



Aspects of the plasma biochemistry and tibia minerals of broilers fed amorphous dolomite as a natural source of calcium and magnesium

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ABSTRACT

One-day-old Cobb 500 broiler chicks (1200) were allocated to 3 experimental treatments, each one had 4 replicates. The treatments differed only by the source of Ca and Mg supplemented as follows: control group contained limestone (as Ca source: 10.0/9.6/9.0 g/kg, for each growth phases) and MgO (as Mg source: 2.36/2.39/2.38 g/kg, for each growth phases); AD1 and AD2 groups – limestone and MgO were replaced with AD as Ca and Mg source. The AD1 diet contains the same levels of Ca as control and Mg levels were 5.8/5.6/5.45 g/kg. The AD2 diet contained reduced levels of Ca (7.5/7.3/6.8 g/kg) and Mg levels were 4.3/4.21/4.12 g/kg, for each of the growth phases. The plasma biochemistry was not influenced by treatments, except Mg concentration which was increased in the experimental groups. Tibia ash, bone Ca and P content were not different in any of the treatment groups. Bone Mg content was increased in the AD groups compared with control. AD can improve weight gain and presented similar plasma metabolic profile, tibia ash and bone Ca and P content with limestone adding MgO.

Key words: Amorphous dolomite, Broilers, Performance, Plasma biochemistry, Tibia minerals

Minerals such as calcium (Ca), phosphorus (P) and magnesium (Mg) have important biological functions and must be provided in adequate amounts in poultry diets (Hurwitz 1995, Klasing 1998, Driver *et al.* 2005 a,b, Blair 2008). Inadequate intake of these minerals may affect hormonal secretion, enzyme activity, muscle function, bone mineral content, other body mineral functions, reduced productivity and loss of resistance to diseases (Whitehead 1995, Qian *et al.* 1997, McDowell 2003, Peters and Mahan 2008). In broilers growth phase, the majority of the Ca is used for the bone formation, allowing the support of the body weight (Williams *et al.* 2000). The P deposition in the bone follows the Ca deposition, characterizing the interdependence between these minerals. Mg is involved in many biochemical processes (Suttle 2010) and is also present in heart, liver, spleen, kidneys, acting in tissue synthesis (proteins, lipids). In addition, it functions in activation of amino acids, synthesis and degradation of DNA, neuro-transmission and immune function (Stryer 1988). Less is known about Mg supplementation of the high producing breeds at various stages of growth and levels of production to maintain health, as well as high production level. Most studies have been focused on the therapeutic

effects of repletion of Mg deficiency or on adverse effects of its excess (McWard 1967, Lee *et al.* 1980 a,b). Stillmak and Sunde (1971 a,b) fed hens just over 1.0% Mg from magnesium carbonate, causing decreased egg production. The same level of Mg from dolomitic limestone (limestone containing 10% or more Mg) had no apparent effect on the hens. Further studies suggested that Mg from the dolomitic limestone being used was less available than Mg from magnesium carbonate, but these studies were done with chicks. Other researchers suggest that requirements of newly hatched broiler chicks for both Ca and 'available' P are much lower than the latest NRC (1994) standards (Henry and Pesti 2002, Driver *et al.* 2005a, Fritts and Waldroup 2006).

Dolomite [$\text{CaMg}(\text{CO}_3)_2$] is a carbonate mineral composed of calcium magnesium carbonate, a sedimentary rock. It is generally formed from limestone by dolomitisation, a diagenetic process involving replacement of calcium in the calcite with magnesium (Baltre 1976). This may occur either soon after limestone deposition, by exchange with seawater, or after lithification by exchange with magnesium-bearing solutions. The process is partly a function of the permeability of the rocks and can therefore be very selective, giving rise to interbedded limestone and dolomite. There is a lack of literature data on the effect of dolomite (in particular amorphous dolomite) as a unique source of calcium and magnesium into diets on the performance and bone quality in broiler chicks. The most

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of the study was carried out on the laying hens. It is assumed that the new breeds of high-producing farm animals (hybrids) require more nutrients and minerals than the former strains (Suttle 2010). The objective of this study was to evaluate the effects of amorphous dolomite as natural Ca and Mg source on broiler performance, plasma metabolic profile and bone minerals.

MATERIALS AND METHODS

Calcium sources and chemical analyses: Two calcium sources were used in this study: amorphous dolomite (AD) provided from Romanian quarries (in the Delnita area of Harghita County, Transylvania) and a limestone provided from Vâlcea County, Romania, Râmnicu-Vâlcea quarry. Each calcium source was crushed and screened to a size of <1mm. The powder thus obtained was weighed and kept in bags prior to its incorporation into diets. The sample of diet and each calcium source were analyzed for trace element contents (Ca, Mg, P), manganese (Mn), copper (Cu) and zinc (Zn). Macro and micro-minerals were estimated after microwave mineralization by hydrochloric acid and hydrogen peroxide, Ca Mg, Mn, Cu and Zn by flame atomic absorption spectrometry using atomic absorption spectrometer at wavelengths of 422.7 nm (Ca), 285.2 nm (Mg), 279.5 nm (Mn) 324.7 nm (Cu) and 213.8 nm (Zn) and P spectrophotometrically as vanadate yellow using an UV/VIS Spectrophotometer at wavelengths of 422 nm. Apparent calcium solubility was determined by following the pH changes, as the percentage H⁺ disappearance after introduction of 10 g of sample in 100 ml of a 0.1N hydrochloric acid solution (Savage 1982). All analyses were performed in duplicate.

Broilers, diets and performance measurements: Day-old 1,200 broiler chickens, obtained from a local commercial hatchery and raised for 42 d were used. In total, 12 floor pens with wood shaving (surface area 7.5 m²) were used, each containing 50 male and 50 female broilers, to give 4 pen replicates and 400 birds / treatment. Diets were formulated as starter, grower and finisher diets in accordance with the feeding recommendations of these strains (Cobb-Vantress 2008). The proximate composition of feed components and the diets (dry matter, crude protein, crude fiber, crude ash, ether extract) were analyzed using the Kjeldahl procedure (AOAC 1990). Metabolizable energy content of the diets was calculated on the basis of the energy content of individual feed ingredients using European Tables equation (Janssen 1989). The diets were calculated to be isonitrogenous, isocaloric, and with similar content of digestible sulphur amino acids (met + cys), lysine and available P for each growth phase. All diets were based on corn, soybean meal and rapeseed meal and differed only by source and level of Ca and Mg supplemented as follows: Control group—supplemented with limestone (as Ca source) at standard levels (10.0/9.6/9.0 g Ca/kg, for the starter/grower/finisher feeding phases, respectively) and magnesium oxide (MgO) as inorganic Mg source (2.36/2.39/2.38 g Mg/kg, for each growth phases); for

experimental group 1 (AD1) and experimental group 2 (AD2) limestone and MgO were replaced with amorphous dolomite as natural Ca and Mg source. Thus AD1 diet contained the same levels of Ca as control group and Mg levels were higher than control (5.8/5.6/5.45 g/kg). The AD2 diet contained lower levels of Ca (7.5/7.3/6.8 g/kg), and higher Mg levels (4.3/4.21/4.12 g/kg) than control for each growth phases. The broilers were given *ad lib.* access to feed and water. A lighting schedule of 23L: 1D was imposed throughout the experimental period. Control parameters, such as temperature, humidity, light, ventilation and vaccination, were the same for all groups. In order to determine the performance of broilers, the body weight gain, feed intake and feed conversion ratio were measured for starter, grower and finisher, as well as for the total experiment period. In cases where mortalities were observed, the numbers and weights of such mortalities were recorded accurately to make necessary corrections in calculating feed intake and feed conversion ratio. Birds were treated in accordance with Romanian legislation for handling and protection of animals used for experimental purposes. This study protocol was approved by the Ethical Committee of The National Research Development Institute for Animal Biology and Nutrition, Balotesti, Romania.

Plasma biochemical parameters: At 42 d of age, twelve broilers per treatment were randomly selected and blood samples were collected from wing vein of the birds in heparinized tubes. Blood samples were subsequently stored in ice, centrifuged at 2,500 rpm for 10 min at 4°C, and the plasma was transferred to a fresh tube, frozen, and stored at -20°C until analyses. Plasma concentrations of glucose, cholesterol, triglycerides, total protein, albumin, total globulin, creatinine, urea, Ca, P, Mg, Fe and the activity of alkaline phosphatase (AP), γ -glutamyl transferase (GGT), glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) were determined on a chemistry analyzer, using kits. Total concentration of immunoglobulin (Ig) subsets was measured by ELISA after plasma dilution: 1/4000 (IgA), 1/60000 (IgG) and 1/6000 (IgM) as previously reported (Marin *et al.* 2006), and according to the manufacturer's instructions. Absorbance was read at 450 nm using a microplate reader and results were expressed as mg/ml of plasma.

Tibia ash and mineral concentrations: The tibia from the left leg was removed, boiled for 10 min. and cleaned of adhering tissue. The bones were harvested from the same broilers used to collect blood samples and frozen (-20°C) until the analysis. Tibias including epiphyses were dried at 105°C for 12 h and extracted with ethyl ethanol for 24 h followed by a 24 h extraction in petroleum ether (Kjeldahl procedure) (AOAC 1990). The dry fat-free bones were ashed in a muffle furnace at 550°C. Ash weight was calculated as a percentage of dry fat-free bone weight. Bone Ca and Mg were analysed by flame atomic absorption spectrometry and total P spectrophotometrically.

Statistical analysis: All data were analysed by the general linear models (GLM) procedure using the SPSS software

version 17. One-way analysis of variance (ANOVA) with the post hoc Duncan's multiple comparison tests was used to evaluate statistical significance of differences among the control and experimental groups. The results are given as means and standard error of the mean (SEM). Differences were considered significant at $P < 0.05$. Replication was considered as the experimental unit for determined performance.

RESULTS AND DISCUSSION

Chemical composition of calcium sources: The calcium supplements (amorphous dolomite and limestone) contained different levels of Ca from 20.61 and 35.13% respectively. In AD source, a higher level of Mg (12.60 vs. 0.03%) was observed compared with limestone and their trace mineral levels fluctuated, especially for Mn (64.81 vs. 105.97 mg/kg) and Zn (41.30 vs. 12.15 mg/kg). Apparent calcium solubility in hydrochloric acid (0.1N solution) differed slightly between the calcium sources. Limestone had the lowest solubility *in vitro* when compared with amorphous dolomites, 10 min after the beginning of the reaction (86.95 vs 92.52%) (data not shown).

Broilers performance: The higher body weight gain was observed in the AD1 followed by AD2 group in starter, grower and finisher, as well as the total (day 42) experiment period, compared to control group (2552.72 and 2564.60g vs. 2479.45g; $P < 0.05$) (data not shown). Similar results have been reported by Gaol *et al.* (2004), that the additional Mg improves feed digestibility and the weight gain of broilers.

Rogler and Parker (1972) showed that the diet formulation for broilers before the age of 28 days must include higher proportions of Mg. On the other hand, Reddy *et al.* (1973), Chou *et al.* (1979) and Lee *et al.* (1980 a,b) also reported that the improper supply of Mg mineral may slow down growth, cause bone malformations and mortality. During the overall period there was no significant difference between the experimental groups regarding feed intake feed conversion ratio and mortality rate ($P > 0.05$) (data not shown).

Plasma biochemistry: There was no significant treatment effect on plasma protein (Table 1) and energy profile ($P > 0.05$). Generally, the broilers fed diets containing AD had slightly higher plasma protein and energy values (except triglyceride and cholesterol levels) than the broilers fed on the control group. Serum urea concentration is a good indicator of protein status in chicks (Kaneko 1997). Accordingly, the present results of plasma urea being within the normal values signified the good ability of diets to supply the protein requirements for chicks. Also, in the present study, broilers fed diets containing AD1 and AD2 had only a slightly decreased in plasma triglyceride and cholesterol levels than those of the control group without significantly differences ($P > 0.05$). Similarly, Sahin *et al.* (2005) demonstrated that Mg supplementation (1 or 2 g Mg/kg of diet) reduced content of plasma lipid and has decreased the effect of heat stress on 10-day-old quail, whether it was given as MgO or magnesium proteinate. In our study there was no significant treatment effect on plasma Ca, P and Fe

Table 1. Effects of sources of Ca and Mg on plasma metabolic profile of broilers at 42 days

Plasma profile	Parameter	Dietary treatment				
		Control	AD1	AD2	SEM	P-value
Energy	Glucose (mg/dl)	198.57	216.79	229.45	7.806	0.262
	Cholesterol (mg/dl)	101.22	86.88	89.35	3.353	0.647
	Triglycerides (mg/dl)	33.84	27.57	29.70	1.346	0.221
Protein	Total protein (g/dl)	2.88	3.10	3.00	0.072	0.591
	Albumin (g/dl)	1.32	1.26	1.28	0.019	0.237
	Total globulin (g/dl)	2.13	2.18	2.27	0.027	0.067
	Creatinine (mg/dl)	0.38	0.44	0.48	0.018	0.063
	Urea (mg/dl)	1.33	1.64	1.67	0.121	0.561
	Calcium (mg/dl)	12.14	11.21	11.74	0.388	0.629
Mineral	Phosphorus (mg/dl)	6.88	6.44	6.47	0.223	0.068
	Magnesium (mg/dl)	2.12 ^b	2.90 ^a	2.83 ^a	0.009	0.001
	Iron (µg/dl)	149.53	162.37	131.92	17.274	0.168
Enzymatic	AP (U/l)	583.67	603.74	604.65	8.675	0.121
	GGT (U/l)	13.42	14.81	14.77	0.961	0.194
	GPT (U/l)	10.67	12.11	11.78	0.227	0.138
	GOT (U/l)	65.77	68.10	67.23	2.456	0.334
	IgA (mg/ml)	0.27	0.29	0.33	0.012	0.073
	IgM (mg/ml)	2.19	2.25	2.28	0.065	0.137
	IgG (mg/ml)	4.34	4.39	4.51	0.087	0.243

^{a,b}Means within a row with the same or no letter do not differ ($P > 0.05$).

concentration ($P>0.05$). However, plasma Mg concentration increased ($P<0.05$) in AD1 and AD2 groups, compared with control group (2.90 and 2.83 vs. 2.12). The treatment did not affect ($P>0.05$) plasma enzymes (AP, GGT, GPT or GOT) values. Also, the broilers fed with diets containing AD did not significantly affect ($P>0.05$) plasma IgA, IgM or IgG values, compared with control group. Our results show normal function of liver, kidney and pancreas and were in agreement with what was reported by Lebarcq-Verheyden *et al.* (1974). Also, the diets did not significantly affect plasma IgA, IgM or IgG values, indicating that the treatment did not affect the immunity of birds and is congruent with the similar reference values reported for chicks by Kaneko (1997). Liver function tests for GPT, GOT and Ig produced similar results in all treatments. Guo *et al.* (2003) reported that supplemental Mg in both the proteinate and oxide forms significantly elevated the activity of hepatic catalase and improved antiperoxidation capacity of broilers. Other researchers found no detrimental effects of excess Mg on bird performance from natural or chemically defined (McGillivray and Smidt 1975) sources. Atteh and Leeson (1983) suggested that the dietary supplements of Mg reduces the stress of the high production level or of the growth stage; it also prevents cannibalism. Recent information showed that Mg supplementation may reduce the consequences of stress in animals (Suttle 2010). Physiologically, this beneficial effect was mediated, at least partly, by restoring the activity of anti-oxidative enzymes (Yang *et al.* 2012). Also, this finding suggested that magnesium supplementation might be a potential strategy to attenuate heat stress induced detrimental effects in broilers raised in summer or tropical areas.

Tibia mineral composition: Information on the mean value for tibia chemical composition of the experimental broilers are given in Table 2. Tibia ash was not significantly different in any of the treatment groups ($P>0.05$). Bone Ca and P content were higher in the broilers fed with control diet, but no significant differences ($P>0.05$) were seen compared to the AD1 and AD2 groups (39.05 vs 38.65 and 38.30; 17.89 vs 17.74 and 17.43, respectively). Similarly, Viveros *et al.* (2002) also found decreases in bone Ca and P concentration in diets with Ca: P at 1.62: 1 in comparison to diets with Ca: P at 2.02: 1. Schouten *et al.* (2003) have found values between 54.8 and 56.5% of tibia ash, 18.8

and 20.1% of Ca in ash, and 10.4 and 11.9% of P in ash from Hubbard broilers at 42 d of age. In our experiment bone Mg content was increased significantly ($P<0.05$) by feeding AD1 and AD2 compared to the control diet. Similarly, Barreiro *et al.* (2009) reported Ca and P values (as % in ash) close to this experiment, but the Mg values were a little lower. Our results do not disagree with Oviedo *et al.* (2006) who reported that it is important to limit the content of Mg to 0.4–0.5% in the compound feeds with dolomite because it may decrease the tibia ash level. In our experimental diets, dietary level of Mg was formulated below the current NRC (1994) recommendations to ensure maximum responses (600 mg/kg). Suttle (2010 citing the NRC, 2005) has placed the tolerable limits of Mg for poultry at 5.0–7.5 g kg⁻¹ DM. On the other hand, recent research suggests that newly hatched broiler chicks can grow unconstrained for 16–21 days with dietary Ca at 5–7 g kg⁻¹ DM with available P at (Henry and Pesti 2002) or below (Driver *et al.* 2005b) the NRC standard: for growers, 3.5 g Ca kg⁻¹ DM can suffice, reflecting the more efficient absorption by older chicks, but requirements for maximal bone ash were slightly higher. Although live performance is an important measure of any dietary changes, our results demonstrated that, plasma and bone mineral concentrations are generally more sensitive than performance factors for evaluating Ca, P and Mg bioavailability; these minerals play a major role in the formation and maintenance of broiler skeleton.

This study showed that the possibility of using an AD as natural Ca and Mg source. It was found that AD supplementation used in the diets of broilers increased body weight gain and presented similar feed conversion ratio, plasma metabolic profile, tibia ash and bone Ca and P content with limestone adding MgO. Generally, the values obtained from plasma constituents indicated normal physiological and healthy status of broilers fed diets containing AD. In summary, our experimental results suggested that Ca and Mg supply in the nutrition of farm animals calls for more attention. The amount of Ca, available P and Mg required by hybrid animals requires re-evaluation.

REFERENCES

- AOAC 1990. *Official Methods of Analysis*. 15th edn. Association of Official Analytical Chemists, Washington DC, USA.
- Atteh J O and Leeson S. 1983. Influence of increasing dietary calcium and magnesium content of the drinking water on performance and bone and plasma minerals of broiler chickens. *Poultry Science* **62**: 869–74.
- Baltre A. 1976. Middle Triassic, Supratidal and Intertidal Dolomites in the Hăghima Massif (East Carpathians): Carbonate Sedimentation and Diagenesis. pp: 65–70. Guidebook Series, 15, Institute of Geology and Geophysics, Bucharest, RO.
- Barreiro F R, Sagula A L, Junqueira O M, Pereira G T and Baraldi-Artori S M. 2009. Densitometric and biochemical values of broiler tibias at different ages. *Poultry Science* **88**: 2644–48.
- Blair R. 2008. *Nutrition and Feeding in Organic Poultry*. pp: 23–66. CAB International, Wallingford, UK. <http://www.cabi.org>.

Table 2. Effects of sources of Ca and Mg on tibia ash and bone minerals of broilers at 42 days

Variables	Dietary treatment				P-value
	Control	AD1	AD2	SEM	
Ash,%	44.58	43.97	43.52	0.984	0.686
Calcium,%	39.05	38.65	38.30	0.209	0.227
Phosphorus,%	17.89	17.74	17.43	0.116	0.400
Magnesium,%	0.79 ^b	0.87 ^a	0.84 ^a	0.008	0.029

^{a,b}Means within a row with the same or no letter do not differ ($P>0.05$).

- Chou H F, Schwartz R, Krook L and Wasserman R H. 1979. Intestinal calcium absorption and bone morphology in magnesium deficient chicks. *Journal of Nutrition* **69**: 88–103.
- Cobb-Vantress 2008. *Broiler Performance and Nutrition Supplement*. Revised version May. Available from: <http://www.cobb-vantress.com>.
- Driver J P, Pesti G M, Bakali R I and Edwards H M Jr. 2005a. Calcium requirements of the modern broiler chicken as influenced by dietary protein and age. *Poultry Science* **84**: 1629–39.
- Driver J P, Pesti G M, Bakali R I and Edwards H M Jr. 2005b. Effects of calcium and nonphytate phosphorus concentrations on phytase efficacy in broiler chicks. *Poultry Science* **84**: 1406–17.
- Fritts C A and Waldroup P W. 2006. Modified phosphorus program for broilers based on commercial feeding intervals to sustain live performance and reduce total water-soluble phosphorus in litter. *Journal Applied Poultry Research* **15**: 207–18.
- Gaal K K, Sáfár O, Gulyás L and Stadler P. 2004. Magnesium in animal nutrition. *Journal of the American College of Nutrition* **23**(6): 754S–57S.
- Guo Y, Zhang G, Yuan J and Nie W. 2003. Effects of source and level of magnesium and vitamin E on prevention of hepatic peroxidation and oxidative deterioration of broiler meat. *Animal Feed Science and Technology* **107**: 143–50.
- Henry M H and Pesti G M. 2002. An investigation of calcium citrate-malate as a calcium source for broiler chicks. *Poultry Science* **81**: 1149–55.
- Hurwitz S, Plavnik I, Shapiro A, Wax E, Talpaz H and Bar A. 1995. Calcium metabolism and requirements of chickens are affected by growth. *Journal of Nutrition* **125**: 2679–86.
- Janssen W M M A. 1989. *European Table of Energy Values for Poultry Feedstuffs*. 3rd edn. Working Group No. 2 of the European Branch, World's Poultry Science Association, Beekbergen, The Netherlands.
- Kaneko J J. 1997. Serum proteins and the dysproteinemias. *Clinical Biochemistry of Domestic Animals*. 5th edn, pp. 117–38. (Eds) Kaneko J J, Harvey J W and Bruss M.L. Academic Press, San Diego.
- Klasing K C. 1998. *Comparative Avian Nutrition*. pp: 277–99. CAB International, Wallingford, UK, <http://www.cabi.org>.
- Lebarcq-Verheyden A M, Vaerman J P and Heremans J F. 1974. A possible homologue of mammalian IgA in chicken serum and secretions. *Immunology* **22**: 165–69.
- Lee S R, Britton W M and Rowland G N. 1980a. Magnesium toxicity: Effect on phosphorus utilization by broiler chicks. *Poultry Science* **59**: 1989–94.
- Lee S R, Britton W M and Rowland G N. 1980b. Magnesium toxicity: Bone lesions. *Poultry Science* **59**: 2403–11.
- Marin D E, Taranu I, Pascale F, Lionide A, Burlacu R, Bailly J D and Oswald I P. 2006. Sex-related differences in the immune response of weanling piglets exposed to low doses of fumonisin extract. *British Journal of Nutrition* **95**: 1185–92.
- McDowell L R. 2003. Calcium and phosphorus. *Minerals in Animal and Human Nutrition*. 2nd edn, Chapter 2, pp: 33–92. Elsevier Science BV. Amsterdam, The Netherlands.
- McGillivray J J and Smidt M J. 1975. Biological evaluation of magnesium sources. *Poultry Science* **54**: 1792–93.
- McWard G W. 1967. Magnesium tolerance of the growing and laying chicken. *British Poultry Science* **8**: 91–99.
- NRC. 1994. *Nutrient Requirements of Poultry*. 9th edn. National Academy Press, Washington, DC, USA.
- Oviedo E O, Ferket P R and Havestien G B. 2006. Nutritional factors that affect leg problems in broilers and turkeys. *Avian and Poultry Biology Reviews* **17**(3): 89–103.
- Peters J C and Mahan D C. 2008. Effects of dietary organic and inorganic trace mineral levels on sow reproductive performances and daily mineral intakes over six parities. *Journal of Animal Science* **86**: 2247–60.
- Qian H, Kornegay E T and Denbow D M. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol and the calcium: total phosphorus ratio in broiler diets. *Poultry Science* **76**: 37–46.
- Reddy CR, Coburn J W, Hartenbower D L, Friedler R M, Brickman A S, Massry S G and Jowsey J. 1973. Studies on mechanisms of hypocalcemia of magnesium depletion. *Journal of Clinical Investigation* **52**: 3000–10.
- Rogler J C and Parker H E. 1972. Effects of excess calcium on a fluoride-magnesium interrelationship in chicks. *Journal of Nutrition* **102**: 1699–1707.
- Sahin N, Onderci M, Sahin K, Cikim G and Kucuk O. 2005. Magnesium proteinate is more protective than magnesium oxide in heat-stressed quail. *Journal of Nutrition* **135**: 1732–37.
- Savage S I. 1982. Specialist notes limestone reaction varies by sources. *Feedstuffs* **37**: 12.
- Schoulten N A, Teixeira A S, Conte A J, Silva H O, Bertechini A G and Fialho E T. 2003. Efeito dos níveis de cálcio da ração suplementada com fitase sobre a deposição de minerais na tíbia de frangos de corte de 22 a 42 dias. *Ciência e Agrotecnologia* **27**: 206–10.
- Stillmak S J and Sunde M L. 1971a. The use of high magnesium limestone in the diet of the laying hen. 1. Egg production. *Poultry Science* **50**: 553–63.
- Stillmak S J and Sunde M L. 1971b. The use of high magnesium limestone in the diet of the laying hen. 2. Calcium and magnesium availability. *Poultry Science* **50**: 564–72.
- Stryer L. 1988. Glycolysis. *Biochemistry*. pp. 352. 3rd edn. W.H. Freeman and Company, NY.
- Suttle N V. 2010. *Mineral Nutrition of Livestock*. pp. 92–122. 4th edn. CAB International, Wallingford, UK.
- Viveros A, Brenes A, Arija I and Centeno C. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. *Poultry Science* **81**: 1172–83.
- Whitehead C C. 1995. Nutrition and skeletal disorders in broilers and layers. *Poultry International* **34**: 40–48.
- Williams B, Solomon S, Waddington D, Thorpm B and Farquharson C. 2000. Skeletal development in the meat-type chicken. *British Poultry Science* **41**: 141–49.
- Yang Y, Gao M, Nie W, Yuan J, Zhang B, Wang Z and Wu Z. 2012. Dietary magnesium sulfate supplementation protects heat stress-induced oxidative damage by restoring the activities of anti-oxidative enzymes in broilers. *Biological Trace Element Research* **146** (1): 53–58.