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Effects of Magnesium Sources and Levels on Some Tissue Magnesium Concentration and Bone Mechanical Properties in Broiler

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ABSTRACT

This experiment was conducted to determine the effect of inorganic and organic sources and levels of magnesium (Mg) supplementation on plasma, tibia, meat and liver Mg concentration and mechanical properties of bones in broilers. A total of one day old 450 broiler chicks were used and assigned to six experiment groups each having five replicate, randomly. There were 15 chicks in each replicates. In the experiment magnesium sulphate ($MgSO_4$) was used as inorganic Mg source and magnesium proteinate was used as organic Mg source. Experimental diets were supplemented provide 0 (control), 0.2 and 0.4 % Mg levels inorganic and organic Mg source of basal ration, and experiment period was six weeks. Main effect of Mg source and source x level interaction effect had not significant on plasma, liver, breast and thigh meat Mg concentration. While treatments did not significant effect on liver, breast and thigh meat Mg concentration, the main effect of Mg levels was significant effect on plasma Mg concentration. Plasma Mg concentration which were fed with ration added 0.2 and 0.4 % Mg level was higher than the control group. Tibia Ca concentration which were fed with ration added organic*0.2 and 0.4 % Mg levels were higher than the other groups. Tibia P concentration of organic Mg source fed with the group were higher than the inorganic source. The highest tibia Mg concentration were found to fed with organic*0.4 % Mg level of group. Tibia shear force and tibia stress which were fed with ration added 0.2 and 0.4 % Mg levels were higher than the control group. Supplementation of Mg in broiler diet was increased plasma Mg concentration and tibia shear force and also, tibia Ca and P concentration of organic Mg source fed with the group were higher than the inorganic Mg source.

1. Introduction

Magnesium (Mg) acts as a cofactor or an activator of many critical enzymes for the reactions involving ATP that energize all major metabolic pathways. Under commercial production condition, Mg deficiency is rare in poultry as Mg content in maize-soybean meal diet is 2 – 4 times the 650 mg/kg Mg requirement put forth by the National Research Council (1994). A reference practical diet for chicks (NRC, 1994) with maize-soybean meal would contain on average 1500-1800 mg/kg feed only from these ingredients. Very little attention has been paid to Mg, and Mg is usually not included in mineral mixtures for poultry. Magnesium deficiency is not to be expected under practical feeding in poultry. Thus, additional Mg supplementation to the diets in commercial practice is unnecessary and even might be detrimental to performance and bone health.

Lee et al. (1980) found that feeding excess Mg reduced growth and bone development of broilers. Tibia ash was reduced in chicks fed 0.9 %Mg. McGillivray and Smidt (1975) used semi-purified diets for biological evaluation of Mg sources in broilers. Anhydrous $MgSO_4$ as the reference standard provided levels of added Mg ranging from 0 to 500 mg/kg. Stillmak and Sunde (1971) chose plasma Mg level as an indicator of Mg availability in chicks because of its positive linear correlation with the level of dietary Mg, as shown by Gardiner et al. (1960). Based on this criterion, the authors concluded that the Mg in dolomite was less available than that contained in $MgCO_3$. Liu et al. (2007), using serum Mg, reported higher bioavailability values

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for organic Mg (L-aspartate) compared to inorganic Mg (MgO) sources. However, serum Mg concentration may be influenced by changes in serum pH, serum albumin, and other anionic ligands (Kimura, 2007). It also should be emphasised that the values based on growth, bone, or blood criteria provide only relative values of Mg availability and are not quantitative. Thus, such values are difficult to consider in feed formulation but can be used for qualitative comparison among Mg sources. Harland et al. (1976) reported that in Japanese quails, between 200 and 1000 ppm Mg there was a linear relationship between concentrations of Mg in the tibia. This suggests that tibia Mg concentration might be useful for bioassay of Mg in feedstuffs.

The objective of the present study was to investigate the mechanism by which $MgSO_4$ and Mg proteinate and supplementation levels of Mg, on some tissues Mg content and bone mechanical traits of broilers.

2. Materials and Methods

A total of 450 1-d-old male broiler chicks (Ross 308) were randomly assigned to six experiment groups each having five replicate. The experimental diets were prepared by adding certain amounts of organic (Mg-proteinate) and inorganic ($MgSO_4$) Mg sources which were provided as 0 (control), 0.2 and 0.4 % Mg in basal ration. Starter and grower diets were formulated according to recommendation in the Ross management manual and NRC (1994). The basal diet composition was showed in Table 1 and Table 2. Broilers were fed with starter diets from 1 to 21 day of age and grower diets from 22 to 42 day of age. Water and feed were supplied *ad-libitum* throughout the experiment. Criteria specified by the National Institute of Health Guide for the Care and Use of Laboratory Animals were followed during the study period.

On the last day (42 days) of the experiment, 4 (two male and two female) broilers from each replicates were randomly selected and slaughtered then were taken blood, tissue samples and tibia for determination of breast, thigh, liver, plasma and tibia mineral concentration. Mineral content of the samples was determined by MarsXpress Technology Inside and Inductively Coupled Plasma Atomic Emission Spectrometer (Vista AX CCD Simultaneous ICP-AES). Approximately 0.20 g of dried sample was put into a burning cup, and 5mL nitric acid, 3mL perchloric acid and 2mL hydrogen peroxide was added. The sample was incinerated in a MARS 5 Microwave Oven (CEM Corp., USA, 3100 Smith Farm Road, Matthews, NC) at 190 ^{o}C temperature and 1.207 kPa pressure, and after diluted 50mL of distilled water. Mineral concentrations were determined by an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (Skujin et al., 1998).

Bone mechanical properties were determined from the load-deformation curve generated from a three point bending test (ASAE Standard S459, 2001) using an Instron Universal Testing Instrument (Model 1122; Instron, Canton, MA) and the Test Works 4 software package (version 4.02; MTS System Corporation, Eden Prairie, MN). The crosshead speed was constant at 5mm per min. The full scale load of the load cell was 5 Newtons (N), and none of the bones failed or fractured at or below 5 N. Shear tests were performed on the tibiae using a double shear block apparatus. The shear force was exerted over a 6.35mm (0.25 inch) section located at the centre of the diaphysis. These tests resulted in the ultimate shear force and shear stress being evaluated for each bone. An average wall thickness (cortex thickness) of the tibia was measured at two points on the central axis of the broken tibia used in determining mechanical properties, using digital calipers with a precision of 0.001 mm. These mechanical properties of bone are described by Wilson and Ruszler (1996).

Data were subjected to ANOVA by using General Linear Model procedure (GLM) in Minitab (2000). Duncan's multiple range tests were applied to separate means (Mstat-C, 1995). The experiment was designed as 2 (Mg sources) x 3 (Mg levels) factorial within a completely randomized design.

Table 1.

Composition of experimental diets (Starter diets, 0-3 weeks)

Ingredients (%)	Control	Inorganic Mg ($MgSO_4$)		Organic Mg (Mg-proteinate)	
		0.2 %	0.4 %	0.2 %	0.4 %
Corn	51.30	47.27	44.00	50.50	48.60
Soybean meal	38.80	39.40	39.80	35.40	32.40
Vegetable oil	6.10	7.50	8.45	7.20	8.80
Limestone	1.00	1.00	1.00	1.00	1.05
Dicalcium phosphate	2.10	2.20	2.10	2.10	2.10
Salt	0.30	0.25	0.25	0.25	0.25
Premix[1]	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.02	---	---	0.10	0.20
DL-Methionine	0.13	0.13	0.15	0.13	0.15
Inorganic Mg ($MgSO_4$)	---	2.00	4.00	---	---
Organic Mg (Proteinat)	---	---	---	3.10	6.20
TOTAL	100	100	100	100	100
Calculated Nutrients					
Crude protein (%)	22.08	22.06	22.00	21.98	21.97
Metabolizable Energy	3104	3107	3098	3098	3108
(kcal/kg)	1.00	1.00	1.00	1.00	1.00
Calcium (%)	0.50	0.50	0.50	0.49	0.49
Available phosphorus (%)	0.48	0.48	0.49	0.46	0.46
Methionine (%)	0.85	0.84	0.85	0.79	0.78
Methionine + Cystine (%)	1.31	1.30	1.30	1.29	1.29
Lysine (%)					

[1]: Provided (per kilogram of diet): Vitamin A, 15000, IU; Vitamin D_3 1500IU; Vitamin K 5,0 mg; Vitamin B_1 3 mg; Vitamin B_2 6 mg; Vitamin B_6 5 mg; Vitamin B_{12} 0,03 mg; Niacin 30 mg; Biotin 0,1 mg; calcium D-pantotenat 12.0 mg; folic acid 1.0 mg; coline chloride 400 mg; Manganese 80 mg; Iron 35 mg; Zinc 50 mg; Copper 5.0 mg; Iodine 2 mg; Cobalt 0.04 mg

Table 2.

Composition of experimental diets (Grower diets, 3-6 weeks)

Ingredients (%)	Control	Inorganic Mg ($MgSO_4$)		Organic Mg (Mg-proteinate)	
		0.2 %	0.4 %	0.2 %	0.4 %
Corn	56.00	51.37	47.50	53.80	51.92
Soybean meal	33.60	34.50	35.00	31.00	28.36
Vegetable oil	6.70	8.30	9.58	8.25	9.60
Limestone	1.20	1.30	1.40	1.25	1.20
Dicalcium phosphate	1.83	1.88	1.82	1.85	1.82
Salt	0.30	0.30	0.30	0.30	0.30
Premix[1]	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.02	---	---	0.10	0.20
DL-Methionine	0.10	0.10	0.15	0.10	0.15
Inorganic Mg ($MgSO_4$)	---	2.00	4.00	---	---
Organic Mg (Proteinate)	---	---	---	3.10	6.20
TOTAL	100	100	100	100	100
Calculated Nutrients					
Crude protein (%)	19.99	20.06	20.01	20.00	20.01
Metabolizable Energy (kcal/kg)	3194	3203	3199	3200	3198
Calcium (%)	0.99	1.04	1.05	1.01	1.01
Available phosphorus (%)	0.44	0.45	0.44	0.44	0.44
Methionine (%)	0.42	0.42	0.44	0.42	0.43
Methionine + Cystine (%)	0.76	0.76	0.79	0.72	0.72
Lysine (%)	1.16	1.15	1.16	1.16	1.17

[1]: Provided (per kilogram of diet): Vitamin A, 15000, IU; Vitamin D_3 1500IU; Vitamin K 5,0 mg; Vitamin B_1 3 mg; Vitamin B_2 6 mg; Vitamin B_6 5 mg; Vitamin B_{12} 0,03 mg; Niacin 30 mg; Biotin 0,1 mg; calcium D-pantotenat 12.0 mg; folic acid 1.0 mg; coline chloride 400 mg; Manganese 80 mg; Iron 35 mg; Zinc 50 mg; Copper 5.0 mg; Iodine 2 mg; Cobalt 0.04 mg

3. Results

Main effect of Mg source and source x level interaction effect had not significant on plasma, liver, breast and thigh meat Mg concentration. While treatments did not significant effect on liver, breast and thigh meat Mg concentration, the main effect of Mg levels was significant effect on plasma Mg concentration (P< 0.05). Plasma Mg concentration which were fed with ration added 0.2 and 0.4 % Mg level was higher than the control group. Effects of Mg sources and levels on Mg concentrations in plasma, liver, breast and thigh meat was showed in Table 3

Table 3.

Effects of Mg sources and levels on Mg concentrations in plasma, liver, breast meat and thigh meat

Diets	Plasma Mg (mg/100ml)	Liver Mg (mg/kg)	Breast Mg (mg/kg)	Thigh Mg (mg/kg)
Mg Sources				
Inorganic	2.17±0.04	596.3±4.69	303.4±2.52	305.4±3.78
Organic	2.18±0.04	593.3±5.14	306.4±3.90	298.3±3.77
Mg Levels, %				
0	2.06±0.03[b]	588.2±5.42	306.5±5.17	306.4±5.40
0.2	2.22±0.06[a]	595.5±4.89	300.6±4.17	302.0±4.14
0.4	2.27±0.03[a]	600.6±7.21	307.6±1.84	297.2±4.43
Source*Level				
Inorganic*0	2.07±0.05	586.8±9.99	310.3±4.16	310.1±5.96
Inorganic*0.2	2.17±0.07	599.2±8.97	293.6±1.25	303.7±7.46
Inorganic*0.4	2.28±0.05	602.8±3.98	306.3±3.12	302.4±7.07
Organic*0	2.05±0.02	589.6±5.57	302.8±9.78	302.6±9.42
Organic*0.2	2.26±0.09	591.7±4.49	307.5±7.20	300.3±4.48
Organic*0.4	2.25±0.04	598.5±4.70	308.9±2.12	292.0±4.97

[a, b] : Means with different minuscule in the same column are significantly different at P<0.05.

Tibia Ca concentration which were fed with ration added organic*0.2 and 0.4 % Mg levels were higher than the other groups (P< 0.05). The highest tibia Mg concentration were found to fed with organic*0.4 % Mg level of group (P<0.05). Tibia P concentration of organic Mg source fed with the group were higher than the inorganic source (P< 0.05). Tibia shear force and tibia stress which were fed with ration added 0.2 and 0.4 % Mg levels were higher than the control group (P< 0.05). Effects of Mg sources and levels on mineral contents and biomechanical properties of tibia were given in Table 4.

Table 4.

Effects of Mg sources and levels on mineral contents and biomechanical properties of tibia

Diets	Tibia Ca (%)	Tibia P (%)	Tibia Mg (%)	Tibia Shear Force (N)	Tibia stress (N/mm^2)
Mg Sources					
Inorganic	25.85±0.249	10.89±0.192[b]	0.513±0.0943	1278±88.1	44.29±2.82
Organic	28.50±0.521	11.74±0.172[a]	0.551±0.0208	1373±84.9	50.10±2.05
Mg Levels, %					
0	26.83±0.303	11.23±0.261	0.510±0.0160	1078±65.9[b]	40.74±2.47[b]
0.2	27.19±0.707	11.03±0.244	0.506±0.0734	1341±112.5[a]	48.83±3.71[a]
0.4	27.51±0.956	11.67±0.274	0.580±0.0168	1556±53.4[a]	52.02±1.63[a]
Source*Level					
Inorganic *0	26.60±0.385[b]	11.01±0.397	0.518±0.0188[b]	1081±65.5	38.18±2.43
Inorganic *0.2	25.47±0.290[b]	10.48±0.166	0.493±0.0148[b]	1293±135.9	44.11±6.90
Inorganic *0.4	25.49±0.417[b]	11.18±0.367	0.529±0.0132[b]	1459±38.7	50.64±2.76
Organic*0	27.06±0.495[b]	11.45±0.360	0.502±0.0375[b]	1075±126.5	43.36±4.23
Organic*0.2	28.92±0.523[a]	11.59±0.219	0.520±0.0060[b]	1389±44.1	53.55±1.40
Organic*0.4	29.54±1.168[a]	12.17±0.228	0.631±0.0721[a]	1654±73.8	53.39±1.85

[a, b] : Means with different minuscule in the same column are significantly different at P<0.05.

4. Discussion

In current study results that dietary Mg levels and sources had no significantly effect on Mg concentration of liver, breast and thigh meat. However, dietary Mg supplementation increased Mg concentrations in plasma of broilers. Plasma Mg is the sensitive index to reflect Mg nutritional status of animals. Previous work with broilers has indicated that diets supplemented with Mg increased plasma Mg concentration (Gardiner et al., 1960). Liu et al. (2007) reported that dietary Mg supplementation increased Mg concentrations in serum and liver of broiler chickens, and the supplemented organic Mg. Georgeta et al. (2014) reported that plasma Mg concentration increased in dietary supplements of Mg groups, compared with control group. However, there was no significant treatment effect on plasma Ca and P concentration. The current study result agrees with that of Zimmermann et al. (2000) who reported that serum Mg of the rodents was highly dependent on dietary Mg level. Hess and Britton (1997) reported that excess Mg (dietary Mg levels of 0.15, 0.36, 0.53, 0.76, and 0.91%) reduced plasma Ca, and increased plasma Mg. It has been shown that blood Mg levels play a role in the control of parathyroid hormone release and that Mg may be as effective as calcium on a molar basis in parathyroid gland function (Sherwood et al., 1970).

The present study results that dietary Mg sources had significantly effect on Ca, P and Mg concentration of tibia. A dietary organic Mg source when the added highest level increased Ca and Mg concentrations in tibia. Tibia P concentration of organic Mg source fed with the group were higher than the inorganic Mg source. In addition, tibia shear force and tibia stress were increased in chicks fed with 0.2 and 0.4 % Mg. This result is in agreement with previous research (Hess and Britton, 1997) in which effect of excess Mg on bone is to increase bone Mg. The increased levels of Mg in bone of the hens fed excess Mg suggest a possible mechanism for the action of the Mg. Nugara and Edwards (1963) showed that 0.32% of diet Mg did slightly reduce bone ash, whereas 0.64 % of diet Mg greatly reduced bone ash. Toba et al. (2000) reported that Mg supplementation increases the dynamic strength of bone. These results indicate that the breaking force and breaking energy of the femur in the rats fed the 0.15% Mg diet were significantly higher than in the rats fed the 0.05% Mg diet. Other researchers, found no detrimental effects of excess Mg until a level of 8.380 mg/kg (Mehring and Johnson, 1965) or even 12.000 mg/kg feed (McWard, 1967) for laying hens. Furthermore, the effect of supplemental organic Mg was more significant than inorganic Mg. This result, along with the reported by others (Gaal et al., 2004; Guo et al., 2003; Şahin et al., 2005), indicates that organic Mg would be more bio-available than inorganic Mg. It could be the higher absorptivity of organic Mg that resulted in more Mg accumulation in the bone tissues of chickens.

In conclusion that supplementation of Mg in broiler diet was increased plasma Mg concentration, however was no effect on liver and meat Mg contents. Tibia P concentration of organic Mg source fed with the group were higher than the inorganic Mg source. A dietary organic Mg source when the added highest level increased Ca and Mg concentrations in tibia. Tibia shear force and tibia stress were increased in chicks fed with 0.2 and 0.4 % Mg.

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