

MINERAL NUTRITION OF MODERN POULTRY GENOTYPES

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Invited paper

Abstract: In the latest research relating to mineral nutrition of poultry, the interest in several nutrients is prevailing for which in ordinary diets additional sources are necessary. In mineral nutrition of layers constantly the calcium (Ca) requirements are reviewed as one of the most important factors influencing the quality of eggs and bones, in regard to adequate level, source and particle size of added Ca, as well as mutual balance with other nutrients, primarily vitamin D, phosphorus (P) and certain micro-elements. Also, efforts are directed towards possibility and justification for use of new source of vitamin D, existing recommendations related to P are reviewed as well as increase of possibility for adoption of phytine bound phosphorus in diets using enzyme phytase, as well as investigation of organic forms of certain micro-element sources. Certain researches which combine the latest studies of the mineral nutrition of poultry are especially interesting also for practical application. Contrary to layers, focus of research of mineral nutrition of broilers is on phosphorus and application of enzyme phytase. In general, researches of the mineral nutrition of modern poultry genotypes are always actual, considering that their objective is to support genetic progress with adequate nutrition, but also to solve increasing problems relating to product quality, metabolic disorders and ecology.

Key words: calcium, phosphorus, micro-elements, broilers, layers, quality of products

Introduction

There are twenty nine elements known which are required by at least one animal species. According to usual division, seven elements are macro-minerals whose requirements or concentrations in organism are expressed by over 100ppm, and 22 elements are micro-minerals in traces whose requirements are bellow 100 ppm, and even can be expressed in ppb values (*McDowell, 2003*). Of course this classification does not reflect important or less important role of these nutrients,

but only necessary quantities in diets or their generally low (or in traces) concentration in tissues. However, in researches relating to mineral nutrition of poultry interest in several minerals is predominant, therefore this study will be focused on several nutrients for which in ordinary diets additional sources are necessary. In this paper a short review of the latest results of calcium, phosphorus and some micro-elements studies which suggest certain practical changes in mineral nutrition of poultry is presented.

Mineral nutrition of layers

Functional and economically most important mineral in nutrition of layers is calcium, primary because of egg production, i.e. forming of the egg shell. Average egg shell contains approx. 2.3g (2.0-2.5g) Ca. This quantity is equivalent to approx. 10% of total calcium content in the skeleton of layer, considering that estimated amount of this macro element in skeleton of layers is approx. 20g. If we take into consideration that during laying cycle layers lay in average one year every 24 to 28 hours, it is clear how significant, efficient and complex this system of Ca metabolism regulation is, since it enables layers to respond to constant challenge of maintaining the Ca balance in organism.

Calcium homeostasis in domesticated layer hens is achieved primarily by balancing the ability and efficiency of intestinal absorption, renal re-absorption and bone resorption of calcium with animals needs and requirements for this element (*Etches, 1987*). In case of layers, process which take place in bones are especially interesting and complex, considering the exceptional dynamics and scope of Ca circulation in organism and unique presence of medular bone responsible for providing of Ca in the time of egg shell calcification, especially when food as primary source of Ca for calcification is not present in digestive tract (*Leach, 2000; Whitehead 2004*). On the other hand, dynamic processes in skeleton Ca depots during laying period in layers can weaken the firmness of certain bones due to certain loss of structural bone. Scope and duration of these changes in quality of bones depend on numerous factors, causing the incidence of osteoporosis in layer bones at the end of laying period and consequent bone fractures, which has been serious problem without easy solution in layers housed in cage system (*Whitehead and Fleming, 2000*).

Calcium as one of the most important factors which influence the quality of eggs and bones must be added to diets for layer hens. Based on literature data, it is considered that (*Roland, 1986b*) already at the end of 19th century it was usual practice to add some concentrated calcium source to diets for layer hens in order to have high quality egg shell. However, requirements of layers for calcium in order to realize high egg production, good quality of egg shell and animal health, are constant topic of research and reviewing, for the purpose of more precise satisfying

of needs of new hybrids of greater genetic potential for production, as well as solving of continuously present problems of egg shell quality and bones of layer hens during exploitation (*Whitehead and Fleming, 2000; Lukić et al., 2008, Lukić, 2009*). *Roland (1986a)*, summarizing in his paper all previous research dealing with requirements and needs of Leghorn layers for calcium and phosphorus for the purpose of realization of maximum production and eggshell quality available in literature in period from 1942-1984, points out great variations of results obtained by different researchers which demonstrates how difficult it is to determine calcium requirements of layers. Main factors which can influence and impede determination of needs and requirements of layers for Ca, as stated by this author, are the following: constant genetic progress of layers, differences in needs of layers of same or different proveniences, mutual co-dependence between Ca and other nutrients, effect of particle size of Ca sources, ability of layers to partially adjust food consumption to their Ca needs, fear of producers or nutrition experts from harmful effects of over doses or inadequate consumption of Ca, as well as fact that many researchers expressed the layer needs in Ca in the form of its % in diet not taking into consideration variations in food consumption and those caused by level of energy, environment temperature, different provenience and/or age of poultry. Author concluded that, based on average of results obtained by different researchers in decades of observed period, the constant increase of Ca requirements is obvious (by 0,70g/layer/day over sixty years, compared to previous period, i.e. 0,29 in seventies and 0,53 in eighties of the last century), whereas, at the same time, phosphorus requirements showed tendency of slight decrease. *Leeson (2006)*, also, points out that nutritive requirements of poultry have changed slightly considering that eggs and meat are of very stable main composition in all birds, but diet specifications for poultry were constantly re-evaluated because of continuous increase of meat and egg production, stating that according to recommendation of the University Guelph in Canada, in diets for layers in 2005 almost double amount of Ca is necessary compared to diets 50 years ago (2,1% in year 1956 compared to 4,0% in 2005).

Requirements for this mineral in maximum laying ability are extremely high, and numerous studies have shown that quality of egg shell depends not only on adequate level, but also on source and particle size of added Ca. Namely it is considered that large size particles of Ca source are maintained longer in muscle stomach and are slowly dissolved during this longer passage through digestive tract, whereas contrary to them, small size particles pass through digestive tract faster and therefore are only partially dissolved (*Roland, 1986b; Zhang and Coon, 1997; Lukić, 2009*). In numerous researches different sources and particle sizes were compared, shares of fine and large particles in diet, as well as time and method of adding in order to determine optimal solutions. *Lukić et al. (2009)* stated that use of 40-60% of coarse ground marble in nutrition of young layers is justified,

since it was observed that they lay eggs with thicker egg shell which are less susceptible to breaking, whereas *Scheideler et al. (2005)* suggested combination of 50% finely ground and 50% large size particles of lime stone can satisfy the requirements of layers for realization of optimal production of eggs and egg shell quality up to 40 weeks of age. *Pavlovski et al. (2003)* pointed out that by substitution of 60-80% of lime stone with large particle size of limestone in mixtures for older layers positive effects can be achieved on quality of egg shell.

In the attempt to solve problems of frequent incidence of osteoporosis in layers, researchers investigated mainly the change in nutrition, housing and movement of layers, as well as genetic improvements through selection. Researches related to nutrition mainly engaged in study of the optimal nutrition with main minerals (Ca, P, Ca : P ratio) and vitamin D. Majority of researchers pointed out that adequate nutrition with this nutrients is essential, but also that the increase of level of these nutrients above recommendations does not improve the quality of bones. *Whitehead and Fleming (2000)* concluded that good nutrition can help minimize osteoporosis, but it can not prevent it. Several studies (*Guinotte and Nys, 1991; Rennie et al., 1997; Lukić, 2009*) indicated that use of large size particles of Ca source in nutrition of layers can have certain effect on improvement of the quality of bones in layers.

Practical implications of previous findings on mineral nutrition of layers with calcium are summarized maybe in the best way in several recommendations by *Roland and Bryant (2000)*, who pointed out that if we want to achieve maximum egg shell quality, it is necessary to provide to layers level of Ca necessary for laying period at least 7 days before the first egg. Authors also suggested that formulating of diet should primarily be based on providing necessary daily intake of Ca in monitoring of food consumption and mass of eggs, and not only on recommendations expressed only as necessary level of diet Ca. In regard to source of added Ca, they stated the need for provision of at least 25% of Ca source in diet in form of large size particles, source of Ca should be highly soluble and not contaminated with other ingredients.

Except calcium, of course, in mineral nutrition of layers it is very important to satisfy needs and to balance other nutrients in diet, primarily vitamin D, phosphorus and certain micro elements, because of their individual importance as well as because of their mutual interaction. Latest researches related to needs of poultry for vitamin D often represent evaluation of possibilities and justification for use of one of the metabolites of vitamin D, 25-hydroxi-calciferol (25-OH D₃), as additional source of this vitamin in broiler and layer nutrition. In regard to needs for phosphorus of modern layer genotypes mainly the possibility of the reduction is considered, as well as reviewing existing recommendations and increase of availability of phytine bound phosphorus in diets using enzyme phytase, primarily in order to decrease the content of excreted phosphorus and its negative effect on

environment. Needs of layers for certain micro minerals are also subject of reviewing because of transfer on organic forms of sources of these micro elements for which it is considered that they are better absorbed than inorganic forms.

Especially interesting also for practical implementation are studies which combine the latest findings related to mineral nutrition of layers. Among many researchers, *Keshavarz (2003)* investigated in two trails individual and mutual effect of different levels of diet Ca (3.34; 4.3; 4.73 and 4.94%) and different sources of vitamin D (vitamin D₃ and 25-OH D₃), i.e. in other trial different levels of non-phytate phosphorus (0.11; 0.21 and 0.41%), presence of phytase (0 and 300 U/kg diet) and different sources of vitamin D (vitamin D₃ and 25-OH-D₃) in diet for layers on production traits and quality of egg shell. He concluded that the level of diet Ca of 3.34%, which was provided to layers, in trial due to realized consumption, by 3.63g Ca per layer daily, was adequate for production and quality of egg shell. Also, used level of non-phytate phosphorus of 0.21% was adequate value, giving results in trial comparable to performance of layers fed 0.41% of non-phytate phosphorus, whereas application of enzyme phytase had no effect on production results but influenced some parameters of egg shell quality, and substitution of vitamin D₃ with 25-OH D₃ had no effect in any of the trails in given conditions.

Huyghebaert and Maertens (2007) investigated the effect of different combinations of 2 forms of vitamin D (vitamin D₃ and 25-OH D₃) and 3 combinations of concentrations of Ca and P (normal Ca/normal P, low Ca/normal P and low Ca/low P) in diets for layers on production characteristics of tibia. Authors reported among other things, that in the first research phase in young layers (20-44 weeks of age), low Ca level in diets (3.1% Ca) induced significant decrease of egg mass and observed parameters of the egg shell quality, as well as increased incidence of damaged (cracked) eggs, and adding of active form of vit. D (25-OH D₃) to diet shoed tendency of compensation of the negative effect of low level of Ca on quality of egg shell.

Very interesting are results of the research carried out by *Steenfeldt et al. (2007)*, who investigated different possibilities for feeding of layers with diets without added phosphates (diets with approx. 0.32% of total P) with increased share of wheat, addition of enzyme phytase or additional nutrition with coarsely ground sea shells. Authors stated that layers at the age of 19 to 35 weeks, fed main diets with 1.8% Ca and 0.32% total P with additional nutrition with coarsely ground sea shells (in average approx. 4.6g per layer daily), which they received separately from main diet two hours prior to dark period, realized equally good production parameters and quality of egg shell like layers from control group fed diets with 3.2% Ca and 0.57% P in diet, pointing out that applied strategy of nutrition with coarsely ground Ca source annulled negative effect of low content of total P in diet.

Mineral nutrition of broilers

Contrary to layers, focus in research of mineral nutrition of broilers is on phosphorus because of several reasons. One of them is significance of its physiological role in organism and constant problems related to quality of broiler bones, with attempts to solve these problems through interventions in nutrition in order to adequately support the obvious genetic progress achieved by poultry hybrid producers from year to year (Lukić et al., 2006). Very important is also ecological aspect, considering that for long time phosphorus is declared as one of the main pollutants of soil and waters. However, main reason for great number of researches relating to mineral nutrition of broilers with phosphorus is current revolution in animal nutrition created by adding of exogenous enzymes into livestock food, i.e. the most prominent member of this revolution – phytase.

Namely, it is well known that lack of phosphorus in nutrition, because of its multiple and very important metabolic roles in organism, disturbs the intensity of the process of bio-synthesis and causes poorer growth and decrease of body mass in poultry in the phase of growth and development, and consequential incidence of irregular/incorrect ossification (Lukić et al., 2005). At the same time, by selection of ordinary, usual feeds for animal nutrition, not all requirements can be satisfied, and in feeds of plant origin phosphorus is mainly present in the phytine form (60-80% of total amount of phosphorus) which can not be utilized by poultry (Sebastian et al., 1998). Problem related to provision of sufficient quantity of phosphorus is solved by use of mineral feeds, but considering low availability of phosphorus, poor availability from feeds of plant origin, and incomplete availability from mineral feeds, great amount of undigested phosphorus is released through faeces into environment. So, concentrating of phosphorus and its excretion through faeces into the environment is serious ecological problem since phosphorus is one of the dominant pollutants (Van der Klis, 1992). For the purpose of solving existing problems in nutrition, enzyme phytase is used and in this way the availability of phosphorus is significantly increased which indirectly reduces the necessary amount of added phosphorus to food (Lukić, 2002). Use of phytase reduces excretion of phosphorus by direct increase of its utilization from plant feeds and/or indirectly reducing the level of non-organic phosphorus added to food. In contribution to the application of phytase are results of the latest studies indicating the positive effect on digestibility and availability of calcium, iron, zinc, magnesium and protein with the use of this enzyme. Also, because of almost universal presence of phytate in grain cereals and oil meals, phytase represents enzyme with the highest potential among other enzymes which can be used in poultry production.

Until now, use of microbial phytase was confirmed in numerous researches, and widely accepted in practice, for release and utilization of phosphorus and other nutrients bound in phytate complex in diets for poultry and pigs. Main condition for regular and successful use of phytase enzyme is that the diet should contain sufficient amount of phytate phosphorus, and with use of phytase the need for additional mineral sources of phosphorus but also calcium are reduced (*Sebastian et al., 1998; Huyghebaert et al., 2005*). Approx. 50% of phytine phosphorus from feeds can be released by use of phytase (*Christensen et al., 1997*), but this value can be significantly lower since efficiency of the use of phytase depends on series of factors. In general, moderate reduction of mineral source of phosphorus, with addition of phytase in diets for broiler is optimal, whereas greater reduction or complete exclusion of mineral phosphorus from diet even with increased concentrations of added enzyme phytase causes lower growth and lower consumption of food in broilers (*Lukić et al., 2005a*).

In regard to practical application of phytase in combination with reduced amounts of inorganic phosphorus sources, due to the effect of many factors on its efficiency, previous checking of the efficiency of selected combination of factors is recommended (type and level of phytase – level of reduction of inorganic sources of phosphorus, type of diet or typical level of phytine phosphorus in diet) through biological test where it would be sufficient to monitor production parameters (body mass, gain, feed consumption) as well as reliable indicators (*Lukić et al., 2005b*).

In broilers, researches of the use of 25-hydroxy-calciferol (25-OH D₃), instead of conventional source of vitamin D in food have shown positive effects and lead to development of new and perspective type of additive in practice (*Whitehead, 2005*). Needs for certain micro-elements in broilers, which are also considered as potential ecological pollutants, after almost 40 years, are being reviewed in the latest studies, especially with the development of mineral proteinates as highly adoptable sources of these micro minerals (*Leeson, 2006*).

Research of the issues relating to phosphorus, calcium, vitamin D and application of phytase in broiler production are equally important and are mutually complementary (*Huyghebaert et al., 2005*). These researches are especially important for the purpose of solving issues and problems which are very frequent and induced by rapid growth of broilers (bone deformations, predisposition to breaking and infections, etc.), as well as poor quality of the product, i.e. bone firmness, which is considered as serious disadvantage in the processing industry, because of frequent breaking of bones during handling, transportation and automatic broiler processing procedures.

Conclusion

Studies of the mineral nutrition of modern poultry genotypes are gaining on interest and significance and their main objective is to support with the adequate nutrition the genetic progress achieved by poultry hybrid producers from year to year, but also to solve growing problems relating to quality of product, metabolic disturbances or disorders and ecology. Modern approach to mineral nutrition includes very specific and precise satisfaction of all requirements of the new poultry proveniences for the purpose of profitable production of high quality food stuffs of animal origin without any harmful substances, and taking into consideration at the same time their health condition and animal welfare and protection of the environment.

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Mineralna ishrana savremenih hibrida živine

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Rezime

U novijim istraživanjima vezanim za mineralnu ishranu živine uglavnom preovlađuje interesovanje za nekoliko nutrijenata za koje su u uobičajenim obrocima potrebni dodatni izvori. Kod mineralne ishrane nosilja stalno se revidiraju potrebe nosilja za kalcijumom (Ca) kao jednim od najbitnijih činilaca koji utiču na kvalitet jaja i kostiju, kako u pogledu adekvatnog nivoa, izvora i veličine čestica dodatog Ca, tako i međusobnog balansa sa ostalim nutrijentima u obroku, pre svega vitaminom D, fosforom (P) i pojedinim mikroelementima. Takođe se radi na mogućnosti i opravdanosti upotrebe novog izvora vitamina D, revidiranju postojećih preporuka za P i povećanju usvojivosti fitinski vezanog fosfora u obrocima upotrebom enzima fitaze, kao i ispitivanju organskih formi izvora nekih mikroelemenata. Posebno interesantna i za praktičnu primenu značajna su pojedina istraživanja koja kombinuju ova novija saznanja vezana za mineralnu ishranu nosilja. Za razliku od nosilja, težište kod istraživanja mineralne ishrane brojlera je na fosforu i primeni enzima fitaze. Generalno, istraživanja mineralne ishrane savremenih genotipova živine ne gube stalnu aktuelnost,

obzirom da za cilj imaju da se ishranom adekvatno prati progresivni genetički napredak, ali i da se reše narastajući problemi vezani za kvalitet proizvoda, metaboličke poremećaje i ekologiju.

References

- CHRISTENSEN L., BECH L., OHMAN A., PETTERSSON D. (1997): Development, production and performance of microbial phytases. Proceedings 11th European Symposium on Poultry Nutrition, Faaborg, Denmark, August 1997, 203-210.
- ETCHES R.J. (1987): Calcium logistics in the laying hen. *Journal of Nutrition*, 117, 619-628.
- GUINOTTE F., NYS Y. (1991): Effects of particle size and origin of calcium sources on eggshell quality and bone mineralization in egg laying hens. *Poultry Science*, 70, 583-592.
- HUYGHEBAERT G., LIPPENS M., LESCOAT P., NYS Y. (2005): The interaction between the macrominerals calcium and phosphorus, vitamin D and phytase in broilers. Proceedings 15th European Symposium on Poultry Nutrition, Balatonfüred, Hungary, September 2005, 146-160.
- HUYGHEBAERT G., MAERTENS L. (2007): The efficacy of 25-OH-cholecalciferol in combination with different dietary Ca-P concentrations for laying hens. Proceedings 16th European Symposium on Poultry Nutrition, Strasbourg, France, August 2007, 701-704.
- KESHAVARZ K. (2003): A comparison between cholecalciferol and 25-OH-cholecalciferol on performance and eggshell quality of hens fed different levels of calcium and phosphorus. *Poultry Science*, 82, 1415-1422.
- LEACH R.M. (2000): Physiology of bone growth and development. XXI World's Poultry Congress, Montreal, Canada, August 2000, Abstract & Proceedings CD.
- LEESON S. (2006): Defining and predicting changes in nutrient requirements of poultry. XII European Poultry Conference, September 2006, Verona-Italy, Abstracts&Proceedings CD.
- LUKIĆ M. (2002): Uticaj fitaze u ishrani brojlera na proizvodne rezultate i zdravstveno stanje. Magistarska teza, Fakultet veterinarske medicine, Beograd.
- LUKIĆ M. (2009): Uticaj deficita kalcijuma i udela krupnih čestica mermera u ishrani na proizvodne rezultate, kvalitet jaja i kostiju kokoši nosilja. Doktorska disertacija, Poljoprivredni fakultet, Beograd.
- LUKIĆ M., PAVLOVSKI Z., ŠKRBIĆ Z., MILOŠEVIĆ N., PERIĆ L. (2008): The effect of housing system and hybrid type of layer hens on egg quality. 1st Mediterranean Summit of WPSA "Advances and Challenges in Poultry Science", May 2008, Porto Carras, Greece, Book of Proceedings, 642-646.

- LUKIĆ M., PAVLOVSKI Z., ŠKRBIĆ Z., CMILJANIĆ R. (2006): The influence of different genotype and producer of day old chicks on production performance and carcass quality of broilers. International conference "Linking up the meat chain: ensuring quality and safety for the costumer." October 2006, Animal Science, 1, Supplement, 192-194.
- LUKIĆ M., SINOVEC Z., PAVLOVSKI Z., CMILJANIĆ R., SPASOJEVIĆ I. (2002): Effect of microbial phytase in nutrition of broilers on production performance and carcass quality. European Poultry Conference, September 2002, Bremen, Archiv für Geflügelkunde, Band, 66, 138.
- LUKIĆ M., SINOVEC Z., PAVLOVSKI Z., ŠKRBIĆ Z., CMILJANIĆ R. (2005a): The reduction of mineral phosphorus source in diets for broiler and addition of phytase in condition of temperature stress. Proceedings 15th European Symposium on Poultry Nutrition, Balatonfüred, Hungary, September 2005, 201-204.
- LUKIĆ M., ŠKRBIĆ Z., PAVLOVSKI Z., SINOVEC Z., CMILJANIĆ R. (2005a): Possibility of prevention of phosphorus deficit by introduction of microbial phytase in fattening of broilers. Biotechnology in Animal Husbandry, 21, 5-6, Book 2, 147-151.
- LUKIĆ M., PAVLOVSKI Z., ŠKRBIĆ Z., JOKIĆ Ž., VITOROVIĆ D., PETRIČEVIĆ V. (2009): Possibility of preventing short term calcium deficit by using large size marble particles in nutrition of young laying hens. Archiva Zootechnica (in press).
- McDOWELL L.R. (2003): Minerals in animal and human nutrition. Elsevier Health Sciences, 1-9.
- PAVLOVSKI Z., VITOROVIĆ D., LUKIĆ M., SPASOJEVIĆ I. (2003): Improving eggshell quality by replacement of pulverised limestone by granular limestone in the hen diet. Acta veterinaria, 53, 1, 35-40.
- RENNIE J.S., FLEMING R.H., McCORMACK H.A., McCORQUODALE C.C., WHITEHEAD, C.C. (1997): Studies on effects of nutritional factors on bone structure and osteoporosis in laying hens. British Poultry Science, 38, 417-424.
- ROLAND D.A. (1986a): Egg shell quality III: Calcium and phosphorus requirements of commercial Leghorns. World's Poultry Science Journal, 42, 154-165.
- ROLAND D.A. (1986b): Eggshell quality IV: Oystershell versus limestone and the importance of particle size or solubility of calcium source. World's Poultry Science Journal, 42, 166-171.
- ROLAND D.A., BRYANT, M. (2000): Nutrition and feeding for optimum egg shell quality. XXI World's Poultry Congress, Montreal, Canada, August 2000, Abstract & Proceedings CD.
- SCHEIDELER S., JALAL M., WEBER T. (2005): Testing the optimum blend of fine : large particle size limestone and dietary calcium level for the Hy Line W-36

and W-98 strains of White Leghorn hens. Poultry Science Association Annual Meeting 2005. Abstract book, 121.

SEBASTIAN S., TOUCHBURN S.P., CHAVEZ E.R. (1998): Implications of phytic acid and supplemental microbial phytase in poultry nutrition: a review. *World's Poultry Science Journal*, 54, 27-47.

STEENFELDT S., HAMMERSHOJ M., POULSEN H.D. (2007): Reduction in dietary phosphorus are achievable in egg layers by different feeding strategies. Proceedings 16th European Symposium on Poultry Nutrition, Strasbourg, France, August 2007, 121-124.

VAN DER KLIS J.D. (1992): The role of minerals in relation to nutrition and pollution. Proceedings 19th World's Poultry Congress, Amsterdam, The Netherlands, September 1992, 2, 227-230.

WHITEHEAD C.C. (2004): Overview of bone biology in the egg-laying hen. *Poultry Science*, 83, 193-199.

WHITEHEAD C.C. (2005): Mechanisms and nutritional influences in skeletal development: Influence of macro- and microelements on bone formation. Proceedings 15th European Symposium on Poultry Nutrition, Balatonfüred, Hungary, September 2005, 137-145.

WHITEHEAD C.C., FLEMING R.H. (2000): Osteoporosis in cage layers. *Poultry Science*, 79, 1033-1041.

ZHANG B., COON C. (1997): The relationship of calcium intake, source, size, solubility in vitro and in vivo, and gizzard limestone retention in laying hens. *Poultry Science*, 76, 1702-1706.

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