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Bone Status and Performance of Broiler Chickens in Response to Fennel Extract and Chelated Manganese-Glycine

Research Article

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ABSTRACT

The present study was conducted to evaluate the effects of fennel hydroalcoholic extract and chelated manganese-glycine on performance and bone status in broiler chickens. A total of 360 of day-old Arbor Acres broilers were randomly arranged according to a completely randomized experimental design (CRD) in a 2 × 3 factorial management with 6 treatments 6 replicates and 10 birds each. Treatments consisted of basal diets as control (include corn and soy bean meal), three levels of fennel (0, 100, or 200 mg/kg diet), and two levels of chelated manganese-glycine (0 or 150 mg/kg diet). Two similar weight of broilers were selected in each replicate and sacrificed by neck dislocation then tibia bones were removed, and freeze-dried and carcass yield and relative weights of internal organs were expressed as the percentages of live body weight. The results have shown that broiler performance was not affected (P>0.05) by the supplementation of fennel extract and chelated manganese glycine (150 mg/kg) in broilers diet but increased the tibia manganese content in this respect. However, the bone manganese reduced significantly (P<0.05) by fennel hydroalcoholic extract at 42 days of age. The result of this study has suggested that improved broiler tibia manganese content by manganese chelated-glycine supplementation.

KEY WORDS bone, broiler chicken, chelated manganese, fennel.

INTRODUCTION

Skeletal disorders are more likely to occur when the rate of growth is high (Wideman *et al.* 2012). Bone fractures damages the tissue around the bone could reduce meat quality for consumers (Gregory *et al.* 1993). High demand of natural compounds such as fennel and other medicinal plants instead of drugs and chemical compounds are preferable. The essential oils, and extracts of medicinal herbs, added to animal diet for improving the animals' health or production aspects (Kim *et al.* 2012; Hosseini *et al.* 2016; Ogbe and Affiku, 2020). Researchers have reported that improvement in the feed intake (FI), feed conversion ratio (FCR) and carcass yield (CY) were obtained by adding aromatic plants in feed and water. It is indicated that increased weight and improved nutritional efficiency by fennel which is one of the aromatic plants in broilers diet. Also, these plants (fennel) are useful for skeletal disorders and osteoporosis treatment (Kim *et al.* 2012). Researchers have shown that manganese requirements are high in poultry and supplementation of manganese in poultry diets is more important than in other animals (Collins and Moran, 1999). Commercial rations often contain large quantities of corn and soybean meal which could not provide enough manganese re quirements and also have a high amount of anti-nutritional, such as phytate. The phytate has a strong binding affinity to minerals such as calcium, magnesium, iron, and manganese and making unavailable and absorption minerals in the poultry intestine. Therefore, it is suggested to use the chelate form of mineral elements with amino acids. The most important characteristics of chelated are bioavailability and the high stability coefficient of these materials. Thus, have a high absorption rate in the intestine, thereby increasing the productivity of consumption in nutritional elements of the diet (Ji *et al.* 2006). Therefore, this study aimed to investigate the effect of chelated manganese-glycine and fennel extract on broilers' bone status and performance.

MATERIALS AND METHODS

Experimental design and diets

A total of 360 Arbor Acres broiler chickens day old were used in this experiment (1 to 42 days). The birds were arranged according to a completely randomized design (CRD) with 6 treatments in a 3 × 2 factorial arrangement, 6 replicates and10 broilers each. Dietary treatments were included three levels of fennel extract (0, 100, and 200 mg/kg diet) and two levels of chelated manganese–glycine (0 and 150 mg/kg diet). In this study, the fennel extract was obtained using the decoction method (the process of boiling a substance in a liquid to extract its active ingredients). Thirty grams of fennel seeds were mixed with 200 mL of 70% ethanol. The mixtures were kept in refrigerator for 24 hours and then filtered through gauze and evaporated under vacuum conditions at 40 ˚C using a rotary evaporator (Kazemi-Fard *et al.* 2013) and the Mn source included Mn-chelate of glycine hydrate (Mn-glycinate) (E.C.O.Trace® Mn; Biochem, Germany). The broiler, feed intake, and feed residue were weighed periodically per pen to calculate weight gain, feed intake, and feed conversion ratio. Mortality was also recorded to correct performance data. The broilers were sacrificed by neck dislocation, carcass yield, and relative weights of internal organs were expressed as the percentages of live body weight at the end of experiment (42 days of age).

Birds and housing

During the six weeks of the experiment, access to feed and water were provided *ad libitum* throughout the 42-d feeding period. The temperature was maintained at 29 to 31 ˚C for the first week and the temperature was decreased as the birds aged, reaching a final temperature of 24 ˚C. The chemical analysis of corn, corn gluten, and soybean meal are presented in Table 1 and experimental diets were managed according to the Arbor Acres recommendation, 2014 (Table 2).

Bone characteristics

The tibia bones were collected, freeze-dried, and frozen at −20 ˚C until mechanical testing. The bone resistance was determined by the length and diameter tibia bone were measured (these characteristics measured by Digital Caliper).

Also, flexural stress (mPa) and flexural strain (%) at a three-point test were carried out with the Instron device (BT1-FR0.5TH.D14, 191483/2010, Germany). Tibia breaking strengths (breaking force divided by bone weight expressed as kilograms per gram) were measured using an Instror3 with 50-kg-load cell at 50-kg-load range (Park *et al.* 2003).

The left tibia were weighed and ashed in muffle furnace at 600 ± 5 ˚C for 4 h and bone Mn was determined using atomic absorption spectrophotometer (Spectr AA 220 VARIAN) (Gajula *et al.* 2011).

Statistical analyses

The data analyzed by SAS software version 9.4 (SAS, 2004) with a general linear models procedure for ANOVA. Duncan's multiple tests were used to analyze the differences between means. The significant difference statements were based on the probability (P<0.05).

RESULTS AND DISCUSSION

Performance and production status

The average weight gain, feed intake, and feed conversion ratio from 1 to 42 days are shown in Table 3. No significant differences were found in weight gain, feed intake andfeed conversion ratioin different levels of fennel and manganese compared with control (P> 0.05). Furthermore, Yan *et al.* (2011) have shown similar reaction of this study by levels of manganese did not have any significant effects on weight gain and feed intake.

Contrary to the results of this study, Gholami *et al.* (2016) have observed that the feed conversion ratio and weight gain of broilers significantly (P<0.05) were improved by diets containing 70 and 150 mg/kg of manganese but no responsed was found by various sources of manganese in diet (Wang *et al.* 2012). Some researchers have indicated that fennel consumption could lead to increase weight gain and improved nutritional efficiency in broiler diets (El Deek *et al.* 2003). Also, then have pointed out that beneficial observed without any adverse effect on the productive performance of Post Molt Broiler Breeder by incorporation of 50 mg/kg fennel extract and 3500 IU/kg Vitamin D3 in the diet exhibit (Kazemi-Fard *et al.* 2013). In addition, increased the performance and growth of broiler chickens were highlighted by the using of fennel with citric acid (Salari *et al.* 2014).

Table 1 experimental chemical composition (%)

Nutrients	C	CG	SBM
Dry matter	90.97	94.94	91.22
Crud ash	1.095	2.3	3.703
Crude Protein	7.85	60.69	46.15
Crud fat	3.5	8.5	0.5
Crud fiber	1.9	1.2	00.7
Profile of amino acids[1]			
Methionine	0.161	1.193	0569
Cystine	0.161	0.817	0.520
Methionine + cystine	0.322	2.01	1.082
Lysine	0.218	0.694	2.498
Threonine	0.253	1.627	1.515
Tryptophan	0.051	0.210	0.552
Arginine	0.342	1.612	3.014
Isoleucine	0.259	2.147	1.840
Lucien	0.846	9.156	3.117
Valine	0.356	2.397	1.935
Histidine	0.225	1.051	1.087
Phenylalanine	0.352	3.356	2.097

[1] Estimated with near infrared spectroscopy (NIR).
C: corn; CG: corn gluten and SBM: soybean meal.

Table 2 Experimental ingredient diet

Ingredient	0-10 d	11-24 d	25-42 d
Corn	52.38	59.22	68.15
Soy bean meal	37.91	33.02	26.44
Corn gluten	3.00	2.00	0.00
Soy oil	2.15	1.68	1.8
DCP	2.05	1.65	1.38
Oyster shell	1.02	1.01	0.9
Salt	0.33	0.33	0.33
DL-methionine	0.31	0.28	0.24
Mineral premix[4]	0.25	0.25	0.25
Vitamin premix[5]	0.25	0.25	0.25
L-lysine	0.25	0.23	0.19
Threonine	0.11	0.09	0.07
Calculated composition			
Metabolism energy (kcal kg^{-1})	2950	3000	3100
crud protein	23.908	21.548	17.924
Calcium	0.9440	0.8420	0.7260
Available phosphorus	0.4720	0.4210	0.3630
Sodium	0.1570	0.1550	0.1550
Potassium	0.9325	0.8561	0.7467
Chlorine	0.2765	0.2729	0.2691
Lysine (SID)	1.2800	1.1500	0.9600
Methionine (SID)	0.6437	0.5854	0.5014
Methionine + cysteine (SID)	0.9500	0.8700	0.7500
Tryptophan (SID)	0.2423	0.2167	0.1807
Threonine (SID)	0.8600	0.7700	0.6400
Arginine (SID)	1.3700	1.2300	1.0300
DCAB	230.669	210.612	183.038

Additive minerals containing (per kg of supplement): Mn: 37000 ppm; Fe: 20000 ppm; I: 400 ppm; Se: 800 ppm and Choline chloride: 8000 mg.
Vitamin supplement containing: Vitamin A: 3600000 UI; vitamin D_3: 80000 IU; vitamin E: 7200 IU; K_3: 800 mg; vitamin B_1: 720 mg; B_2: 2640 mg; B_3: 4000 mg; B_5: 12000 mg; B_6: 1182 mg; B_9: 400 mg; B_{12}: 6 mg; H_2: 40 mg; Choline chloride: 12000 mg and Antioxidant: 400 mg.

Table 3 Broiler chicken performance in response to various levels fennel and manganese chelated at 42 days of age

Treatments (mg/kg)	BWG	FI	FCR
Fennel			
0	2755.129	4508.168	1.61
100	2715.153	4400.626	1.645
200	2718.531	4333.303	1.59
SEM	40.910	95.614	0.044
Manganese			
0	2742.873	4569.712	1.691
150	2716.336	4296.589	1.606
SEM	33.401	78.068	0.036
Fennel-manganese			
0-0	2794.520	4733.300	1.69
0-150	2715.740	4398.916	1.644
100-0	2730.940	4643.799	1.727
100-150	2699.360	4329.866	1.630
200-0	2703.150	4330.281	1.626
200-150	2733.910	4337.746	1.612
SEM	57.853	135.218	0.063
P-value			
Manganese	0.751	0.429	0.130
Fennel	0.585	0.112	0.127
Fennel × manganese	0.647	0.421	0.510
Treatment	0.866	0.318	0.447

BWG: Body weight gain; FI: feed intake and FCR: feed conversion rate.
SEM: standard error of the means.

Table 4 Percentage of relative weight of internal organs (percentages per live weight) at 42 days of age

Treatments (mg/kg)	GT	PR	FP	LI	TM	Pan	EG	FG	B	S	H
Fennel											
0	5.070^{a}	0.414	1.46	2.18	0.47	0.26	1.685	2.288	0.0	0.1	0.47
100	5.112^{a}	0.403	1.62	2.05	0.49	0.27	1.583	2.182	0.0	0.1	0.45
200	4.358^{b}	0.449	1.52	2.07	0.46	0.23	1.659	2.308	0.0	0.1	0.49
SEM	0.192	0.025	0.09	0.04	0.04	0.01	0.036	0.073	0.0	0.0	0.02
Manganese											
0	4.829	0.410	1.55	2.08	0.49	0.25	1.626	2.218	0.0	0.1	0.46
150	4.864	0.434	1.51	2.12	0.45	0.26	1.658	2.300	0.0	0.1	0.48
SEM	0.157	0.020	0.08	0.03	0.03	0.00	0.029	0.060	0.0	0.0	0.01
Fennel-Manganese											
0-0	5.000	0.399	1.42	2.2^{a}	0.50	0.2^{ab}	1.551^{b}	2.122	0.0	0.1	0.48
0-150	5.139	0.430	1.49	2^{ab}	0.44	0.23^{b}	1.819^{a}	2.453	0.0	0.1	0.47
100-0	5.106	0.413	1.52	1.9^{b}	0.48	0.2^{ab}	1.638^{b}	2.260	0.0	0.1	0.43
100-150	5.118	0.393	1.72	2.1^{a}	0.50	0.30^{a}	1.528^{b}	2.104	0.0	0.1	0.46
200-0	4.380	0.417	1.71	2^{ab}	0.50	0.23^{b}	1.689^{ab}	2.273	0.1	0.1	0.46
200-150	4.336	0.480	1.33	2^{ab}	0.42	0.2^{ab}	1.629^{b}	2.344	0.0	0.1	0.52
SEM	0.272	0.035	0.13	0.06	0.05	0.01	0.050	0.104	0.0	0.0	0.03
P-value											
Manganese	0.876	0.415	0.72	0.44	0.40	0.64	0.446	0.351	0.1	0.6	0.30
Fennel	0.028	0.426	0.51	0.15	0.83	0.12	0.150	0.451	0.4	0.6	0.39
Fennel × manganese	0.942	0.519	0.12	0.03	0.64	0.03	0.005	0.102	0.5	0.4	0.53
Treatment	0.155	0.579	0.33	0.06	0.83	0.06	0.017	0.233	0.3	0.7	0.51

GT: gastrointestinal tract; PR: proventriculus; FP: fat pad; LI: liver; TM: thymus; Pan: pancreas; EG: empty gizzard; FG: full gizzard; B: bursa; S: spleen and H: heart.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The percentage of liver weight was significantly decreased by supplementation of 100 mg of hydroalcoholic extract of fennel in kg diet at 42 days of age but no affected were found by other levels of fennel and manganese in this respect. The weight of empty gizzard significantly increased by supplementation of 150 mg/kg manganese cheated (P<0.05) in comparison to control but no response was found by different levels of fennel and manganese in this case. These results were comparable with the results of other researchers who have explained that the liver and gizzard weight of broiler were not affected by fennel extract (Rath *et al*. 2000).

However, the percentage of the gastrointestinal tract was significantly decreased by 200 mg/kg of fennel in the diet. Moreover, Ragabet *et al.* (2013) have reported that no significant (P>0.05) effect on live body weight, abdominal fat, gizzard, bursa, and thymus were indicated by fennel seed in the broiler diet.

The relative weight of carcass composition is shown in Table 5. The relative weight percentage of breast, thigh, and carcass were not affected by various levels of fennel and manganese. In contrast, reduced tibia manganese content at 42 days of age by 150 mg/kg of chelated manganese and 100 mg/kg of hydroalcoholic extract of fennel. Also, researchers have stated that broiler carcass characteristics were not significantly affected by 1, 2, and 3g/kgfennel in the broiler diet.

No significant effect on the femur, breast, and internal organs weight was caused by fennel seed in the broiler diet (Mohammed and Abbas, 2009). This supports our results.

No significant effect onbone weight, length, small and large diameter were found (P>0.05) by various combined treatments, fennel and chelated manganese-glycine (Table 6). The measurement of flexural stress and bone strain at 42 days of age are indicated in Table 7. Bone strength, defined by stress and strain, is an intrinsic property of bone (Turner, 2006). Stress is a measure of load per unit of area; whereas a strain is a measure of linear or shear deformation expressed as a percentage (%) of change in dimension (Ammann and Rizzoli, 2003; Wang and Puram, 2004). The results of this study have shown on Flexural stress and Flexural strain were not (P>0.05) affected by different levels of fennel and chelated manganese-glycine and the treatments composition. The bone resistance and bone manganese content are presented in Table 7. It is noticeable that the ultimate tibia resistance was not affected by the various levels of fennel extract and manganese-glycine (P<0.05). Also, the results have shown that a gradual decrease in tibia bone manganese content was found by the increase in fennel concentrations and the averages of manganese content were decreased in comparison to the control treatment at 42 days of age (P<0.0001). In the present study, bone manganese was not affected by dietary manganese.

Table 5 Relative weight of carcass components (percentages per carcass weight) at 42 days of age

Treatments (mg/kg)	Breast	Thigh	Carcass
Fennel			
0	38.336	31.320	69.307
100	39.194	30.172	68.799
200	37.443	31.931	69.477
SEM	0.647	0.461	0.485
Manganese			
0	38.492	31.264	69.130
150	38.156	31.049	69.258
SEM	0.528	0.376	0.396
Fennel-Manganese			
0-0	39.303	30.743[ab]	69.687
0-150	37.368	31.897[a]	68.927
100-0	38.079	31.375[a]	68.800
100-150	40.308	28.968[b]	68.797
200-0	38.095	31.582[a]	68.903
200-150	36.791	32.280[a]	70.050
SEM	0.915	0.651	0.686
P-value			
Manganese	0.660	0.734	0.823
Fennel	0.202	0.054	0.603
Fennel × manganese	0.087	0.036	0.404
Treatment	0.155	0.042	0.692

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Characteristics tibia bones in broiler chickens at 42 days of age

Treatments (mg/kg)	TW (g)[1]	BL (mm)[2]	LD (mm)[3]	SD (mm)[4]
Fennel				
0	21.161	109.001	9.047	7.572
100	19.823	108.693	8.814	7.358
200	20.028	98.133	8.717	7.648
SEM	0.875	4.631	0.224	0.279
Manganese				
0	20.417	102.629	8.804	7.628
150	20.258	107.921	8.914	7.424
SEM	0.715	3.781	0.183	0.228
Fennel-Manganese				
0-0	20.622	107.792	8.972	7.547
0-150	19.242	107.853	8.720	7.175
100-0	20.405	109.532	8.902	7.542
100-150	21.700	110.210	9.115	7.597
200-0	20.225	90.565	8.525	7.795
200-150	19.832	105.700	8.908	7.502
SEM	1.238	6.549	0.317	0.394
P-value				
Manganese	0.8773	0.3419	0.6770	0.5396
Fennel	0.5264	0.2095	0.5792	0.7532
Fennel × manganese	0.6643	0.4338	0.6734	0.8548
Treatment	0.8088	0.5486	0.8202	0.9267

TW: Tibia weight; BL: bone length; LD: large diameter and SD: small diameter.
SEM: standard error of the means.

Table 7 Bone strain and stress,resistance and manganese contentat 42 days of age

Treatments (mg/kg)	Flexural stress (mPa)[1]	Flexural strain (%)[2]	UTR (kg)	TMC (mg/kg)
Fennel				
0	49.257	8.248	47.2570	14.3854[a]
100	56.066	7.356	54.003	10.7708[b]
200	61.859	7.768	59.6930	6.2500[c]
SEM	5.179	0.532	3.2594	1.4389
Manganese				
0	52.859	8.237	50.2470	9.0902
150	58.999	7.136	57.0540	11.8472
SEM	4.228	0.435	2.2943	0.8815
Fennel-Manganese				
0-0	49.201	9.614	46.530	12.8333[b]
0-150	49.313	6.882	47.980	15.9375[a]
100-0	47.249	7.66	44.250	8.8958[c]
100-150	66.091	6.722	63.760	12.6458[b]
200-0	62.126	7.731	59.960	5.5417[d]
200-150	61.593	7.804	59.430	6.9583[d]
SEM	7.323	0.753	7.438	1.6208
P-value				
Manganese	0.3742	0.3127	0.2843	0.1999
Fennel	0.3148	0.6580	0.2839	< 0.0001
Fennel × manganese	0.4451	0.5254	0.3635	0.0004
Treatment	0.4635	0.6563	0.6552	< 0.0001

[1] External force is inserted perpendicular to bone length.
[2] Changes in bone length relative to initial length.
UBR: ultimate tibia resistance and TMC: tibia manganese content (mg/kg).
SEM: standard error of the means.

However, the amount of tibia bone manganese in the treatment by 150 mg/kg manganese was higher than in the other treatments. Also, the researchers have stated that different levels of dietary manganese have the highest effect on the bone marrow, liver, and kidney weight (Black *et al.* 1984). Moreover, it was reported that the increased significantly the amount of bone manganese content by supplementation of two types of proteins source and manganese oxide, (Gholami *et al.* 2016).

CONCLUSION

The results of this study have indicated that the tibia manganese content increased by supplementation of 150 mg/kg manganese-glycine chelated in the diet, however, broiler performance is not affected by this supplementation.

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