Ann. Anim. Sci., Vol. 17, No. 3 (2017) 645–669 DOI: 10.1515/aoas-2016-0098

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INFECTIOUS AND NON-INFECTIOUS FACTORS ASSOCIATED WITH LEG DISORDERS IN POULTRY – A REVIEW

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Abstract

Broiler chicken welfare, health and performance are strictly linked with skeleton development. Lameness compromises welfare of broiler chickens and causes considerable economic loss since lame birds have difficulty accessing feed and water, become dehydrated and eventually die. Leg disorders are therefore considered to be one of the main factors associated with in-field mortalities between 21–42 d in broiler rearing at European poultry farms. In chickens and other farm animals, bone development is strictly correlated with dietary content of inositol hexaphosphate (IP6), as well as calcium and phosphorus availability. However, lameness is also associated with many other factors, such as diseases, genetics, species, gender, growth, aging, as well as physical loading, rearing period and management. Therefore, the aim of the current paper is to review selected non-infectious and infectious factors, which contribute to bone quality in poultry.

Key words: poultry, leg disorders factors, bones, nutrition, management

In the last 50 years, owing to intense genetic selection, body mass growth rates in broiler chickens have increased by more than 300% (Knowles et al., 2008). Quick growth, development and maturation of the skeleton are not accompanied by development of sufficiently strong legs, fully capable of supporting a heavier than ever body, which causes their deformation (Fleming, 2008). Breeding programmes aimed at achieving the greatest muscle mass in birds interfere with their health. It is caused by an inverse correlation between the increase in wing muscle mass and the decrease in leg muscle circumference. For the above reasons, excessive bone loading causes different leg pathologies, such as weakening, contusion, deformity, infections and osteoporosis (Rath et al., 1999).

According to UK estimates, as many as 27% of birds were affected by locomotion problems in the pre-slaughter period and 3.3% were unable to walk (Knowles et al., 2008). In addition, tibial dyschondroplasia affected 30% of meat-type chickens and 90% of turkeys (Derakhshanfar et al., 2013). It has been estimated that 12.5 billion birds worldwide experience leg problems annually (FAO, 2010). The most frequent form of tibial dyschondroplasia is sub-clinical stage (Crespo and Shivaprasad, 2011). What is more, economic loss caused by these disturbances in growth and development of bones and skeletal system in poultry reached 150 million dollars in the USA alone (Sullivan, 1994; Cook, 2000; Oviedo-Rondón and Ferket, 2005). Almeida Paz et al. (2010) noted that bone problems could indirectly account for reduced profit from further chicken meat processing and thus for gross profit reduction (10–40% of costs).

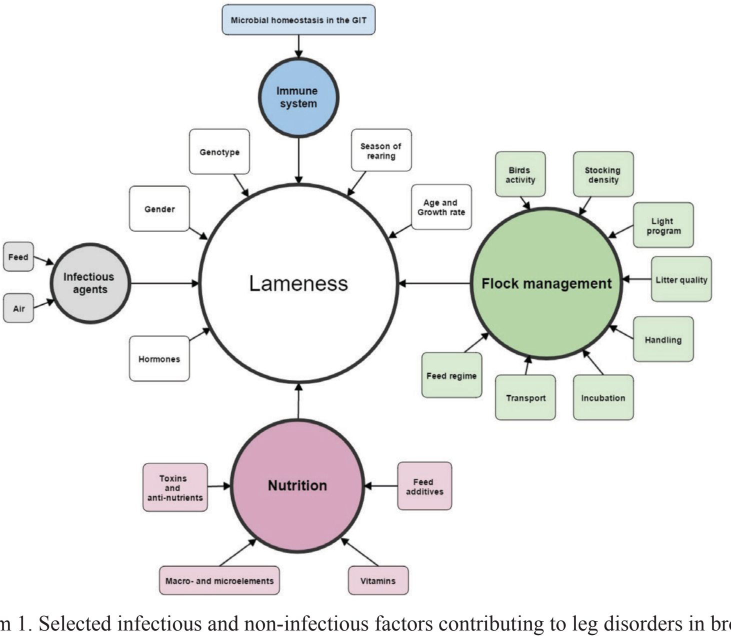

Diagram 1. Selected infectious and non-infectious factors contributing to leg disorders in broiler chickens

The strength of leg bones depends on many factors beginning from genetic determinants, through species, sex, age, nutrition, rearing period to infectious agents or endocrine system functions (Diagram 1). Knowles et al. (2008) also highlighted the significance of flock management practices which are often disregarded or limited only to individual aspects, which can contribute to the worsening of leg bone quality.

Bone structure

Of all the known vertebrate classes, the bone system of birds is characterised by a specific functionality. Being adapted to active flight, it entails many anatomical modifications. Pneumatised bones, reduced amount of bone marrow, no teeth, horny beak, and enlarged orbits in relation to the whole skull cause body mass reduction. The forearm and hand bones and all pelvic bones are the only non-pneumatised bones. Of special note from the aspect of bone and skeleton development and function, is the degree of mineralisation of the *os femoris*, *tibia*, *fibula* and *skeleton pedis* due to their supporting character (Langenfeld, 1992).

The skeleton of birds is composed of a mineral part (70%), organic part (20%) and water (10%). The majority of the mineral structure of bones is composed of calcium and phosphorus built in hydroxyapatite (Turek, 1984). For the above reasons, crude ash is a prevailing component of bones of birds and the ash content is thought to be a good indicator of the mechanical strength and quality of bones. Mechanical bone breaking strength is defined as a sum of factors/forces causing bone fracture (Nigg and Grimstone, 1994). Bone density, defined as the mass-to-volume ratio, is another criterion of bone quality evaluation (Rath et al., 2000). It is indicative of the completion of structure building and mineralisation (Boskey et al., 1999). It was shown that density was not always dependent only on mineral content but partially relied on osseomucoid (Knott and Bailey, 1998). Bone composition is also influenced by intermolecular collagen crosslinking, interaction of collagen with proteoglycans and other noncollagenous proteins and by glucooxidative changes. Hence, bone microarchitecture has a significant effect on their mechanical strength (Gorski, 1998).

Long bones are built by trabecular tissue with a lesser degree of calcification, participating in metabolic processes and under constant remodeling, and by compact tissue (Seifert and Watkins, 1997). The organic matter of bones is composed mostly of collagen which improves bone toughness and supports its mineral components (Riggs et al., 1993). Disturbances in collagen synthesis impair the biomechanical strength of bones. Bone tissue is strengthened by calcification, fibrillogenesis, hydroxylation and crosslinking processes. It was shown that dense pyridinoline networks (a component of intermolecular bonds of mature collagen) contribute to the increased bone strength, while in osteoporotic birds their content was reduced (Knott et al., 1995). Apart from collagen, the organic substance contains proteoglycans, lipids and noncollagenous proteins (osteocalcin, osteonectin, and osteopontin). Bone marrow, due to its role in osseous tissue remodeling (osteoblasts) and production of cellular blood components, is particularly important for keeping homeostasis in the animal organism. For this reason disturbances in leg bone formation not only can worsen the motor performance of animals but can also reduce the functional efficacy of the immune system. It is particularly important during bone marrow infection, *inter alia* due to the production of α- and β-defensins responsible for innate and acquired immunity, which is dependent on production of leukocytes (Derache et al., 2009). Bone marrow, as a natural source of these peptides, is also the place of their greatest expression (Lynn et al., 2004). It should be remembered that during generalised infections, leukopoiesis in bone marrow increases twofold (Klasing, 1998). Thus, it can be expected that during bone development disturbances caused by pathogenic bacteria penetration, leukocyte synthesis will be limited. On the other hand, Rajput et al. (2014) demonstrated that supplementation of probiotic strains (*Saccharomyces boulardii*, *Bacillus subtilis* B10) had a beneficial effect on the immunity of broiler chickens, by increasing cytokine production by dendritic cells in bone marrow. Moreover, the immunosuppressive properties of viruses such as Chicken Anemia Virus (CAV) and Infectious Bursal Disease Virus (IBDV) can aggravate necrosis of the head of the femoral bone in broiler chickens (Thorp et al., 1993; McNamee et al., 1999; McNamee and Smyth, 2000).

Non-infectious factors contributing to leg disorders

Mineral nutrition

Due to strong interactions between the levels of available calcium and phosphorus, an optimal Ca:P ratio in chicken feeding is 2:1; however, for laying hens this proportion is much higher, reaching 12:1. In commercial feeds for chickens, the Ca:P ratio is maintained by using fodder phosphates, fodder chalk and exogenous enzymes – phytase, and also other unconventional sources of these macroelements. For the above reasons the use of calcium-containing preparations in drinking water can severely disturb its availability in relation to phosphorus, since an excessive supply of one of these elements worsens the assimilability of both of them. The poor quality of water which contains more than 75 mg of Ca/L may negatively affect nutrients, as well as medicines absorption (Mituniewicz, 2014). Jamroz et al. (2007) demonstrated that from the calcium and phosphorus intake of 100 g each, chickens assimilate 60–72 g Ca and 35–54 g P, depending on their age. Other studies have revealed a significant role of the vitamin 25-hydroxycholecalciferol (Hy-D) (Koreleski and Świątkiewicz, 2005) in improving calcium use. It is possible to add D_3 or 25-hydroxy vitamin D_3 to the water for reducing rachitic episodes associated with low calcium or malabsorption (Pattison, 2008). Experiments with alternative calcium sources in chicken diets showed no differences in the crude ash content and Ca, P, Zn and Mg concentration in the tibia between groups with and without supplementation of snail and oyster shells (Ajakaiye et al., 2003; Rao et al., 2006; Oso et al., 2011). It was also shown that charcoal used as a Ca source lowered its bioavailability with a concomitant increase in tibial phosphorus content. Thus, a risk of leg pathologies increased (Oso et al., 2011). It should be highlighted that excess Ca concentration in the layer's diet may negatively affect the retention of other essential minerals or reduce phytase efficacy (Pastore et al., 2012; Englmaierova et al., 2014)

Both the structure of the used component and the time of its availability in the digestive tract of birds are significant factors determining optimal use of Ca. Scott et al. (1971) suggested that larger particles of a calcium source prolonged the retention time in the crop and gizzard in contrast to ground forms, thus the availability of this macroelement was prolonged. This fact seems to be even more important because, during 8–9 hours of darkness, when laying hens do not feed, demand for Ca increases due to the formation of the egg shell (Etches, 1987). Moreover, it was shown that larger particles of the calcium-containing feed component had a positive effect on bone quality in laying hens (Rennie et al., 1997; Fleming et al., 1998; Saunders-Blades et al., 2009) which was demonstrated by the increased mechanical resistance and crude ash content in the tibia (Guinotte and Nys, 1991). The physical form of mineral components and diet supplementation with 25-hydroxycholecalciferol can also be conducive to reducing the prevalence of keel bone deformities in laying hens housed in cages with perches (Soares et al., 1995; Abrahamsson et al., 1996; Fleming et al., 1998). However, the role of nutritional factors in the prevention of the mentioned deformities and fractures is limited. This was evidenced by the lack of differences in the crude ash content in the tibia and keel bone in the birds with deformities compared with healthy ones (Fleming et al., 2004). The genetic traits of birds, housing system (cage vs. free range) and perch material (plastic vs. metal) will have a greater influence in respect of this (Fleming et al., 2006; Käppeli et al., 2011).

Plant-derived feed components contain *ca.* 70% of phosphorus in the form of phytic acid unavailable to poultry. Phosphorus excess in the diet is excreted by the kidney, thus having a disadvantageous effect on the metabolism of birds and the natural environment. Phytase hydrolysing phytic acid to ortho-phosphate, *myo*-inositol and phospho-inositol derivatives (Swick and Ivey, 1990) is one of the most commonly used enzymes in the feed industry. There are many scientific reports confirming the significant effect of phytase on proper bone mineralisation in broiler chickens. Pintar et al. (2005) demonstrated that diet supplementation with phytase increased Fe and Mg concentration in the tibia while Yi et al. (1996) noted an enhanced Zn utilisation. In addition, Ca, P, Mg and Zn retention was elevated during feeding 3- and 6-week-old broiler chickens with phytase-supplemented feed (Viveros et al., 2002). On the other hand, Ptak et al. (2013) documented the significance of the kind of exogenous phytase in feed on tibia mineral composition in broiler chickens. It should be remembered that the digestive tract of birds has a limited ability to hydrolyze phytates (Iqbal et al., 1994), which is especially important for *myo*-inositol release. The action of exogenous phytase is specifically stimulated by endogenous microflora and wall enzymes, which was confirmed by different forms of phospho-inositol present in the crop and small intestine of birds. In addition, many studies have suggested a synergistic action of phytase and fibrolytic enzymes, e.g. xylanase or β-glucanase.

Unfortunately, common phytase use in the feeding of non-ruminants can have disadvantageous aspects. This problem seems especially important in the feeding of presently-used hybrid breeds of broiler chickens. In recent years the body mass in this group of animals has increased along with a concomitant decrease in feed consumption per kg of body mass growth. In addition, manufacturers of genetic material recommend the use of lower levels of calcium and phosphorus and assimilable forms of these elements are produced mostly by the activity of exogenous phytase. For the above reasons, both phytase overdose and its losses during granulation can worsen bone mineralisation.

Flock management in the rearing of broiler chickens is another important factor influencing phytase action in the avian digestive system. Exogenous phytase operates principally in the bird's crop in which feed remains from several to several tens of minutes. Therefore, rapid intestinal transit induced, for instance, by lighting programmes used at the farm reduces exposure of phytates to this enzyme, causing lower phytate phosphorus use (Svihus et al., 2010; Svihus et al., 2013).

Apart from the above-mentioned macroelements, microelements also significantly influence bone mineralisation. Fluorine is beneficial for bone density in poultry, contributing to an improvement in bone quality (Lundy et al., 1992; Rennie et al., 1997). Wilson and Ruszler (1998) demonstrated that boron supplement in feed was beneficial for bone strength. On the other hand, too low a copper content in a bird's diet shrank the collagen network structure and reduced the mineralisation intensity (Osphal et al., 1982). Further, aluminium caused growth depression (Huff et al., 1996) and decreased the mechanical strength of bones (Johnson et al., 1992). Świątkiewicz and Koreleski (2008) revealed that Zn and Mn supplementation in an organic form – instead of an inorganic form – to the diet of laying hens did not affect rearing efficiency and bone quality but contributed to alleviation of the negative effect of age of laying hens on the mechanical resistance of the egg shell. On the other hand, a zinc deficit (10 mg/kg) in young fowls had a detrimental effect on bone formation (Wang et al., 2002). In broiler chickens, increasing the zinc level to 100 mg/kg of feed resulted in a significant improvement of bone strength and a reduction of the risk of locomotor disturbances (Štofaníková et al., 2011). The bioavailability of different mineral components changes when they are added in the form bound to either organic or inorganic carriers. The bioavailability increases with the change in the mechanism of the absorption process, namely the transport through the cell membrane by diffusion is much less efficient than the transfer of mineral components bound with an amino acid (Sun et al., 2012; Świątkiewicz et al., 2014). It should also be mentioned that there are specific interactions between macro- and microelements which can result both in their antagonism or cooperation.

Moreover, the role of vitamins are crucial in tibial dyschondroplasia prevention in poultry flocks. Apart from cholecalciferol which was described above, vitamins such as retinol (Vit. A), ascorbic acid (Vit. C), as well as menaquinone (Vit. K) affect the chondrocytes maturation, synthesis of collagen and its cross-links or stimulate calcification process, respectively (Horvath-Papp, 2008). However, the vitamin A overdosing may be the cause of rickets or keel bone deformities.

Feed quality

It is well known that mycotoxins have a negative effect on animals growth performance, reproduction, and health. It was proven that trichothecen toxin (*Fusarium roseum* 'Graminearum') increases tibial dyschondroplasia prevalence (Lee et al., 1985). Furthermore, aflatoxin may interact with Vit. D deficiency and escalate the rickets occurrence in chickens (Hamilton et al., 1974). The study of Huff et al. (1980) confirmed that aflatoxin and ochratoxin have harmful influence on bone properties in scope of decreased mechanical strength of tibia and increased its flexibility. The use of diet contaminated with mycotoxins (aflatoxin, ochratoxin) may enhance appearance of lameness in broiler chicken flocks from 2.3% up to 25% (Okiki et al., 2010). Aflatoxin B_1 experimentally added *in-ovo* impaired embryonic development of the tibial growth plate, thus birds are more vulnerable to legs abnormalities during rearing (Oznurlu et al., 2012). Fumonisin B_1 was considered as an etiological factor of leg deformity and rickets, malabsorption through diarrhoea (reduced efficiency of mineral metabolism), as well as liver and kidney lesions which are involved in cholecalciferol conversion. However, fumonisin by itself is not sufficient to induce leg problems (Wu et al., 1995). Additionally, less common toxins such as fusarochromanone (TDP-1) cause leg deformities as well (Pattison, 2008). It must be highlighted that the wheat and other cereals may be contaminated with more than one mycotoxin. Up to 69% of samples studied by Bryła et al. (2016) contained between 3 and 8 mycotoxins. Thus, the additive or synergistic activity of mycotoxins may enhance the adverse impact on the avian skeletal system.

Dyschondroplasia may be induced by pesticides also. In this case, Rath et al. (2011) noticed the negative role of dithiocarbamates, which are widely used in agriculture as fungicides or pest repellents. It is well known that thiram and disulfiram increase the incidence of tibial dyschondroplasia. Rath et al. (2004) show that even a short posthatch exposure (1–2 d) of birds to thiram causes the enhanced presence of tibial dyschondroplasia. Moreover, Subapriya et al. (2007) found that the negligible levels of thiram (15 ppm) affect the health and growth performance of broilers. Consecutive trial of Rath et al. (2007) emphasised that other pesticides like disulfiram, ferbam, as well as ziram are potentially factors causing tibial dyschondroplasia.

It is well known that imbalanced diet may cause several negative effects from growth depression and health problems to economic losses. As described by Orth et al. (1992), amino acids in the diet, especially sulfur amino acids such as cysteine, cystine, homocysteine may induce tibial dyschondroplasia, except methionine. Andrews et al. (1989) noticed that histidine may cause tibial abnormalities as well.

The use of feed supplements from the aspect of skeletal system building

Antibiotics can either improve or worsen the skeletal system structure in birds. Studies on diet supplementation with virginiamycin (15 ppm) in broiler chickens demonstrated its beneficial effect on Ca and P content in the tibia and in the blood of birds. Further studies revealed that penicillin use had an advantageous effect on the calcium content in bones. However, its action was tightly correlated with vitamin D level in the diet (Ross and Yacowitz, 1954). It was also noted that bambermycin and oxytetracycline supplement elevated Mn concentration in bones (Henry et al., 1987). Avilamycin was efficient in increasing crude ash content in bones with a concomitant improvement in the immunological status of chickens (Chowdhury et al., 2009). However, there are also reports of a negative effect of enrofloxacin, and ciprofloxacin on the development of tendons, cartilage and bones in embryos. Disturbances of bone formation at the egg stage increase mortality in fowls, caused by their inability to successfully hatch (Lemus et al., 2009). It should also be remembered that the use of any type of antibiotic affects the development of the digestive tract microbiome in birds, thus indirectly influencing retention of mineral components in the body (Ziaie et al., 2011). For this reason, in certain cases, e.g. after antibiotic therapy, probiotic microflora supplement to the diet can have a positive effect on the structure and function of the skeletal system. This assumption was confirmed by studies by Mutuş et al. (2006), who indicated a positive effect of *Bacillus licheniformis* and *Bacillus subtilis* in the diet of laying hens on the crude ash content and phosphorus level in the tibia. Studies of Abdelqader et al. (2013) demonstrated that *B. subtilis* increased the mass and density of bones in broiler chickens and elevated the inorganic matter content. Moreover, Nahashon et al. (1994) noted that the addition of probiotic bacteria of the genus *Lactobacillus* could improve calcium and phosphorus use and increase the egg size. *Lactobacillus sporogenes* applied in broiler chicken diet caused an increase in bone inorganic substance and improved the bones' mechanical strength (Panda et al., 2006). The use of *Aspergillus niger* (Fermacto®, PetAg Inc., Hampshire, IL 6014, USA) as a feed supplement in turkey hatchling flocks significantly influenced bone mineralisation parameters and their mechanical strength (Reginatto et al., 2011). The experiment conducted by Houshmand et al. (2010) proved that broiler chicken diet supplementation with probiotic, prebiotic and synbiotic preparations and organic acids can constitute a strategy to increase production, concomitantly alleviating bone problems in chickens. Furthermore, it was noted that the use of beer yeast in chicken nutrition reduced the prevalence of tibial dyschondroplasia and increased the mechanical strength of bones (Plavnik and Scott, 1980). Other authors have noted that *Mitsuokella jalaludinii* (native to the rumen of ruminants) used as a supplement to the diet with low non-phytic phosphorus concentrations increased rearing efficacy and improved bone mineralisation in broiler chickens.

The application of short-chain fatty acids (SCFA) in laying hen nutrition and their combination with medium-chain fatty acids (MCFA) showed a positive effect on mineral retention (Świątkiewicz et al., 2010). These substances enhance Ca and P bioavailability by lowering pH in the upper parts of the digestive tract. Experiments in broiler chickens proved that the use of organic acids was beneficial for the intestinal villus height (Garcia et al., 2007). The increase in Ca use induced by organic acids is underpinned by a reduction of insoluble forms of the calcium phytate complexes and making Ca available in the form of chelates (Boling et al., 2000). Irani et al. (2011) noted the beneficial effect of butyric acid supplement to broiler chicken diet as it increased crude ash content, calcium and phosphorus level; however, statistically significant differences were not achieved. When butyric acid is added to the diet, it should be remembered that 60% of this substance is absorbed in the crop of birds (Bolton and Dewar, 1965). To be able to achieve a greater efficacy of this acid, it should be used in combination with mineral carriers and also estrified by glycerol or used in a microcapsulated form (Irani et al., 2011). At the same time, as suggested by Katono et al. (2008), butyric acid is a stimulator of bone formation by the production of osteoprotegerin (OPG) and bone sialoprotein (BSP). What is more, the use of a mixture of butyric, formic, propionic and lactic acid salts significantly reduced the number of broken eggs which could be related to an increased serum Ca concentration (Soltan, 2008).

Due to rapid intestinal transit in chickens lasting *ca.* 12 h on average (Svihus et al., 2010), the choice of appropriate diet components is a crucial aspect of poultry nutrition. The use of ground herbs does not produce such good effects as the use of their extracts. The experiment of Deng and Hou (2003) involving supplementation of Gushukang (a herb mixture containing *Herba Epimedium*, *Rhizoma Drynariae*, *Rhizoma Atractylodis* and *Radix Astragali*) revealed that it significantly increased the contents of mineral components in the bones of pullets. The supplement of the above preparation to the diet of 55-week-old laying hens significantly improved egg production and reduced the percentage of cracked eggs. Gushukang significantly influenced tibial, fibula and humeral bone mass, bone mass-to-body mass ratio (bone index) and bone density. For the tibia, a positive effect of the preparation on its mechanical strength vs. the control group was evidenced by appropriate measurements (Zhou et al., 2009).

Management

Environmental conditions, both during incubation and production, are crucial for bone system development in poultry. During the prenatal period, the choice of a correct incubation programme in an incubator is the most important issue. However, control of housing conditions during chicken rearing seems to be of key significance for optimal bone quality. The most important managerial factors include: litter quality, the lighting programme, stocking rate, distance between drinking line and feeding line, supplements to drinking water, ventilation, installation of perches in cages for laying hens and vaccination schedule. In addition, a direct contact, sometimes contributing to wing or leg fracture, is an often disregarded but very important component of poultry management programmes.

The knowledge about physiological changes occurring during rearing of various bird species is crucial from the point of view of their management. It should be emphasised that in the case of meat type Japanese quails (*Coturnix coturnix japonica*) tibiotarsal bone density is decreasing in 6-week-old birds (Charuta et al., 2013 a). In the case of 9-week-old turkeys, the lowering density of proximal metaphyses of tibiotarsal bone may cause leg disorders (Charuta et al., 2012 a). The same attenuation may be observed in the 4-week-old broiler chicken stocks (Charuta et. al., 2013 b). Moreover, for Peking ducks (*Anas platyrhynchos* var. *domestica*) the loss of bone mineral content was observed in the period from 4 and 6 weeks of rearing (Charuta and Cooper, 2012). The lowest value of tibia density was noticed in 6-week-old males of growing domestic geese (*Anser domesticus*), which was correlated with deformities and fractures (Charuta et al., 2012b). Above-mentioned data may constitute useful information for preventing leg abnormalities in poultry.

Incubation

The most significant stress factors which affect the developing embryo during incubation include the inappropriate setting of temperature, humidity and ventilation (Meijerhof, 2002; Hulet, 2006). It was noted that an increase in temperature by 1 degree above 37°C and hypoxia (below 19% oxygen) during the final 4 d of incubation impairs the development of bones and collagen type X and increases asymmetry of the skeleton in broiler chickens (Oviedo-Rondón et al., 2008). However, it was also shown that temperature increase from 37.5 do 38.5°C from 4 to 7 d of incubation caused elongation of the tibia and tarsal bone in Leghorn chickens (Hammond et al., 2007). On the other hand, Oviedo-Rondón et al. (2008) noted the longest tibia in broiler chicks incubated at 38°C compared with 36, 37 and 39°C. It is thought that an optimal incubation temperature of 37–38°C (Wilson, 1991) allows for achieving the maximal hatching rate, but future health is rarely taken into consideration (Decuypere and Michels, 1992), especially in terms of bone system development. Increasing of early incubation temperatures may induce the tibial dyschondroplasia due to delayed heat-shock protein 90 (Hsp90) driven chondrocyte differentiation (Yalçin et al., 2007; Genin et al., 2012). However, the studies of Christensen et al. (1994) and French (1994) suggested that temperature requirements differed depending on poultry hybrid and egg size, which hindered the proper setting of incubators and precluded the development of universal solutions. For instance, the Cobb embryo developed faster in the first 4 to 5 d of incubation, in comparison to the Ross which grow more rapidly in the 2nd week (Tona et al., 2010).

Every temperature increase during incubation results in a change in hatching date and hatchling body weight. It was shown that incubation duration was essential for bone formation process in poultry. Prolongation of incubation from 505 to 520 h shortened tibial bone length from 61.04 to 59.25 mm (Shim, 2010). Groves and Muir (2016) observed that tibial dyschondroplasia occurs less frequently in the case of the Cobb 500 broilers hatch after 498 h of incubation. Moreover, it was noted that the time between hatching and setting was another stress factor which could influence chick leg health (Shim and Pesti, 2011). Worthy of note, different incubation systems are used in practice which can significantly affect the prevalence of leg deformities. As demonstrated by Oviedo-Rondón et al. (2009 a), the incubation of embryos in a multistage system can reduce the prevalence of crooked toes and increase the locomotor activity of birds.

The highest bone growth rate during the prenatal period in chicks occurs mostly in the last phase of the incubation and several days after hatching (Church and Johnson, 1964; Applegate and Lilburn, 2002). Due to the fact that currently embryos are characterised by a high metabolism rate (Tona et al., 2004), some nutrients can be deficient during the final incubation days. For instance, in this period, available P, Zn, Cu and Mn reserves are limited (Yair and Uni, 2011). The postnatal period seems to be the next critical time for an incompletely developed bone system due to weak mineralisation, immature digestive system and negligible feed intake in hatchlings (Angel, 2007).

The environment in an incubator appears to be particularly important because it affects the chicken organism for over a half of its life (58%) taking into account 21-d incubation and 36-d rearing.

Transport

Up to now the optimal transport conditions for chickens have not been well established. However, it is known that they can minimise (temperature, ventilation) the first week mortality and contribute to rearing success (Xin and Rieger, 1995; Xin and Harmon, 1996; Joseph and Moran, 2005). At present, it is suggested that even a short-term exposure of animals to stress related to temperature deviation from the optimum during transportation from hatchery to the farm can disturb chicken leg health, especially with regard to development of twisted legs (Oviedo-Rondón et al., 2009 b)

Lighting

The lighting regimen in poultry production is an important factor stimulating reproduction, growth and activity of the animals (Phillips, 1992). It has been proven often that an increase in locomotor activity of birds reduced the risk of bone system defects (McLean et al., 1986). Prayitno and Phillips (1997) demonstrated that red light increased the frequency of pecking of the substrate and other birds (uninjurious) and wing stretching compared to blue and green light. In turkeys, blue light reduced the activity of the animals compared to white, green or red light (Levenick and Leighton, 1988). It was proven that an enhanced light intensity reduced bone pathologies in fowl, like tibial and tarsal bone deformities, talus enlargement and dyschondroplasia (Newberry et al., 1988; Classen et al., 1991). The lighting programme seems to be the most important factor in the management of poultry production. It is commonly known that an intermittent lighting system increases body mass growth in chickens and feed conversion ratio compared to a continuous system (Ogan et al., 1999; Ingram et al., 2000). Due to the fact that *ad libitum* feeding system causes reduction of physical usage of the crop (Kierończyk et al., 2016), as well as longer resting period used for the digestion and assimilation of nutrients. Too long a light phase results in excretion of high protein concentrations in faeces (North and Bell, 1990). On the other hand, a prolonged exposure of birds to darkness reduces the chick growth rate but also decreases the risk of leg pathologies and metabolic diseases (Simmons, 1982; Wilson et al., 1984; Classen and Riddell, 1989). Brickett et al. (2007) noted that a longer resting time (12L:12D) increased the concentration of minerals in bones, as was confirmed by Scott (2002), based on a comparison of the systems 16L:8D and 23L:1D. These chickens were characterised by a higher crude ash content in the toe. Yang et al. (2012) noticed that 4L:4D schedule improve growth performance of broilers, blood (total protein), as well as tibia parameters (bone elastic modulus) in comparison to 2L:2D photoperiod. However, due to the interaction between sex and lighting programme, cockerels, usually having more problems with leg health, will gain more benefits from a proper lighting regimen (Pierson et al., 1981; Classen et al., 1991).

Ambient temperature

Temperature is a significant factor contributing to the increased prevalence of bone and skeleton disorders in chickens. It was repeatedly shown that too high a temperature had a negative effect on feed intake and body mass growth in these animals (Deaton et al., 1978; Charles et al., 1981; Deaton et al., 1984). Therefore, it caused deficits of nutrients in the body which participate in bone building. Concomitantly, thermal conditions can lead to changes in the absorption and retention of mineral components (El Hussieny and Creger, 1981; Wolfenson et al., 1987; Belay et al., 1992). It was proven that thermal stress could reduce bone mass and the bones' mechanical strength (Siegel et al., 1973). The prevalence of leg disorders can also be related to too low a temperature, since, as suggested by Hulan and Proudfoot (1987), a reduction of the blood circulation rate can decrease the absorption of mineral substances.

Moreover, literature data suggests that the presence of potentially pathogenic bacteria in the environment can be associated with temperatures during specific periods of the year. Butterworth and Halsam (2005) noted that the probability of the appearance of *E. coli* and/or Enterococci was the lowest in December, in contrast to June which was characterised by the highest prevalence of these bacteria. Temperature dependence was also observed for the gait score, which was low in March and the highest in September.

Space allowance

An increase in floor space in broiler chicken production increases the activity of the animals. Reiter and Bessei (1995) proved that an intensification of broiler chicken locomotor activity influenced their bone development. Hence, raising the distance between the drinking line and feeding line (Reiter and Bessei, 1996) or construction of barriers (Bizeray et al., 2002) can improve leg health. Interestingly, in the study of Kaukonen et al. (2016) broilers did not use the perches in comparison to platforms which positively contributed to reducing tibial dyschondroplasia occurrence. In addition, it was demonstrated that decreasing the stocking rate made chickens walk longer distances (Lewis and Hurnik, 1990). Škrbić et al. (2009) suggested that the stocking density had a greater effect on tibial quality than the lighting programme. However, it should be noted that it is possible to alleviate the negative impact of overstocking by the use of an intermittent lighting regimen.

Litter

Up to now numerous experiments have been conducted to find the most beneficial substratum for poultry production (Petersen and Jensen, 1983). Wiedmer and Hadorn (1996) suggested that when wood chips were used as a litter material, the birds showed a greater activity than when housed on wheat straw, which could influence leg bone health. However, no effect of litter material, i.e. straw, wood chips or hemp on tibial dyschondroplasia was noted. Poor quality, wet or ammonia-contaminated litter is the main cause of foot-pad dermatitis and hock burn in broiler chickens (Tucker and Walker, 1992). It is thought that in large-scale production, exactly the substratum quality, temperature and humidity are more important for leg problems than overstocking (Dawkins et al., 2004). The studies of Su et al. (2000) confirmed that the use of wood chips efficiently reduced the prevalence of foot-pad dermatitis (FPD). However, a direct link between the development of bone pathologies and FPD has not been established. Nevertheless, bone pathologies were noted to accompany 'shaky leg' syndrome in turkey flocks. This condition could have been caused by the feeling of leg pain indirectly caused by FPD (Laing, 1976; Wise and Ranaweera, 1978; Martland, 1984).

Restrictive feeding

The slow development of the tibial bone in broiler chickens compared with ducks or turkeys increases the risk of biomechanical leg problems in these animals (Lilburn, 1994). Alternative measures aimed at reducing bone deformities in poultry involve a restricted supply of nutrients (Kirn and Firman, 1993) or energy (Toghyani et al., 2011). A reduction of feeding frequency in commercial poultry production has been applied mostly to breeding flocks of broiler chickens. The restrictive feeding regimen during rearing reduces growth rate, thus lowering the probability of leg pathologies (Mench, 2002). The reduction of feed intake to 40% *ad libitum* reduces the tibial length and width without an effect on its mechanical strength (Bruno et al., 2000). Nielsen et al. (2003) showed that the implementation of a period with restricted diet increased the activity of birds, which had a beneficial effect on bone strength and reduced bone disorders (Falcone et al., 2004). The studies of Su et al. (1999) confirmed that a restricted diet could reduce the prevalence of leg defects. However, it should be noted that the use of a diet involving restricted access to feed can have a detrimental effect on chickens, leading to polydipsia and stereotyped behaviour manifested by object pecking (Hocking, 1993; Savory and Maros, 1993; Savory et al., 1996). Fortunately, Sandilands et al. (2005) noted that qualitative diet restriction could reduce its disadvantageous effects on animals. The most common methods used to reduce the intake of energy or nutrients from feed comprise supplementation of appetite suppressants or diet dilution (Pinchasov and Elmaliah, 1995; Savory et al., 1996).

Infectious factors contributing to leg disorders

Infectious agents, most often of a bacterial origin, are a separate problem affecting the growth and development of the bone and skeletal system (Table 1). They can significantly impair motor performance of broiler chickens, their health and consequently selection at the farm. Bacterial infections of the bone and skeletal system have been observed in the USA, Canada and Europe for many years. Many microorganisms can cause bone and skeletal system dysfunctions in poultry. The most important of these are *Enterococcus* sp. (Kense and Landman, 2011), *Staphylococcus aureus* (McNamee et al., 1998), *Salmonella* spp. (Padron, 1990) and *Escherichia coli* (Dinev, 2009). The etiology of infection-based lameness has not been fully elucidated. However, numerous literature data indicates that chickens contract infection with microorganisms responsible for chondronecrosis through the respiratory and digestive system, and the infection spreads by way of the bloodstream (Diagram 2).

Table 1. Infectious and non-infectious skeletal system disorders of meat-type and layer-type fowl. Based on Pattison (2008)

Item	Skeletal disease	Aetiological factor
1	2	3
Meat-type fowl		
infectious	Arthritis, tenosynovitis	*Staphylococcus aureus, Staphylococcus epidermidis*, aviadenoviruses, reoviruses
	Bacterial chondronecrosis with osteomyelitis	*Staphylococcus aureus, Staphylococcus hyicus, E. coli, Enterococcus cecorum*
	Femoral head necrosis	Osteomyelitis, *Staphylococcus* sp., Gumboro virus, Rickets, dyslipidemia, physical injuries
	Spinal osteomyelitis	*Staphylococcus* sp.; *Enterococcus caecorum*
	Hock joints	*Staphylococcus* sp., stress e.g. coccidiosis, lack of perches
	Mycoplasma	*Mycoplasma synoviae*
non-infectious	Spondylopathies	Displacement of the fourth thoracic vertebra which may compress the spinal cord
	Rotational and angular deformity	Low mineralisation, insufficient physical activity
	Rickets	Deficiency of Ca or P with insufficient Vit. D
	Dyschondroplasia	Low Ca:P ratio, metabolic acidosis (electrolyte imbalance in feed), high level of chloride in feed, Cu deficiency, excess dietary cysteine or homocysteine, mycotoxicosis, pesticides

Table 1 – contd.

1	2	3
	Chondrodystrophy	Deficiency of Mn, choline, niacin, Vit. E, biotin, folic acid, pyridoxine
	Fracture	Mechanical trauma, simultaneously with osteodystrophies, dyschondroplasia, mycotoxicosis, osteomyelitis, Vit. C deficiency, aluminium excess, bone marrow lymphomas
	Spiral fracture	Overweight, Ca deficiency or its short retention time in the gizzard
	Foot-pad dermatitis	Litter condition, methionine, biotin deficiency, protein digestibility, high unsaturated fats, diarrhoea, litter management
	Degenerative joint disease	Improper handling, *Mycoplasma*, inflammatory arthropathy
	Osteochondrosis	Pathology of the trochanter and antitrochanter
	Deep pectoral myopathy; Nonspecific lameness	Mycotoxins, deficiency in antioxidants, ionophore toxicity, Infectious Bronchitis Virus (IBV)
Layer-type fowl		
non-infectious	Osteoporosis	Decrease in mineralised structureal bone (Ca mobilisation in eggshell formation)
	Osteopenia	Consequence of osteoporosis, Deficiency of Ca and/or P
infectious	Osteopetrosis	*Retroviridae*, avian leukosis/sarcoma virus, high level of alkaline phosphatase in serum
	Amyloidosis	*Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, Enterococcus faecalis*

The studies of Wideman et al. (2012) threw a new light on this problem due to the application of a special experimental model that allowed for the simulation of lameness in laboratory conditions. Thus, it was possible to test solutions aimed at alleviating these diseases. The hallmark of this model is that birds are not experimentally infected, as in experiments of a 'challenge' type in which the birds are infected with known pathogens inducing leg diseases. In the studies of Wideman et al. (2012), the animals were housed in pens measuring 3.7 × 2.5 × 2.5 m (which did not restrict their movements), equipped with constant ventilation (6 m^3/min). Drinkers and feeders were placed on opposite sides so that the birds had to always move when they consumed feed or drank water. In this model, bone system dysfunctions were caused by keeping animals in cages with a slatted floor which much more frequently induces disturbances in the structure and development of the leg bones. It is caused by chronic joint loading which leads to microinjuries of cartilages, making them a good medium for development of potentially pathogenic microorganisms. In the model under discussion, wire panels were used in order to obtain the above effect. Wideman et al. (2012) noted that the use of different types of floor significantly contributed to the development of lameness. Diet supplementation with probiotic bacteria was beneficial for the motor performance of broiler chickens. A positive effect was also observed when only one type of floor, i.e. a slatted floor was used. It is possible that the improvement of the bone and skeletal system development in birds after probiotic supplementation was achieved due to secretion of antibacterial peptides of ribosomal origin, i.e. bacteriocins by these microorganisms. *Staphylococcus aureus*, causing necrosis of the head of the femur, was susceptible to bacteriocin synthesised by *Staphylococcus epidermidis*. In addition, experiments involving spraying an aerosol containing *S. epidemidis* reduced the number of *Staphylococcus aureus* bacteria and decreased the frequency of lameness in turkeys and broiler chickens (Nicoll and Jensen, 1987 a, b).

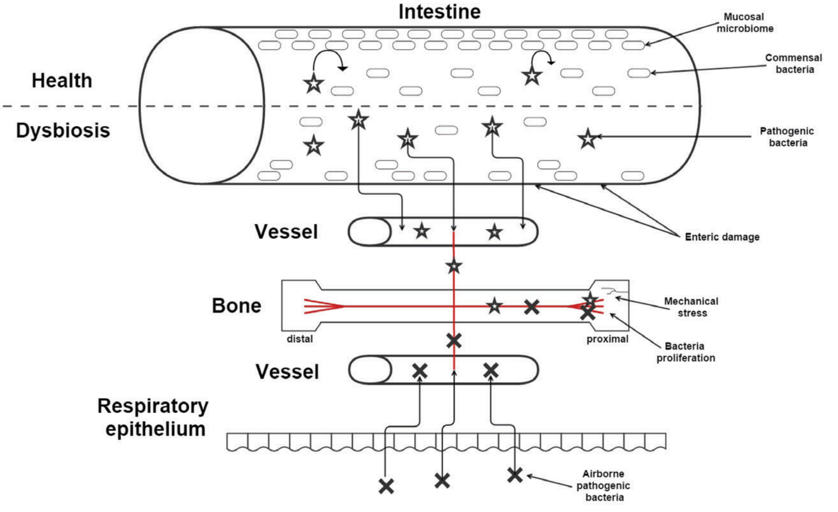

Diagram 2. Infectious pathways contributing to bone abnormalities. Based on Mutalib et al. (1983), Wideman et al. (2012) and Pastorelli et al. (2013)

Interestingly, it is possible to observe no signs of discomfort, lameness or leg weakness during rearing of birds which have severe lesions. It may be explained that birds have an ability to mask symptoms of distress due to avoiding aggressive behaviour of flock mates (Wideman et al., 2014).

The bone and skeletal system disturbances in broiler chickens are caused not only by pathogenic bacteria but also by viruses. As suggested by Van der Heide et al. (1981), infection with reovirus (Connecticut strain S1133 avian reovirus) and, indirectly, with reovirus-induced enteritis can lead to impaired absorption of nutrients in the digestive tract, which may contribute to osteoporosis. Moreover, the reovirus isolated from the alimentary tract of broiler chickens with diarrhoea symptoms can induce lesions of tenosynovitis and, in turn, femoral head necrosis and brittle bone disease.

Immunosuppressant viruses are often used to develop experimental conditions for studies on virulence of e.g. *Staphylococcus aureus* causing bone diseases in poultry. This model is based on the hypothesis that the incidence of bacterial chondronecrosis with osteomyelitis (BCO) is much higher when the bird's organism is exposed to viruses (Thorp et al., 1993). However, McNamee et al. (1999) suggested that in spite of the fact that adenovirus and reovirus were isolated from bone material, their presence was not directly related to leg defects. Nevertheless, in the infection model with *Staphylococcus hyicus*, (CAV) and (IBDV) significantly increased (from 9.1 to 23.1%) the incidence of BCO in chickens (McNamee, 1998). Moreover, Butterworth (1999) also distinguished the Laryngotracheitis virus, Pox viruses and Marek's disease as the agents potentially able to significantly contribute to an increased BCN frequency. Rosenberger and Olson (1991) reported that 4- to 7-week-old chickens did not show considerable virus-induced mortality (<5%) but revealed significant morbidity. Unequivocal literature data indicates that further studies of this problem are required. However, from a practical perspective the use of vaccines against one pathogen, e.g. *S. aureus* may prove inefficient because, as suggested by McNamee and Smyth (2000), an efficient control and prevention strategy should be based on limiting the role of different potentially pathogenic bacteria and immunosuppressant viruses.

Summary

There are many factors in the rearing of broiler chickens that influence the development and function of the bone and skeletal system. A properly balanced diet is only one of them. Undeniably care for the welfare of birds and appropriate hygienic conditions seems to be the key issue for intensive growth rate of bone tissue. For the above reasons it should be emphasised that when motor dysfunctions in broiler chickens have been noticed, a correct diagnostics is of crucial significance because only when the factor responsible for worsening of bone and skeletal system function is identified can the problem be efficiently resolved.

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Received: 3 I 2017
Accepted: 2 III 2017