



## **FEED ADDITIVES REGULATING CALCIUM HOMEOSTASIS IN THE BONES OF POULTRY – A REVIEW\***

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### **Abstract**

The strength of leg bones is not only genetically determined but it also depends on the gender, age, health condition and nutrition of slaughter birds. Calcium ions deficit in bones results in the deterioration of skeleton structure and reduction of bone strength. The presented work compiles the results of studies concerning the effect of feed additives on the level of calcium in the bones of broiler chickens, published during the past 10 years. From the analysis of available literature it follows that some additives had a positive effect on the accumulation of calcium (e.g. vitamin D, probiotics, prebiotics and synbiotics), some were not very explicit (e.g. ascorbic acid and phytase), while others did not have a significant effect on the accumulation of calcium in bones (e.g. herbs and chelates). It is concluded from our collected information that the use of probiotics, prebiotics and synbiotics offers the best advantages for poultry. These additives, apart from stimulating the accumulation of calcium in bones, also benefit animal health.

**Key words:** feed additives, calcium, accumulation, bones, broilers

The about 50-year long intensive genetic selection aiming to increase weight gain in broiler chickens made it possible to achieve more than 300% higher effects (Knowles et al., 2008). However, such a rapid growth rate prevents the skeletal system from reaching full maturity, which means that the legs are not completely capable of supporting heavy bodies of broilers, which in turn results in various types of pathological conditions of legs, including deformities, infections and osteoporosis (Rath et al., 2000; Fleming, 2008). The strength of leg bones is not only genetically determined but it also depends on the gender, age, health condition and nutrition. Therefore, studies are carried out to increase the strength of the broilers' leg bones.

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The deficit of calcium ions in bones deteriorates their structure, which is often a stage of osteoporosis, and reduces their strength (Dolińska et al., 2009). The optimum ratio of available calcium and phosphorus in feeding broiler chickens is 2:1, which is conditioned by strong interactions between these elements (Coto et al., 2008). An excessive supply of one of these elements causes a deterioration in the assimilability of both. In complete feed mixtures for broilers the Ca : P ratio is primarily achieved by adding phosphates, calcium carbonate and exogenous phytase. Also, the effectiveness of other substances, e.g. vitamin D, magnesium, inulin, non-digestible saccharides, and short-chain fatty acids was measured. The presented work compiles the results of studies regarding the effect of feed additives on the mineralisation of bones in broiler chickens, published over the past 10 years. The content of calcium was considered the basic ratio of mineralisation.

### Absorption of calcium

The absorption of calcium is affected by many factors such as age, health condition and nutrition. Most calcium is supplied with feed as salts or complexes involving other nutrients. To enable the absorption of calcium by the intestinal epithelial cells the links must be degraded and the released Ca must be transformed into ions, which takes place in the proventriculus of the birds. Complete absorption of calcium is determined by two processes: active transport (involving calcium-binding protein CaBP) and passive diffusion through the intestinal wall (Booth and Camacho, 2013). The paracellular transport of Ca is carried out through close intracellular connections according to the electrochemical concentration gradient. Calcium penetrates into the epithelial cell through the calcium-selective channel, TRPV5 or TRPV6, in the brush-border membrane. Within the cell calcium is transported by calbindin which is also a buffer protecting the cell against the adverse effect of Ca. Calcium is removed from the cell via the calcium pump PMCA1b and Na<sup>+</sup>/Ca<sup>2+</sup> (NCX1) exchanger, both located in the basolateral membrane (Hoenderop et al., 2005). The efficiency of calcium absorption, the absorbed percentage of calcium supplied with diet, is determined by several factors, including the presence of other nutrients, level of calcium and presence of an active form of vitamin D, as well as the form in which calcium was supplied (Dolińska et al., 2009).

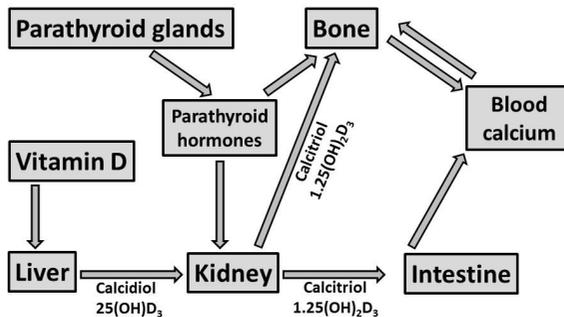


Figure 1. Calcium metabolism (according to Brown, 2007; Holick, 1996)

Calcium management in the body is controlled by calcitriol hormone, 1,25-dihydroxycholecalciferol, an active metabolite of vitamin D, parathormone and calcitonin (Figure 1). Calcitonin prevents resorption of calcium from the alimentary tract and bones and increases its excretion with urine. Calcitriol and vitamin D<sub>3</sub> increase the absorption of calcium from the alimentary tract, prevent its excretion with urine and cause bones to release calcium (Martin-Malo et al., 1996).

### **Vitamin D<sub>3</sub> and related metabolites**

The fundamental function of vitamin D in the human body is the control of calcium and phosphate management as well as bone modelling and mineralisation. Thanks to the discovery of active vitamin D receptors (VDR) in the intestinal epithelial cells, renal tubules and in the osseous tissue cells, the cells were identified as target cells for vitamin D (Tukaj, 2008). Blood-borne vitamin D can be either endogenous (cholecalciferol) or exogenous (ergocalciferol). In the liver cholecalciferol is hydroxylated to 25(OH)D<sub>3</sub> (calcidiol) with the participation of 25-hydroxylase, and then in the kidneys to 1,25(OH)<sub>2</sub>D<sub>3</sub> (calcitriol) with the participation of 1 $\alpha$ -hydroxylase (Tukaj, 2008; Garcia et al., 2013). Calcitriol, biologically active dihydroxylated vitamin D, and its analogues, interact with the target cells through vitamin D receptor (Eisman and Bouillon, 2014). The most important function of calcitriol is bone mineralisation control. Calcitriol is also a PTH (parathormone) secretion regulator interacting through feedback (Tukaj, 2008).

The supplementation of vitamin D to feed mixtures for poultry increases intestinal absorption of calcium and phosphorus and stimulates the production of calcium-binding proteins in the mucous membrane. Few results of studies verifying whether respective metabolites of vitamin D<sub>3</sub> have a different effect on the mineralisation of the bones of poultry are available. The results achieved are inconsistent. Michalczuk et al. (2010) carried out studies involving Cobb 500 chicks and proved that the content of Ca and P in femoral bones and the mineral thickness of these bones was higher in broilers receiving the mixture in which cholecalciferol was partly replaced with calcidiol, whereas the higher the share of calcidiol, the better the results (Table 1). Similar results were obtained by Gomez-Verduzco et al. (2013). These authors found that simultaneous supplementation of the feed mixture administered to Ross 308 broiler chickens with cholecalciferol and calcidiol increased the degree of calcification of the tibia (Table 1). In turn, studies carried out by Garcia et al. (2013), involving Cobb chicks, showed that metabolites of vitamin D (cholecalciferol, 25-hydroxycholecalciferol, 1,25-dihydroxycholecalciferol, 1 $\alpha$ -hydroxycholecalciferol) equally stimulated the absorption of Ca in tibia and femoral bones. These findings confirm the results of studies involving Hy-Line W36 laying hens where no significant effect of the form of vitamin D<sub>3</sub> (cholecalciferol, 25-hydroxycholecalciferol, 1,25-dihydroxycholecalciferol) on the content of Ca in blood was recorded (Nascimento et al., 2014).



Phytase	Ross 308 broiler chick; low P (5.1 g/kg of feed) diet plus phytase (1,000 units/kg of feed), adequate P (7.7 g/kg of feed) without phytase and very low P (4.0 g/kg) without phytase; at 22nd day of experiment the chicks were killed	Tibia	↑ bone mineral content ↑ ash percentage	Onyango et al., 2004
	Male and female Ross broilers; corn-soybean meal based diet with reduced Ca and nonphytate P plus phytase, or without phytase; at 43rd day of experiment the chicks were killed	Tibia	ns ash ns Ca, P, Fe, Cu, Zn, Mn	Shelton and Southern, 2006
	BUT-9 type turkeys; basal commercial diet plus phytase (750 FTU/kg) or wheat bran (10%) or wheat bran (10%) + phytase (750 FTU/kg); at 10th week of experiment (16th week of age) the turkeys were killed	Femoral	↓ crude ash ↓ Ca, P, Mg, K	Kwiecień et al., 2007
	Male Ross broiler chickens; 2 levels of dietary Ca (0.6 and 1.0%) and 3 levels of dietary phytase (0, 500 and 1 000 PU/kg of feed); at 21st day of experiment the chicks were killed	Tibia	↑ Fe, Mg ns Ca, P, Cu, Zn	Pintar et al., 2005
Probiotics, prebiotics, symbiotics	Chicks (Avian × Avian); a combination of <i>Bacillus licheniformis</i> and <i>Bacillus subtilis</i> added to starter (3 weeks) and finisher (3 weeks) diets at 500 g/kg of feed	Tibia	↑ percentage ash ↑ P	Mutus et al., 2006
	Japanese quails; commercial prebiotic and commercial symbiotic added to the basal diet; at 42nd day of experiment the quails were killed	Tibia	↑ ash ↑ Ca, P	Vahdatpour et al., 2014
	Cobb broiler chicks; FloraMax B-11 commercial probiotic culture consisting <i>Lactobacillus salivarius</i> and <i>Pedococcus parvulus</i> ; experiment 1: at days 1, 7, 14 and 21, treated chickens received the probiotic with drinking water (at 10 <sup>6</sup> cfu/chick, final dose 1 g/100 birds), at 30th <sup>h</sup> day of experiment the chicks were killed; experiment 2: at days 1, 12, 23, 34 and 45, treated chickens received the probiotic with drinking water (at 10 <sup>6</sup> cfu/chick, final dose 1 g/100 birds), at days 7, 28 and 45 of experiment the chicks were killed	Tibia	↑ total ash ↑ Ca, P	Gutierrez-Fuentes et al., 2013
	Male Ross 308 broiler chickens; basal diet plus probiotic (100 or 150 mg/kg) or commercial herbal blend (450 mg/kg); at 42nd day of experiment the chicks were killed	Tibia	↑ ash percentage ↑ Ca, P	Ziaie et al., 2011
	Turkey poult; basal diet plus prebiotic – dried <i>Aspergillus</i> meal; at 30th day of experiment the turkeys were killed	Tibia	↑ ash percentage ↑ Ca, P	Reginato et al., 2011
	White Lohmann Selected Leghorns (LSL-CLASSIC) laying hens; basal diet plus <i>Bacillus subtilis</i> (1 g/kg) and/or inulin (1 g/kg) for 12 weeks	Tibia	↑ ash and Ca levels in inulin and inulin + <i>B. subtilis</i> groups	Abdelqader et al., 2013
	Male Ross 308 broiler chickens; basal diet plus probiotic (100 or 150 mg/kg) or commercial herbal blend (450 mg/kg) or organic acid (400 mg/kg; commercial mixture of propionic acid, NH and sodium bentonite); at 42nd day of experiment the chicks were killed	Tibia	↑ ash percentage ↑ Ca, P	Ziaie et al., 2011

Table 1 – contd.

1	2	3	4	5
Chelates	Ross 308 broiler chicken; basal commercial diet plus inorganic form of Fe (control group) as $\text{FeSO}_4$ (50 or 100% of the total requirement) or organic form of Fe as Fe-glycine chelate (50 or 100% of the total requirement) for 42 days	Femur	ns Ca, P, Mg, Zn, Cu, Fe	Kwieceń, 2013 a
	Ross 308 broiler chicks; basal commercial diet supplemented with Cu in organic form (control) as glycinate chelate (25, 50 or 100% of the total requirement) or in inorganic form (control group) as $\text{CuSO}_4$ (25, 50 or 100% of the total requirement) for 42 days	Tibia	ns crude ash ns Ca, P, Mg, Zn, Cu, Fe	Kwieceń, 2013 b
	Ross 308 male broiler chicks; basal commercial diet plus organic form of Cu (control) as glycinate chelate (25, 50 or 100% of the total requirement) or inorganic form of Cu as $\text{CuSO}_4$ (25, 50 or 100% of the total requirement) for 42 days	Femur	ns crude ash ns Ca, P, Mg, Zn, Cu, Fe	Kwieceń et al., 2014
Herbs	Ross broiler chickens; control commercial diet contained antibiotic Flavomycin (0.1%), experimental diets (without antibiotic) contained dry pansy (1% or 3%) or dry nettle (1% or 3%) for 42 days	Tibia	ns crude ash ns Ca, P, Mg	Kwieceń and Jaśkiewicz, 2011
	Ross 308 broiler chickens; basal commercial diet contained 2% of dry hop, lime, lemon balm, pansy, nettle or peppermint; control diet contained antibiotic (Flavomycin) and dried grass silage; the experiment took 42 days	Tibia	ns crude ash, P, Ca ↓ Mg in lemon balm, pansy, peppermint and nettle groups	Kwieceń and Winiarska-Mieczan, 2009

ns – no significant effect compared to control; ↑ – increased compared to control; ↓ – decreased compared to control.

### **Ascorbic acid**

Ascorbic acid, also known as vitamin C, is a 2,3-dehydro-L-gulonic acid  $\gamma$ -lactone formed in plants from D-glucose or from D-galactose. In the bodies of animals which synthesize ascorbic acid independently for their own needs this vitamin is formed from D-glucose (Hacışevki, 2009). Ascorbic acid has an influence on processes related to proliferation and differentiation of osteoblastic cells and to accelerating their growth and extending their lifespan. The results of vitamin C deficiency include distortion of collagen helix, reduced number and distorted structure of collagen fibres, which in turn leads to many pathological lesions of bone tissue (Hacışevki, 2009). This is due to the fact that vitamin C is a co-factor in reactions where proline is hydroxylated to 4-hydroxyproline forming a significant element of the collagen structure. As a result of disturbances in hydroxylation the formed collagen has improper structure and it does not have proper biological and mechanical properties. Völker and Weiser (1993) found that vitamin C participates in the process of hydroxylation of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol as well as in the hydroxylation of proline and lysine which take part in the biosynthesis of collagen.

Yildiz et al. (2009) demonstrated the positive effect of ascorbic acid administered to broilers with water (200 mg/l) on the content of crude ash, Ca and P in the tibia (Table 1). Furthermore, Lohakare et al. (2005) obtained similar results by supplementation of 10, 50, 100 and 200 ppm of vitamin C, which caused an increase in the content of ash, Ca and P in the tibia. In another work Lohakare et al. (2004) proved that the content of Ca and P in the tibia of broilers does not depend on whether vitamin C is administered in water or in feed. In turn, Keshavarz (1996) in his studies involving laying hens did not find any statistically significant effect of adding vitamin C (0, 125, 250, 100 or 1000 ppm/kg feed) on the concentration of crude ash in the tibia. Konca et al. (2009) obtained similar results in their studies involving Ross broilers receiving 150 or 300 mg of L-ascorbic acid with feed.

### **Probiotics, prebiotics and synbiotics**

Probiotics are preparations containing strictly defined live germs which, used as feed additives, have a positive effect on the microflora of the specific location in the body while prebiotics are non-digestible components which selectively stimulate the growth or activity of selected strains (derivatives of galactose, fructose or inulin). The combination of a probiotic with a prebiotic is a functional whole and is referred to as a synbiotic. Probiotics, prebiotics and synbiotics have influence on the animal body through supporting metabolic processes and improving digestion and absorption of nutrients (Younis et al., 2013; Saminathan et al., 2011). They stimulate the assimilation of essential minerals and electrolytes such as calcium, sodium, magnesium and potassium (Scholz-Ahrens et al., 2007), thanks to which they increase the degree of mineralisation and development of bones. Probiotics support calcium absorption primarily by the effect of microorganisms: they produce metabolites and enzymes and take part in the synthesis of vitamins, some of which participate in the metabolism of Ca (Scholz-Ahrens et al., 2007). The positive effect of prebiotics on the absorption of calcium is primarily due to the increase in the solubility of calcium

in consequence of reducing the intestinal pH as a result of fermentation of fructooligosaccharides in the colon (Suzuki and Hara, 2004), production of short-chain fatty acids in this section of the intestine which reduce the pH and activate the mechanism of exchange of hydrogen ions into calcium ions (de Sousa et al., 2011; Martin et al., 2010) and the effect of these carbohydrates on increasing the concentration of calbindin D9k (Regassa and Kim, 2014), calbindin being the protein responsible for transporting calcium within cells (Hoenderop et al., 2005).

Available literature contains many examples of a positive effect of probiotics, prebiotics and synbiotics on the mineralisation of poultry bones (Table 1). Feeding broilers with various types of preparations containing probiotic bacteria contributes to increasing the content of calcium in bones, whereas positive results were obtained both during continuous exposure (Reginatto et al., 2011; Ziaie et al., 2011) and when the preparations were used every few days (Gutierrez-Fuentes et al., 2013). The increased content of Ca in bones was observed when probiotics were administered both with feed and with water (Table 1). In White Lohmann laying hens receiving feed with probiotic strains of *Bacillus subtilis* and/or inulin amounting to 1 g/kg feed not only the content of ash, Ca, and density of the tibia increased but also the quality of egg shells improved, including size, thickness, density and content of calcium. Vahdatpour et al. (2014) demonstrated the results of Japanese quails, in which the content of Ca and P in the tibia following the use of a commercial synbiotic was dependent on the sex of the bird – statistically significant results were observed in males only.

### Phytase

Phytic acid (IP6) which contains 6 phosphate groups and its derivatives which contain from 3 to 5 phosphate groups (IP3, IP4 and IP5) occur in grains and seeds of leguminous crops. This compound forms insoluble complexes with divalent ions of metals (Ca, Mg, Zn, Fe) and with phosphorus, reducing their bioavailability and thus increasing the requirement of such components. The availability of compounds chelated by phytic acid is increased by adding microbiological phytase to the feed since the body of birds produces only small amounts of this enzyme (Coto et al., 2008; Junqueira et al., 2011). The mechanism by which phytase affects availability is the hydrolysis of phytic compounds to inorganic compounds (phosphates and inositol) followed by the release of minerals and reduction in the molar ratio between such minerals and phytic acid.

The use of phytase in mixtures fed to broiler chickens increases the apparent digestibility of calcium by ca. 4–6% (Saima et al., 2009). Moreover, Wang et al. (2013) demonstrated a statistically significant increase in the concentration of Ca in the plasma of Ross 308 broiler chickens receiving feed in which phytase corresponded to 0.02% and 0.03% (5000 u/g) of the mixture compared to those not receiving the enzyme. According to Ptak et al. (2013) in Ross 308 broiler chickens, which over 42 days of the experiment received mixtures deficient in calcium (by 1.8 g/kg) but were supplemented with phytase, the level of Ca in the tibia was identical as in birds receiving an adequate amount of calcium with feed. Supplementing phytase mixtures with a reduced content of calcium improved the mineralisation of the tibia in Ross

broiler chickens (Powell et al., 2011). Lan et al. (2002) carried out an interesting experiment by using cultures of phytase-producing bacteria *Mitsuokella jalaludinii* naturally occurring in the alimentary tract of ruminants. Results showed an increase in the content of ash and calcium in the tibia of birds receiving bacteria compared to the control group. The increase was particularly noticeable when the level of bacteria was medium and high (equivalent of 500, 750 and 1 000 U phytase/kg feed). In turn, according to Pintar et al. (2005) and Shelton and Southern (2006), phytase has no significant effect on the content of Ca in the tibia. Moreover, Kwiecień et al. (2007) found that the content of calcium in the tibia of turkeys receiving phytase with feed was reduced (Table 1).

### Mineral chelates

The proper balance of minerals is significant for bone mineralisation processes, since incorrect proportions between them disturb the bone-forming processes. Magnesium, boron, manganese, copper and zinc are the necessary co-factors, for example, for enzymes taking part in the synthesis of collagen which forms an essential part of the bone matrix. Collagen determines the most important processes occurring within bone tissue, e.g. its formation, mineralisation and development of adequate mechanical properties (Scholz-Ahrens et al., 2007). It was proved that copper deficiency in the diet of birds causes a deterioration in the mineralisation of bones (Kwiecień et al., 2014), and its excessive supply disturbs the absorption of magnesium and other minerals important for forming the bone matrix (Brink et al., 1992). Magnesium is believed to be one of the most important factors affecting the absorption of calcium, for instance due to the fact that it stimulates metabolism and the activity of vitamin D (Laird et al., 2010), involved in maintaining calcium homeostasis and forming hydroxyapatite (Kannan and Ferreira, 2006).

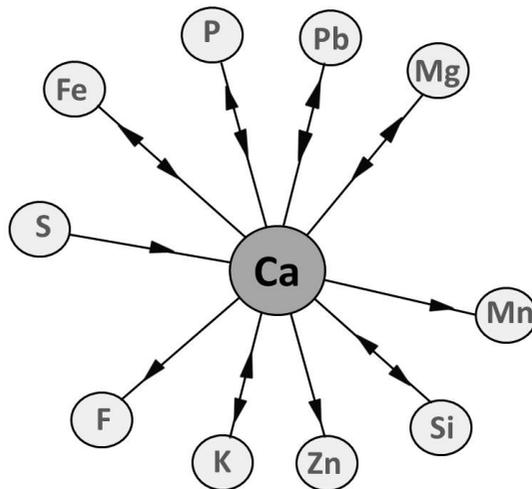


Figure 2. Interactions between calcium and other minerals (according to Watts, 1990; Kotarbińska, 1995)

The assimilability of minerals by animals is generally conditioned by their chemical form. Minerals administered as mineral and organic chelates (metal ion + amino-acid ligand), in the form of multi-functional complexes, are better assimilated by poultry than these components administered as mineral salts, therefore organic minerals can be fed to birds at smaller amounts (Nollet et al., 2007). Upon this assumption, studies investigating the effect of mineral chelates on the degree of calcification of poultry bones were carried out. However, the effects of available experiments did not reveal a significant effect of Fe and Zn on the content of calcium in the tibia and femurs of broilers (Table 1), despite the proven relationship between these minerals and calcium (Figure 2).

### Herbs

The effect of active ingredients of herbs on the calcification of poultry bones is poorly documented. However, it is known that bioactive polyphenolic compounds, and in particular isoflavones and lignans, naturally occurring in herbs have a positive effect on the structure of bones, mostly since they are active antioxidants. A negative effect of oxidative stress on the degree of bone mineralisation was demonstrated (Basu et al., 2001; Sharma et al., 2015). Flavonoids, phytosterols and glycosides occurring, among other plants, in the cones of hop, in garlic and in ginseng have a positive effect on bone metabolism and mineralisation (Xu et al., 2005). Monoterpenes present, among other things, in mint, salvia, rosemary, thyme and hop inhibit bone demineralisation through their immediate effect on the osteoclasts (Mühlbauer et al., 2003; Dolder et al., 2006).

The few available results of studies involving broiler chickens concerning the effect of herbs on the content of calcium in bones are ambiguous. No significant effect of dried pansy, nettle, hop, lime, lemon balm and mint on the content of calcium in tibias was demonstrated (Table 1). In turn, other studies showed a significant increase in the content of Ca in the femurs of Ross broiler cocks receiving feed with 2% dried hop, lime, lemon balm, pansy, mint and nettle while the experimental factor had no effect on the level of Ca in the femurs of broiler hens (Kwiecień and Winiarska-Mieczan, 2007).

### Feed yeasts

Feed yeasts are a source of necessary amino acids and vitamins from group B for animals, thus they have a positive effect on the physiological and immune processes in the body (Sacakli et al., 2013). Live yeast cells show a probiotic effect since they produce, among other things, glucan and mannan oligosaccharides (Zhou et al., 2011); the stimulating effect of probiotics on bone mineralisation was proved (Suzuki and Hara, 2004; Scholz-Ahrens et al., 2007; de Sousa et al., 2011). In addition, it was found that the use of *Saccharomyces cerevisiae* in the feeding of poultry increases the availability of Ca and P from the feed as a result of high content of vitamins from group B, trace elements and oligosaccharides (Akhavan-Salamat et al., 2011). Based on this information it could be expected that yeasts used in the feeding of broilers would have a positive effect on the content of minerals in bones. However, the few available results of studies did not show any significant effect of feed yeasts on the content of Ca in the bones of poultry (Table 1).

## Summary

Numerous studies point to the necessity of using feed additives which stimulate the assimilability of minerals in poultry. Using these additives improves the mineralisation of bones, which is particularly important when the birds grow intensely and are very heavy. The deficit of calcium ions in bones negatively affects their structure and reduces strength. The analysis of available literature showed that currently numerous feed additives designed to improve the assimilability of calcium are being tested. Some additives without any doubt have a positive effect on the accumulation of this mineral in bone – these include vitamin D, probiotics, prebiotics and synbiotics. However, the results are not so clear-cut for some additives, for example, ascorbic acid. Also, based on the available results of studies, the beneficial effect of phytase on the assimilability of calcium in poultry cannot be clearly confirmed. The production of new generation microbiological phytases provides new options as a result of genetic modification of microorganisms; however, relevant studies are not advanced. In addition, it should be noted that some of the studied feed additives had no significant effect on the accumulation of calcium in the bones. Based on the collected information it can be stated that the largest advantages for poultry are offered by the use of probiotics, prebiotics and synbiotics. These additives, apart from the stimulating effect on the accumulation of calcium in the bones, are also healthy for the animal (Saminathan et al., 2011).

## References

- Abdelqader A., Al-Fataftah A.R., Das G. (2013). Effects of dietary *Bacillus subtilis* and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production. *Anim. Feed Sci. Tech.*, 179: 103–111.
- Akhavan-Salamat H., Ghasemi H.A., Khaltabadi-Farahani A.H., Kazemi-Bonchenari M. (2011). The effects of *Saccharomyces cerevisiae* on performance and nutrients digestibility in broilers fed with diet containing different levels of phosphorous. *Afr. J. Biotechnol.*, 10: 7526–7533.
- Basu S., Michaelsson K., Olofsson H., Johansson S., Melhus H. (2001). Association between oxidative stress and bone mineral density. *Biochem. Biophys. Res. Commun.*, 288: 275–279.
- Booth A., Camacho P. (2013). A closer look at calcium absorption and the benefits and risks of dietary versus supplemental calcium. *Postgrad. Med.*, 125: 73–81.
- Brink E.J., Beynen A.C., Dekker P.R., van Beresteijn E.C., van der Meer R. (1992). Interaction of calcium and phosphate decreases ileal magnesium solubility and apparent magnesium absorption in rats. *J. Nutr.*, 122: 580–586.
- Brown E.M. (2007). Clinical lessons from the calcium-sensing receptor. *Nat. Clin. Pract. Endoc.*, 3: 122–133.
- Coto C., Yan F., Cerrate S., Wang Z., Sacakli P., Halley J.T., Wiernusz C.J., Martinez A., Waldroup P.W. (2008). Effects of dietary levels of calcium and nonphytate phosphorus in broiler starter diets on live performance, bone development and growth plate conditions in male chicks fed a corn-based diet. *Int. J. Poultry Sci.*, 7: 638–645.
- de Sousa V.M.C., dos Santos E.F., Sgarbieri V.C. (2011). The importance of prebiotics in functional foods and clinical practice. *Food Nutri. Sci.*, 2: 133–144.
- Dolder S., Hofstetter W., Wetterwald A., Mühlbauer R. C., Felix R. (2006). Effect of monoterpenes on the formation and activation of osteoclasts *in vitro*. *J. Bone Miner. Res.*, 21: 647–655.

- Dolińska B., Mikulska A., Ryszka F. (2009). Factors enhancing calcium's absorption. *Ann. Acad. Med. Siles.*, 63: 76–83.
- Eisman J.A., Bouillon R. (2014). Vitamin D: direct effects of vitamin D metabolites on bone: lessons from genetically modified mice. *BoneKEy Reports*, 3. doi:10.1038/bonekey.2013.233.
- Fleming R.H. (2008). Nutritional factors affecting poultry bone health. *P. Nutri. Soc.*, 67: 177–183.
- Garcia A.F.Q.M., Murakami A.E., Duarte C.R. do A., Rojas I.C.O., Picoli K.P., Puzotti M.M. (2013). Use of vitamin D<sub>3</sub> and its metabolites in broiler chicken feed on performance, bone parameters and meat quality. *Asian-Austral. J. Anim. Sci.*, 26: 408–415.
- Gómez-Verduzco G., Morales-López R., Avila-Gozález E. (2013). Use of 25-hydroxycholecalciferol in diets of broiler chickens: effects on growth performance, immunity and bone calcification. *J. Poultry Sci.*, 50: 60–64.
- Gutierrez-Fuentes C.G., Zuñiga-Orozco L.A., Vicente J.L., Hernandez-Velasco X., Menconi A., Kuttappan V.A., Kallapura G., Latorre J., Layton S., Hargis B.M., Téllez G. (2013). Effect of a lactic acid bacteria based probiotic FloraMax-B11 on performance, bone qualities, and morphometric analysis of broiler chickens: An economic analysis. *Int. J. Poultry Sci.*, 12: 322–327.
- Hacısevkd A. (2009). An overview of ascorbic acid biochemistry. *J. Fac. Pharm. Ankara*, 38: 233–255.
- Hoenderop J.G., Nilius B., Bindels R.J. (2005). Calcium absorption across epithelia. *Physiol. Rev.*, 85: 373–422.
- Holick F.M. (1996). Vitamin D and bone health. *J. Nutr.*, 126: 1159–1164.
- Junqueira O.M., Ferreira Duarte K., Assuena V., da Silva Filardi R., de Laurentiz A.C., Ferreira Menegucci Praes M.F. (2011). Effect of phytase supplementation on performance, bone densitometry and carcass yield in broilers chicks. *Acta Sci. Anim. Sci.*, 33: 301–307.
- Kannan S., Ferreira J.M.F. (2006). Synthesis and thermal stability of hydroxyapatite-β-tricalcium phosphate composites with cosubstituted sodium, magnesium, and fluorine. *Chem. Mater.*, 18: 198–203.
- Keshavarz K. (1996). The effect of different levels of vitamin C and cholecalciferol with adequate or marginal levels of dietary calcium on performance and eggshell quality of laying hens. *Poultry Sci.*, 75: 1227–1235.
- Knowles T.G., Kestin S.C., Haslam S.M., Brown S.N., Green L.E., Butterworth A., Nicol C.J. (2008). Leg disorders in broiler chickens: prevalence, risk factors and prevention. *PLoS One*, 3(2): e1545. doi: 10.1371/journal.pone.0001545
- Konca Y., Kirkpınar F., Mert S., Yurtseven S. (2009). Effects of dietary ascorbic acid supplementation on growth performance, carcass, bone quality and blood parameters in broilers during natural summer temperature. *Asian J. Anim. Vet. Adv.*, 4: 139–147.
- Kotarbińska M. (1995). Minerals. In: Additives in feedingstuffs for pigs, Kotarbińska M., Grela E. (eds). The Kielanowski Institute of Animal Physiology and Nutrition, Jabłonna, Poland, pp. 23–36.
- Kwiecień M. (2013 a). The effect of the level and form of Fe on the quality of femur bones in broiler chicken. *Ann. UMCS, Sec. EE*, 4: 24–34.
- Kwiecień M. (2013 b). Effect of copper glycine chelate on the quality of the tibia bones in broiler chicken. *Med. Weter.*, 69: 492–498.
- Kwiecień M., Jaśkiewicz T. (2011). Effect of different amounts of pans and nettle in feed mixtures for chickens on physical, chemical and strength parameters of their bones. *Med. Weter.*, 67: 258–262.
- Kwiecień M., Winiarska-Mieczan A. (2007). Effect of herbal addition to feed mixtures on mechanical and chemical parameters of the broiler chicken femoral bone. *Polish J. Environ. Stud.*, 16: 179–184.
- Kwiecień M., Winiarska-Mieczan A. (2009). Effect of addition of herbs on body weight and assessment of physical and chemical alternations in the tibia bones of broiler chickens. *J. Elementol.*, 14: 705–715.
- Kwiecień M., Makarski B., Zadura A. (2007). Effect of phytase and wheat bran addition to feed mixtures on the resistance parameters and chemical composition of the broiler femoral bones. *Pol. J. Environ. Stud.*, 16: 174–178.

- Kwiecień M., Winiarska-Mieczan A., Zawisłak K., Sroka S. (2014). Effect of copper glycinate chelate on biomechanical, morphometric and chemical properties of chicken femur. *Ann. Anim. Sci.*, 14: 127–139.
- Laird E., Ward M., McSorley E., Strain J.J., Wallace J. (2010). Vitamin D and bone health; Potential mechanisms. *Nutrients*, 2: 693–724.
- Lan G.Q., Abdullah N., Jalaludin S., Ho Y.W. (2002). Efficacy of supplementation of a phytase-producing bacterial culture on the performance and nutrient use of broiler chickens fed corn-soybean meal diets. *Poultry Sci.*, 81: 1522–1532.
- Lohakare J.D., Chae B.J., Hahn T.W. (2004). Effects of feeding methods (water vs. feed) of vitamin C on growth performance and carcass characteristics in broiler chickens. *Asian-Austral. J. Anim. Sci.*, 17: 1112–1117.
- Lohakare J.D., Ryu M.H., Hahn T.W., Lee J.K., Chae B.J. (2005). Effects of supplemental ascorbic acid on the performance and immunity of commercial broilers. *J. Appl. Poultry Res.*, 14: 10–19.
- Martin B.R., Braan M.D., Wigertz K., Bryant R., Zhao Y., Lee W., Kempa-Stecko S., Weaver C.M. (2010). Fructo-oligosaccharides and calcium absorption and retention in adolescent girls. *J. Am. Coll. Nutr.*, 29: 382–386.
- Martin-Malo A., Rodriguez M., Martinez M.E., Torres A., Felsenfeld A.J. (1996). The interaction of PTH and dietary phosphorus and calcium on serum calcitriol levels in the rat with experimental renal failure. *Nephrol. Dial. Transplant.*, 11: 1553–1558.
- Michalczyk M., Szara T., Bereznowski A., Serwa U., Winkowski M. (2010). Effect of administration of two forms of vitamin D in a feed mixture on the strength traits of the femur bone of broiler chickens. *Rocz. Nauk. PTZ*, 6: 77–83.
- Mutuş R., Kocabagli N., Alp M., Acar N., Eren M., Gezen Ş.Ş. (2006). The effect of dietary probiotic supplementation on tibial bone characteristics and strength in broilers. *Poultry Sci.*, 85: 1621–1625.
- Mühlbauer R.C., Lozano A., Palacio S., Reinli A., Felix R. (2003). Common herbs, essential oils, and monoterpenes potentially modulate bone metabolism. *Bone*, 32: 372–380.
- Nascimento G.R., Murakami A.E., Guerra A.F.Q.M., Ospinas-Rojas I.C., Ferreira M.F.Z., Fanhani J.C. (2014). Effect of different vitamin D sources and calcium levels in the diet of layers in the second laying cycle. *Braz. J. Poultry Sci.*, 16: 37–42.
- Nollet L., van der Klis J.D., Lensing M., Spring P. (2007). The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. *J. Appl. Poultry Res.*, 16: 592–597.
- Onyango E.M., Bedford M.R., Adeola O. (2004). The yeast production system in which *Escherichia coli* phytase is expressed may affect growth performance, bone ash, and nutrient use in broiler chicks. *Poultry Sci.*, 83: 421–427.
- Pintar J., Bujan M., Homen B., Gazić K., Sikirić M., Černý T. (2005). Effects of supplemental phytase on the mineral content in tibia of broilers fed different cereal based diets. *Czech J. Anim. Sci.*, 50: 68–73.
- Powell S., Bidner T.D., Souther L.L. (2011). Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. *Poultry Sci.*, 90: 604–608.
- Ptak A., Józefiak D., Kierończyk B., Rawski M., Żyła K., Świątkiewicz S. (2013). Effect of different phytases on the performance, nutrient retention and tibia composition in broiler chickens. *Archiv. Tierzucht*, 56: 1028–1038.
- Rath N.C., Huff G.R., Huff W.E., Balog J.M. (2000). Factors regulating bone maturity and strength in poultry. *Poultry Sci.*, 79: 1024–1032.
- Regassa A., Kim W.K. (2014). Effects of age, vitamin D<sub>3</sub> and fructooligosaccharide on correlation of different bone parameters in broiler chickens. *Int. J. Poultry Sci.*, 13: 160–167.
- Reginatto A.R., Menconi A., Londero A., Lovato M., Pires Rosa A., Shivaramaiah S., Wolfenden A.D., Huff W.E., Huff G.R., Rath N.C., Donoghue A.M., Hargis B.M., Tellez G. (2011). Effects of dietary *Aspergillus* Meal prebiotic on turkey pullets production parameters and bone qualities. *Int. J. Poultry Sci.*, 10: 496–499.
- Sacaklı P., Koksall B.H., Ergun A., Ozsoy B. (2013). Usage of brewer's yeast (*Saccharomy-*

- ces cerevisiae*) as a replacement of vitamin and trace mineral premix in broiler diets. *Rev. Med. Vet.*, 164: 39–44.
- Saima M.Z., Khan U., Jabbar M.A., Ijaz M., Qadeer M.A. (2009). Efficacy of microbial phytase at different levels on growth performance and mineral availability in broiler chicken. *J. Anim. Plant Sci.*, 19: 58–62.
- Saminathan M., Sieo C.C., Kalavathy R., Abdullah N., Ho Y.W. (2011). Effect of prebiotic oligosaccharides on growth of *Lactobacillus* strains used as a probiotic for chickens. *Afr. J. Microbiol. Res.* 5: 57–64.
- Scholz-Ahrens K.E., Ade P., Marten B., Weber P., Timm W., Acil Y., Gluer C.C., Schrezenmeir J. (2007). Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *J. Nutr.*, 137: 838–846.
- Sharma T., Islam N., Ahmad J., Akhtar N., Beg M. (2015). Correlation between bone mineral density and oxidative stress in postmenopausal women. *Indian J. Endocr. Metab.*, 19: 491–497.
- Shelton J.L., Southern L.L. (2006). Effects of phytase addition with or without a trace mineral premix on growth performance, bone response variables, and tissue mineral concentrations in commercial broilers. *J. Appl. Poultry Res.*, 15: 94–102.
- Suzuki T., Hara H. (2004). Various nondigestible saccharides open a paracellular calcium transport pathway with the induction of intracellular calcium signaling in human intestinal Caco-2 cells. *J. Nutr.*, 134: 1935–1941.
- Tukaj C. (2008). Adequate level of vitamin D is essential for maintaining good health. *Postep. Hig. Med. Dosw.*, 62: 502–510.
- Vahdatpour T., Ebrahimnezhad Y., Vahdatpour S. (2014). Effects of dietary functional additives on characteristics and minerals of tibia bone and blood parameters of Japanese quails (*Coturnix coturnix japonica*). *Int. J. Plant, Anim. Environ. Sci.*, 4: 690–695.
- Völker L.H., Weiser H. (1993). The relevance of vitamins D<sub>3</sub> and C for bone metabolism in poultry. In: Maryland Nutrition Conference for Feed Manufacturers, Maryland, USA, pp. 42–54.
- Wang W., Wang Z., Yang H., Cao Y., Zhu X., Zhao Y. (2013). Effects of phytase supplementation on growth performance, slaughter performance, growth of internal organs and small intestine, and serum biochemical parameters of broilers. *Open J. Animal Sci.*, 3: 236–241.
- Watts D.L. (1990). Nutrients interrelationships. Minerals – vitamins – endocrines. *J. Orthomol. Med.*, 5: 11–20.
- Xu M., Dick I.M., Day R., Randall D., Prince R. L. (2003). Effects of a herbal extract on the bone density, strength and markers of bone turnover of mature ovariectomized rats. *Am. J. Chin. Med.*, 31: 87–101.
- Yildiz H., Gunes N., Gezen S.S., Ozcan R., Petek M., Yilmaz B., Arican I. (2009). Effects of ascorbic acid and lighting schedule on tibiotarsus strength and bone characteristics in broilers. *Archiv. Tierzucht*, 52: 432–444.
- Younis T.M., El-Shafei A.A., Al-Gamal M.A., El-Sayed A.L. (2013). Effects of commercial probiotics on productive and physiological performance of broiler chickens. *J. Appl. Sci. Res.*, 9: 6643–6654.
- Zhou Z.L., Rath N.C., Huff G.R., Huff W.E., Rasaputra K.S., Salas C., Coon C.N. (2011). Bone characteristics of 16 wk-old-turkeys subjected to different dietary supplements and simulated stress. *International J. Poultry Sci.*, 10: 332–337.
- Ziaie H., Bashtani M., Karimi Torshizi M.A., Naeemipour H., Farhangfar H., Zeinali A. (2011). Effect of antibiotic and its alternatives on morphometric characteristics, mineral content and bone strength of tibia in Ross broiler chickens. *Glob. Vet.*, 7: 315–322.

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