodium zeolite supplementation increased serum Ca level and decreased P level. They concluded that decreased serum P level might be related to the sodium zeolite addition. Contrary to that earlier study, tibia P level was decreased numerically in comparison to control on d 42. However tibia P level was significantly lower (P=0.001) for the birds fed clinoptilolite and phytase supplemented diet versus to control on d 21. It can be assumed that dietary level of 2% clinoptilolite and phytase supplementation decreased P utilization, therefore it might useful to decrease clinoptilolite level in phytase supplemented diets at starting period.

Phytase supplementation had no consistent effect on tibia parameters on d 21 and d 42. Contrary to our findings, Chung et al.[28] showed that both fungal and bacterial phytases improved bone mineral density of tibia and femur of broilers on d 21. Discrepancies between the studies might be related to the source of phytase and analytical methods.

Generally, findings from the studies were inconsistent [2,45,48] and the effect of clays is changed according to the kind of clay and obtaining area, physical or chemical properties, dose used in the experiment of the clay. Also diet type and environment (existence of pathogen or toxin etc.) may play a role on the results. It is concluded that there might be a positive effect of 2% clinoptilolite on the performance of male broilers after starting period.

Further studies are needed to determine optimum dose of clinoptilolite and/or phytase combination for starter period. Effectiveness of the clinoptilolite should be examined in suboptimal conditions such as toxin or pathogen existence. Also effect of clinoptilolite and phytase combination should be determined in diets with different Ca levels.

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