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Bem-estar de poedeiras e a osteoporose

Welfare of laying hens and osteoporosis

Bien estar de gallinas ponedoras y la osteoporosis

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Resumo

O objetivo desse trabalho foi revisar os aspectos relacionados com o comportamento natural das aves, o estresse sofrido quando submetidas ao confinamento, a importância do estudo da densidade mineral óssea e o entendimento das causas da osteoporose e medidas cabíveis a serem adotadas para atenuar a severidade desta doença e contribuir para o bem-estar animal. Portanto, vê-se a importância de pesquisas na área de mineralização óssea, adaptações ao ambiente e dietas com níveis adequados cálcio, vitamina D e fósforo para manutenção da qualidade do tecido ósseo das aves.

Palavras-chave: Comportamento; Densitometria óssea; Produção avícola; Estresse.

Abstract

The objective of this review aspects concerned with the natural behavior of birds, the stress suffered when subjected to confinement, the importance of the study of the mineral bone density and the understanding of the causes of osteoporosis and appropriate measures to be taken to mitigate the severity of this disease and contribute to animal welfare. Therefore, the importance of research in the area of bone mineralization, adaptations to the environment and diets with adequate levels of calcium, vitamin D and phosphorus for maintenance of the quality of the bone tissue of birds is seen.

Keywords: Behavior; Bone densitometry; Poultry production; Stress.

Resumen

El objetivo de esta revisión bibliográfica fue revisar aspectos relacionados con el comportamiento natural de las aves, el estrés sufrido al estar encerrados, la importancia del estudio de la densidad mineral ósea y la comprensión de las causas de la osteoporosis y las medidas apropiadas para tomar mitigar la gravedad de esta enfermedad y contribuir al bienestar animal. Por lo tanto, se ve la importancia de la investigación en el área de mineralización ósea, adaptaciones al medio ambiente y dietas con niveles adecuados de calcio, vitamina D y fósforo para el mantenimiento de la calidad del tejido óseo de las aves.

Palabras clave: Comportamiento; Densitometría óssea; Producción avícola; Estrés.

1. Introduction

The Brazilian egg production chain has high technology, conquered by the knowledge and advancement of genetics with the use of high-yielding strains, nutrition, health and ambience and management with the use of automated poultry farms in cage battery systems leading poultry production to industrial levels (Vieira, 2014).

The breeding of laying birds in battery cages known as the conventional system is the most widespread in Brazil and has become one of the most discussed topics in animal production today and one of the greatest controversies about bird welfare due to the growing conviction of consumers that food-producing animals should be treated well (Praes et al. 2012).

The technological evolution of poultry farming has resulted in several disputes about animal welfare, which are related, among others, to the reduced space offered; and the lack of management with environmental enrichment in the conventional system that make it impossible or limit the set of activities regarded as important for birds, besides the management practices employed as high density, forced molting, pecking, among others, have been challenged or even banned in several countries (Mazzuco, 2008; Cabrelon, 2016).

With the exception of Europe, where legislation already exists prohibiting the use of unenriched cages since January 2012 (COUNCIL DIRECTIVE, 1999; IACA, 2013), in a battery cage system, known as conventional, it is predominant in the housing of laying birds (Tauson, 2005). The Brazilian Institute of Geography and Statistics (IBGE) only takes into account the numbers of egg production of hen farms with at least 10,000 laying birds, i.e. many small producers are out of these statistics **(**Amaral et al., 2016). In 2018, approximately 115.6 million laying chicks were housed in Brazil (Ovo online, 2018), and production in this year was a historical record of 3.6 billion of dozens of hen eggs (IBGE, 2018).

The search for the implementation of new breeding systems which aim at the welfare of birds has gained worldwide prominence and more recently in the national poultry sector, seeking to induce them to satisfactory conditions of quality of life, providing behavior near-close to the natural one within the facilities and with a high yield (Oliveira et al., 2014).

Thus, this research aims to show the scenario of the poultry industry and the desire for profitability to the detriment of welfare of layers, due to the high stocking densities and the impossibility of the expression of the natural behavior of birds, resulting in stress and bone fragility, focusing on osteoporosis, with a fall in performance and weakness of the birds.

2. Methodology

This is a literature review article quantitative (Pereira et al., 2018), highlighting the serious problem that occurs in the production of laying hens, which are the bone problems found, especially because these birds remained longer in the production system, with the extended cycle. The way animals are raised and animal welfare have been widely discussed in the scientific community and the consumer market. Therefore, this article would address aspects of the production system, its interaction with the bone quality of animals and the main parameters of bone quality to be known.

The more importants government documents, books, theses, dissertations, scientific articles in research bases such as Google Scholar and Scielo were analyzed.

3. Results and discussions

3.1 Animal Welfare

In 1964, the Englishwoman Ruth Harrison played a pivotal role at the beginning of the modern animal welfare movement by publishing “Animal Machines: The New Farming Factory Industry” (Harison, 1964)**.** The author criticized intensive animal husbandry practices, particularly the use of industrial chicken cages, calf cages and the large-scale production of broiler chicken, which had become increasingly common after World War II. There was an intense public repercussion, sensitizing the British Government, to the establishment of a group appointed by the British Ministry of Agriculture to examine animal welfare issues. The committee, chaired by Professor Roger Brambell, presented the 85-page Report of the Technical Committee on the Welfare of Animals Kept Under Intensive Livestock Husbandry Systems, “The Brambell Report” (Command paper 2836, 1965).

The official concept of Animal Welfare was then first cited by the Brambell Committee in 1965 to assess the conditions under which animals were kept in the intensive production system in that country. According to the committee, Animal Welfare is an encompassing term that concerns both physical and mental well-being. Therefore, any attempt to assess the welfare of an animal should consider physical (physiological) as mental (behavioral) aspects (The official concept of Animal Welfare was then first cited by the Brambell Committee in 1965 to assess the conditions under which animals were kept in the intensive production system in that country. According to the committee, Animal Welfare is a broad term that concerns both physical and mental well-being. Therefore, any attempt to assess the welfare of an animal should consider physical (physiological) as mental (behavioral) aspects (Command paper 2836, 1965).

The Brambell Commission report was often cited in terms of freedom of movement, motion and mental welfare of animals, but many other aspects of welfare discussed in the report were often overlooked (Gonyou, 1994). The group gave rise to the creation, under the British Government, of the Farm Animal Welfare Advisory Committee, later replaced by the Farm Animal Welfare Council (FAWC, 1993), perhaps to correct an imbalance in the report of the suggestions of the Brambell Committee.

The Five Freedoms were created to allow the qualitative assessment of the physical, mental and natural aspects of well-being (Brambell Committee, 1965; FAWC, 2009), where the animal must be free of pain, injury and disease; free of discomfort (environmental stress); free from hunger, thirst and malnutrition; free from fear and anguish (mental stress); and free to express its natural behavior. A concept that complements the five freedoms, first published in 1993 and later mentioned and discussed in 2009 (FAWC, 2009). Later, another concept of animal welfare was presented by Broom (2014), who defined it as the state of the individual in relation to its attempts to adapt itself to its environment, and Siegel (1989) related animal welfare to the physiological and behavioral harmony that the animal experiences.

Welfare aims to study, identify and recognize the basic needs of animals, with a view to their measurement and applicability (Keeling et al., 2011) and its definition has become a topic of worldwide interest, which includes the care of the human beings towards animals and production of better quality products, in addition to the tangible benefits to ensure animal welfare (Cabrelon, 2016).

In practical terms, the BEA establishes a scale that includes the physical, mental health, behavior of animals and their social interactions and their adaptation to the environment and that ranges from very good to very bad, where the physical, physiological, psychological, behavioral needs, social and environmental aspects of an animal are (Grandin and Johnson, 2010).

As a result of the extensive differences to the physiological and behavioral responses to the problems, it is necessary that any well-being assessment includes a wide range of measurements. As an instance, the physiological measures, or the existence of neuromas (Gentle et al., 1997). The Welfare Quality Project was created in 2004 to improve knowledge of the forms of associations between the different variables and their consequences in relation to the severity of the problem and it identifies four principles for the evaluation of the welfare of farm animals, with twelve independent criteria.

According to Grandin (2015), the recognition of pain in animals can be categorized into serious problems, with situations that generate suffering of the animal, such as handling, transport and high density. Once the problem is identified, it must be corrected immediately. The recognition of the pain or suffering of the animal is easily perceived when the biology and behavior of the species is known (Viñuela-Fernández et al., 2011).

There are many welfare indicators, which vary according to the species studied (Zanella, 1995). Increased heart rate, adrenal activity, adrenal activity after challenge with adrenocorticotropic hormone (ACTH), or reduced immune response after a challenge may indicate that well-being is more reduced than in individuals who do not show such changes. Results of physiological measurements should be interpreted with caution, as should those of other measurements (Bright et al., 2007).

The diagnosis of behavioral abnormalities (etiopathies, psychopathies, sociopathies), one of the most studied being stereotypical conduct, self-mutilation, feather pecking on birds or excessively aggressive behavior indicate that the animal is under conditions of poor welfare (Fraser, 2009). A specific example of the effect of facilities which leads to poor welfare is the consequence of the severe reduction of the possibility of exercise. The literature reports that hens, turkeys and quails are pre-disposed to aggressivity reactions such as pecking and cannibalism, and such behaviors become more evident when birds are housed at high population density (Moraes, 2018).

The answers to the disputes are that they need definitions, solutions and scientific approaches, since there is a positive correlation between animal welfare and yield. (Mazzuco, 2006a; UBA, 2008).

3.2 Adaptation for flight X Confinement

The skeleton of birds has two striking characteristics regarding flight adaptation, lightness and strength (Feduccia, 1986). Lightness occurs through the process of pneumatization of the bones by the extensions of the air sacs (Feduccia, 1986). The air sacs are extensions of the bronchial system and occupy the coelomic cavity in particular, along with the abdominal and thoracic viscera. The degree of pneumatization is directly related to the effectiveness of the flight of birds, having greater advance in those with higher flight capacity (Dyce et al., 1997).

As far as resistance is concerned, this occurs by the fusion and, most of the times, elimination of bone tissue with reduced cortical thickness and less amount of spongy tissue relative to mammals. The fusion is visible in the pelvic cingulate and in the cranial bones. The pelvic limb and distal bones of the wing present integrated fusion and elimination (Feduccia, 1986). Added to this, the existence of high mineral content (Dyce et al., 1997), enables the aspect of hardness to the bones, thus contributing to increase their strength (Sanni, 2017).

Much of the normal behavior of birds is hindered and made impossible by confinement. Mating, hatching and caring behavior are prevented, keeping only egg-laying activity (Singer, 1991).

Layers have needs that derive from their basic biological functioning, such as search or nesting; obtaining nutrients and water; interaction with other birds; its growth and its maintenance; exploitation and response to signs of potential danger; ability to minimize an event of pain, frustration and fear; cleaning and dusting behavior in the sand; display of food-seeking movements and possess satisfactory exercise. And such needs are met by means of the carrying out of different activities, of the maintenance of certain physiological states, and of the response to stimuli (Broom, 2001).

The consequences of the lack of exercise for prolonged period fall upon the muscles and bones. When birds are coming from battery-caged systems, they present increased brittleness and lesser bone weight, compared to birds with greater possibility of movement (Meyer and Sunde, 1974), noticing higher number of birds with lameness when housed in cages, Kraus (1978), and significantly higher degree of osteoporosis and osteomalacia in birds housed in cages than in group-dwelling or free-range birds (Loliger, et al. 1981). Fractures occur most often in hens housed in cages with limited space for movement and motion (Mazzuco, 2005). According to the author, a type of osteoporosis occurs that develops due to the absence of the muscle strength exerted on the bones, leading to reduced bone formation and maintenance and decreased bone mass.

Fractured bones in the process of removing birds destined to slaughter **are** evidence of poor animal welfare. Simonsen (1983) found the incidence of bone fractures in the wings upon arrival at the slaughterhouse of 0.5% for birds produced in systems with freedom of movement and motion while those housed in cages increased to 6.5%. Gregory and Wilkins (1989) dissected 3,115 layers housed in battery cage systems that were slaughtered at the end of the production cycle in the UK and found that around 29% had fractured bones prior to arrival at the electrical waterbath stunning at the slaughterhouse. The points that presented the greatest damage were the removal of the animals from inside the cages and the hanging on the overhead poultry conveyor. According to Knowles and Broom (1990), birds housed in battery cages are less active than those that live in terrace system or in a shed com perch and sufficient forces to fracture the humerus and tibia are lower for cage birds.

Calcium and vitamin D are fundamental factors for bone development in the layer diet. However, despite the nutritional balance of the diet, the lesser activity in the battery cage system increases the possibility of occurrence of fractures. In addition to the diet, genetics also determine the basic morphology of the skeleton, but the final mass and bone architecture are modulated by adaptive mechanisms sensitive to mechanical factors (Forwood, 2014). In several studies, 25 to 40% of the battery-caged birds in the final laying phase showed at least one fractured bone after management prior to stunning and 98% of the carcasses had a fractured bone (Gregory and Wilkins, 1989; Gregory et al., 1990, 1991). In the skeleton, the clavicle, wings and pubic bones are the most affected (Clark et al., 2008). The number of fractures has been reduced in perched sheds and poultry houses, although bone fractures occur in overcrowded or improperly designed sheds. In situations with insufficient opportunity for the birds to exercise**,** bone strength in the legs and wings was reduced. When in cages that made it impossible for the birds to flap their wings, their bones held only half the strength when compared to birds housed in perched sheds, which could flap their wings (Knowles and Broom, 1990; Norgaard-Nielsen, 1990).

3.3 Stress and suffering of birds

Animals need to meet their physiological, behavioral and psychological needs to survive in an environment that is constantly changing. Friend (1991) reported that animals are subject to a wide variety of stressing factors, exogenous or endogenous, and the ability to deal with acute and chronic stress and the capacity of interacting and responding to these changes is what enables adaptation and survival.

Therefore, stress is a physiological response of the organism caused by the alteration of homeostasis, which seeks to provide the body with subsidies to act and adapt to these changes. If there is a prolongation of the stressful process, there will be disorders in the body, resulting in production, reproductive, behavioral and psychic changes. The occurrence of production disorders in domestic animals, or due to stressors, favors decreased production, reproductive problems, behavioral disorders and significant physiological changes. Therefore, before any behavioral, autonomic or neuroendocrine biological measure can be used to measure stress, it must be established that the change caused by stress in that measure has a significant correlation in well-being (Kjaer et al, 2011).

Birds may feel the variations of the environment they live in, being induced to the state of stress, with subsequent blood release of adrenocorticosteroid hormones and changes in the leukocyte cell numbers (Sturkie, 1986). In this sense, its environment should be considered as one of the main success points in breeding, because during stress, especially by heat, physiological (electrolyte imbalance), hormonal (costicosterone, T3 and T4) and molecular misfits that weaken birds, impairing performance and making them susceptible to opportunistic diseases (Quinteiro-Filho, 2008).

Research results have reported the consequences of changes in poultry physiology due to stress from various sources, such as decreased immune response and productive performance as laying rate, weight loss, reduced feed intake. Primary manifestations that may or not progress to disease and, consequently, death (Mazzuco, 2006b).

Adrenal hypertrophy, increased corticosterone in the circulation, immunosuppression are stress indices (Siegel, 1995). Cortisol levels in mammals or corticosterone in birds and rodents can be monitored by means of the plasma, saliva, feces, hair/feathers or urine. Some (authors consider glucocorticoid levels as indicators of AWF. This hormone is released by the adrenal cortex and becomes multifunctional in both comfort and stress states of birds. Changes in behavior, metabolic patterns, endocrine and immune functions are triggered by corticosterone, which ensures the physiological balance (homeostasis) compared to several situations (Cheng and Muir, 2004).

Blood corticosterone levels of birds kept on floor proved high, or low, or even without significant difference when compared to levels of birds kept in cages. In another study in which a group of birds was kept in conventional cages or on floor with the same densities of 7.430 cm^2/bird, the results showed similar blood corticosterone concentration (Koelkebeck and Cain, 1984).

3.4 Densidade or stocking rate in cages

In commercial farms, in general, laying birds are kept in cages in limited space at the various production phases. In view of this, several studies have identified welfare problems in birds kept in this breeding system, standing out that population density as the number of birds per cage increases, the performance of layers is harmed, caused by the competition pressure for space. (Moinard, 1998). According to Struwe et al. (1992), high densities may result in an unfavorable environment for bird welfare and may even become a stressful factor.

The European Union has approved, within the CEC Directive (1999), the banning of conventional cages from the production system since 2012, allowing no investment in this type of cage since 2003 (Appleby, 2003), recommending that birds housed in enriched cage systems, at least 750 cm^2 area/bird with 600 cm^2 the minimum usable area, available in the same type of cage (Mazzuco, 2008).

In Asia and the United States, densities of around 400 cm^2 per bird are used and in Brazil, it lies in the range of 350 to 450 cm^2 per bird. In some egg-producing units, it is possible to find densities below this (Vieira et al., 2014).

The space below 350 cm^2 per bird in cages has a negative impact on laying, in addition to increasing flock mortality (Garcia et al., 2015), but commercial egg producers, aiming to increase net profit, exploit the maximum capacity of the production systems. Thus, they tend to increase the number of birds per cage; based on the belief that increased egg yield per cage maximizes profit and compensates for the negative effects of high density (Rocha et al., 2008).

The recommendation made by the Brazilian Poultry Union for bird densities in cages is 375 cm^2/bird (white) and 450 cm^2/bird (red) basing on a cage measuring: 45 cm x 50 cm = 2250 cm^2 (UBA, 2008).

Several studies have shown that the increase of the cage density and reduction of the feeder area caused fall in the performance of laying hens and significant decline in egg /bird/ day yield, egg weight, feed intake and increase in bird mortality (Rocha et al., 2008).

Anderson et al. (1992) evidenced losses in the performance of laying hens, when the space was reduced from 482 to 361 cm^2 per bird and Silva et al. (2006), observed that the housing of light laying hens at density of 375 cm^2 affected egg yield, suggesting that severe space restriction should be avoided, as the aggression mortality was 11%. Contrary to these results, Garcia (2003) found no impairment in weight gain and live weight with increasing density of semi-heavy laying hens during cria and recria and white layers (Garcia et al, 1993). Similarly, Pavan et al. (2005) did not find negative effects of the stocking rate of 375 cm^2 to 563 cm^2/bird in the cage on egg yield, egg weight and feed intake.

In an extensive review on the subject, Lay Jr (2011) e Vieira et al. (2014) concluded that one of the most important issues during the last decades is the need to improve the housing conditions of laying hens, however, as can be observed in the works mentioned by the authors, that none of the systems is the ideal for welfare, aspects of egg yield production and quality together and should therefore be investigated.

3.5 Bone condition of birds housed on floor and in cages

The diseases that confined animals may present in their locomotor system are of paramount economic importance for animal production, resulting in worsened performance and welfare (Almeida Paz et al., 2009).

The floor of the cages for being of wire can cause by deformities in the feet, feathering problems and bone fragility (Tauson, 2005).

Normally, birds raised on floor exhibit better muscle and bone strength. However, Michel and Huonnic (2003) stated that breeding in this system can aggravate such a condition, in addition to the rates of muscle injury are higher in this system, as well as that the mortality rate having been higher on floor than in cages.

Andrews et al (1990) compared plastic-wrapped wire floors presenting 1.5 x 1.7 cm openings. They made use of the combination 2/3 of this floor plus 1/3 of bed and the same combination with wooden slatted flooring. The results showed that the yield of poultry kept on bed floor was relatively higher when compared to the other different types of flooring. Birds housed in the other systems presented fertility, hatchability and mortality rates similar to the litter system; however no foot injury was detected in all systems.

The countries of the European Union have focused on developing cage-free commercial laying systems, called alternative systems. These own a more complex system where birds are raised on the ground in large groups and with a minimum space of 1111 cm^2/bird or 9 birds/m^2 (Paixão, 2005). Laying hens have access to nests, perches or slats at various heights from the ground, area with bedding for sand-bath and can still present an open part with access to pasture. For contributing toward the increased motion and movement of birds, such systems improve the strength of their bones, but due to greater exercise opportunity, raise the occurrence of fractures, resulting from accidents such as perch fall when pushed by the other birds or beats during flight (Whitehead and Fleming, 2000).

Nicol (1990) with the purpose of improving the housing conditions of laying hens, introduced perches, nests and recreation area (sand), with system of large cages, housing up to 60 birds, this system has been known as the "get-away". Currently, the cages have a volume of $1m^3$, housing between 15 to 40 birds, overlapping on two floors. The perches are arranged on two levels, so that birds do not defecate over each other, allowing for greater movement and flapping with greater frequency. Such behavior strengthens the tibial and wing bones by at least 15% and improves the skeleton. However, the mortality rate and egg yield are lower compared to the conventional system, probably justifying the larger number of birds. On the other hand nails, wear out naturally by means of the act of scratching on sand through as well as the feathers, largely meeting the wishes of the ethologists. (Tauson, 1998).

Experiments were conducted in order to evaluate the higher demand of physical activity on bone tissue (Bizeray et al., 2000), as for instance, Fleming et al. (1994) noted that cage-raised layers presented lower values of mineral density when compared to those raised on the bed, indicating that the environment may influence the quality of the bone tissue of animals.

3.6 Study of the mineral bone density

For the evaluation of mineral bone density, the animal restraining becomes necessary, which can be manual, indicated for quiet birds (Williams Walsh, 2002). However, it presents as a disadvantage the increase of the exposure to the radiation of the personnel involved (Lavin, 1994). Physical restraining occurs through the use of tools such as ropes and adhesive tapes. Manual and physical restraining methods are not indicated for excited birds, due to the likely occurrence of injuries to them. Thus, injectable sedatives and inhaled anesthetics are used, being considered safer than radiographic examinations (Lavin, 1994).

Historically, changes in bone integrity have been measured by using invasive techniques such as biomechanical tests for break strength, mineral analysis of ashes, bone ashes and histomorphometry (Mazzuco, 2005).

The methodologies used for the analysis of mineral bone density are: radiographic photometry, radiographic photodensitometry, ultrasonography, neutron activation analysis, computed tomography, direct photon absorption-SPA and dual energy radiographic absorptiometry (DXA) and optical densitometry in radiography (Grier et al., 1996).

To quantify bone mass, both invasive and noninvasive methods can be used. Among the Invasive ones are microradiography, histomorphometry, ash analysis, atomic emission spectroscopy, and “back-scatter” electron microscopy. Such techniques do not allow the sequential evaluation of the bone mineral density of the same individual, besides being necessary to euthanize the animals in significant quantity for the conduction of the experiment (Markel et al., 1994).

On the other hand, the non-invasive methods are divided into non-radiological and radiological methods. Non-radiological methods refer to ultrasound and magnetic resonance techniques (Chilvarquer, 2000). The radiological ones include single energy photon absorptiometry (SPA), dual energy photon absorptiometry (DPA), dual energy x-ray absorptiometry (DXA), quantitative computed tomography (QCT) and radiographic optical densitometry. This last technique has lower cost than the others and presents greater accessibility. (Louzada et al., 1997; Mautalen & Oliveri, 1999).

3.7 Osteoporosis

Poultry osteoporosis has been recognized as a multifactorial metabolic disease in commercial poultry for over 45 years. Laying hens are susceptible to osteoporosis due to the high laying rates maintained during an egg production cycle. Modern laying hen strains produce more eggs than their ancestors, in addition to low feed intake due to reduced appetite, which resulted in increased utilization of calcium stored in bone for deposition in the shell. (Mazzuco, 2006b).

Genetics, the environment and nutrition have additional and independent effects on the condition of the bones of layers suffering from osteoporosis. Genetics is the most effective component, followed by the environment and subsequently nutrition (Fleming et al., 2006). Despite osteoporosis having no prevention as to diet, the good quality of the diet can reduce disease severity (Fleming et al., 1998). However, the timing of the dietary intervention becomes critical with the increase in the rates correct of vitamin D, calcium and phosphorus during the laying period, as it extends the peak of bone quality before increasing bone resorption in the laying phase, in which a high number of active osteoclasts is directed towards calcium resorption and mobilization from trabecular, medullary and cortical bones (Fleming, 2008). Osteoporosis is characterized by bone fragility, since, despite bone mineralization looking normal, the bone matrix is restricted and the trabeculae are often thin due to the imbalance between bone formation and bone resorption (Knowles & Wilkins, 1998).

Conventional cages represent a concern for well-being because limited space restricts bird movement and activity, preventing them from presenting normal behavior, causing stress and contributing to the occurrence of osteoporosis. (Whitehead & Fleming, 2000, Vits et al., 2005).

3.8 Alternatives for the maintenance of bone tissue

Vitamin D is essential for shell formation, maintenance of egg production and calcium homeostasis. Cholecalciferol, a form of vitamin D, is produced in the body and displaced to the outer layer of the skin. When absorbed, it is transported to the liver and gives rise to metabolite 25-hydroxycholecalciferol (Leeson & Summers, 2001; Pesti et al., 2005). Soares et al. (1995) found that 25-hydroxycolecalciferol metabolite is 2.5 to 4.5 times as active as cholecalciferol, being important for preventing bone problems and eggshell thickness. 25-hydroxycholecalciferol may be up to 200 times more effective in intestinal calcium absorption than cholecalciferol (Leeson & Summers, 2001). The recommended levels for laying are around 300 to 2,500 IU/kg of feed (Rostagno et al., 2005). With regard to vitamin C, supplementation in diets has been found to improve the condition of leg bones in birds under stress (Leeson & Summers, 2001).

Other nutritional strategies are important aiming at the maintaining the health of the bone tissue, among them a very important mineral is calcium. About 99% of the total of this mineral that the chicken possess, is located in the bones and have a direct participation in the quality of the bone tissue and shell of the eggs produced (Olgun & Aygun, 2016). Nascimento et al. (2014) found that bone strength increased linearly when Ca levels were increased (from 2.85% to 5.28%) in layer diets (80 weeks old), not only the amount of calcium supplied in the diet is important, but also the source and particle size of the limestone. Oliveira (2012) using 240 Hisex White laying hens at 82 weeks of age and in the second laying cycle, distributed in a randomized design in a 5x2 factorial scheme, resulting in 10 treatments with four replications of six birds. The following factors were investigated: two types of lighting, with and without artificial light and five inclusions of coarse limestone in proportion to the fine limestone of the diet (0, 25, 50, 75 and 100%). At the end of the experiment, the birds were euthanized and the tibias were used for bone quality analysis. In the data analysis, there was no significant effect ($p > 0.05$) of the coarse limestone levels, of the use of artificial light or of the interaction between these factors on bone mineral density measured by the Seedor index and mineral amount in the tibia. However, break strength of the tibia and deformity had a significant influence due to the change in the particle size of the calcium source of the laying diet, due to the increase of the levels of coarse limestone. With the increase in the proportion of coarse limestone in the diet, bone deformity increased, reaching an estimated maximum of 63.33% of coarse limestone (0.60 mm) in substitution of fine limestone (0.23 mm), while for bone strength, the estimated maximum point was 58.88% of coarse limestone.

The ratio between calcium and phosphorus may also influence bone quality, Almeida et al. (2012), studying 378 Hy-Line W-36 chickens during the second laying cycle, distributed the birds in a randomized block design, with a total of seven blocks, in a 3 x 3 factorial scheme, composed of three levels of calcium and three calcium: available phosphorus ratios, amounting to nine treatments with one replication per block and six birds per experimental unit. At the end of the experiment, 72 birds (two per replication of each treatment) were slaughtered and the right and left tibias were removed. There was no significant interaction (p> 0.05) between the calcium levels and calcium: available phosphorus ratios for bone parameters, indicating that calcium levels and calcium: available phosphorus ratios behave independently. The variation in calcium levels was significant (p <0.05) on the absolute value of the mineral matter in the tibia, in spite of no effect on the percentage of mineral matter (p> 0.05) having been demonstrated. The increase of the dietary calcium level raised linearly the mineral matter of the tibia of the birds, which may have been a reflection of the increase in tibia weight due to the increase of the dietary calcium level.

Such results are similar to those found by Almeida Paz et al. (2009), who when working with two levels of calcium (1.8 and 3.8%) for semi-heavy laying hens, from 17 to 40 weeks of age, found an increase in the value of mineral matter in the femur and tibia of the birds. However, these results disagree from those obtained by Velasco et al. (2010) and by Safaa et al. (2008) who did not notice any increase in the value of laying mineral matter of the tibia of layers with increasing calcium content of the diets.

Microminerals can also lead to an improvement in bone quality. Zinc is an essential mineral for osteoblastic activity, collagen synthesis and alkaline phosphatase activity (Palacios, 2006), higher dietary concentrations for poultry showed improvement in bone quality (Swiatkiewicz & Koreleski, 2008). Similar results were found with the mineral manganese, however, studies show that boron is the element with the greatest significant impact on mineral metabolism and bone quality through interactions with Ca, P and magnesium (Olgun & Aygun, 2016).

Providing a perch contributes to increased bone strength (Abrahamsson and Tauson, 1993). Perches need to have suitable designs, without sharp corners, and cannot be too thin or too thick to prevent foot disorders (Moe et al., 2004). Tauson and Abrahamsson (1997) observed that a flattened circular perch on the top made of stiff wood with a diameter of 38 mm presented a lower incidence of foot injuries. Bird preference for perches and their positive effects in reducing deformities, injuries and bone strength denote improved animal welfare, especially solid floor perches rather than wire (Yngvesson et al., 2004).

The failure to verify nail and beak growth can harm birds housed in battery cages. If there is no possibility of wear, they can grow to become severely deformed. The increment of an abrasive strip in the cage can solve this issue (Tauson, 2003). Other deformities or injuries can be solved by improving the cage model (1985), Tauson and Holm (2003) studied these problems and suggested alternatives to circumvent them. Floors with inclination of 23% or more have triggered levels of deformities in the feet, since they slip as far as the wires, but making use of inclination with the maximum of 12%, that fact can be avoided.

3.9 Key techniques used to evaluate bone mineralization of birds

Concerning of conventional radiographic examination, it is not possible to evaluate changes in bone mineralization of less than 30%. Therefore, the application of more sensitive technologies is necessary to identify minimal variations in the bone mineral content of animals (Garton et al., 1994).

Radiographic optical densitometry (DOR) is defined as the method for measuring bone mineral density which, by photochemical action of light on sensitive emulsions, determines the ratio between the amount of light received by the sensitive film and the amount of silver salt that will be undergo reduction by direct blackening or developer (Lobel & Dubois, 1973). In order to reduce the variation of the observed values, the technique used requires accurate standardization (Delaquarriere-Richardson, 1982). This technique has been used to determine bone mineral density in birds, dogs, horses and cats, and presents reliable, accurate methodology and low operating cost, since the conventional radiographic apparatus is used (Louzada et al, 1997; Santos, 2002). The accuracy of this method occurs when the measurements made are sequential from the same anatomical area of the same animal (Quarles & Lyles, 1992). The non-uniformity of the X-ray field, milliamperage, kilovoltage and exposure time are variables that contribute to make this technique difficult (Vogel & Anderson, 1971).

Imaging diagnosis has advanced favoring more accurate diagnosis through non-invasive methods (Shores, 1993). Densitometry done on radiographs is a method that derives from absorption cytophotometry, which is translated in the measure of the absorption of light that a cell has at a given wavelength. The same definitions and principles employed in cytophotometry can be used for light absorption analysis when passes through a photographic film (Louzada, 1988).

DXA is a scanning technique for measuring different attenuations of two x-rays that pass through the body (Paiva et al., 2002). The device emits two x-ray beams under the body of the animal lying in prone position on the table. After the passage through the animal, the attenuated x-rays are measured by means of a discriminating energy detector. DXA performs cross-sectional evaluations of the body at 1-cm intervals from head to toe. Such a technique is noninvasive, considered safe, with the possibility of measuring three body components: fat-free mass; lean mass and bone mass (Cintra et al., 2004). There are three commercially available dual-energy X-ray models, namely, Hologic QDR, Lunar DPX and Norland XR. Each model is based on a software and hardware configuration (Lohman, 1996).

According to Marques et al. (2008), DXA has been recognized as a reference method in the study of body composition. Its limiting factors are due to the high cost of the equipment and radiation exposure. When examined using this technique, radiation exposure is considered low, ranging from 0.05 millirem (1.5 mrem) to 1.5 mrem, depending on the speed and equipment used. However, there is low ionizing radiation, in the distance of up to one meter away from the equipment during the examination. Also, as it is an area measure, the standardization of proper patient positioning is critical (Kerr et al., 2016).

Invasive techniques require the animal to be slaughtered to remove bones, and further, a greater number of animals are needed for significant sampling. Despite the discoveries made by this methodology, modern analytical techniques and available equipment stimulated the use of noninvasive tests as a way to investigate the bone integrity of birds. DXA™ equipment (“Dual-energy X-ray Absorptiometry”) is a diagnostic method for analyzing bone mineralization at different ages without the need for euthanasia (Hester et al., 2004). In addition, genetic selection programs are favored, since predictor factors of bone integrity that are measured “in vivo” by DXA ™ will be able to have their identification and replication in the measurements on the same animal. Mineral density refers to the mass of bone material that was measured by volume and includes the mineral and organic material existing in the bone. Considering the fact that the main component of the extracellular bone matrix is the inorganic matrix, the bone mineral density reflects the degree of skeletal mineralization (Mazzuco, 2005).

DXA examination involves a set of point-to-point measurements of the bone mineral density along the chosen anatomical site. The pixels are grouped together and determine the bone area in square centimeters (cm^2) (Blake et al., 2014). Quantitative variations in the mineral content and density of bones of birds on calcium-deficient diets were identified by Schreiweis et al. (2003) through the use of DXA ™ technology. Laying hens when subjected to forced molting and observed through DXA ™ showed decreased bone integrity in relation to the control birds that did not undergo this process (Mazzuco et al., 2003).

In laying hens, a phenomenon due to the presence of the spinal cord takes place, where any loss in bone mineral density due to age can be masked by the rise in the medullary component, as it has use reduced when in the process of eggshell calcification owing to the fall in the laying rate that happens with age. The thickness of the shell is also reduced, while there is an increase in tibial mineralization, concluding that failures occur in the mechanisms of calcium mobilization in layers at the end of laying (Scolari, 2007).

When using DXA to assess bone mineral density in birds, it is necessary to know that any loss of structural bone due to aging is masked in the face of spinal cord formation (Hester et al., 2004). Elevation in the tibia mineralization of layers in the second production cycle was detected by means of bone densitometry and the tibial medullary component possibly contributed to such an increase in mineral content (Mazzuco and Hester, 2005).

The study of bone mineral density of poultry of economic interest is fundamental for poultry production, for being a technique that allows the monitoring of the bone mass variables with modern technology and reduced cost, contributing to a better evaluation and understanding of the mineralization process of bones (Louzada, 1997).

3.10 Interpretation of the results on osteoporosis

In a study conducted with six birds of the Dekalb strain from different lots at different laying stages coming from a commercial poultry farm in the state of Minas Gerais, Brazil, Braga et al. (2012) verified the occurrence of resorption by osteoporosis at 23, 31 and 46 weeks of age. The affected birds showed clinical signs of prostration and inability to move or stand up. Euthanasia was undertaken for subsequent necropsy. The results were that the humerus, femur, tibia, ribs, and vertebra bones easily broke and could be severed. Lesions were similar in all the birds; however, birds from 23 to 31 weeks of age presented more severe lesions. The 46-week-old birds exhibited femur and humerus with incomplete and complete fractures and hemorrhage in the adjacent musculature. The birds examined showed increased bone resorption represented by the great osteoclastic activity and hypertrophy of the osteocytes.

According to a study by Schreiweis et al. (2003) about the humeral and tibial bone mineral density of white Leghorn birds fed diets with different calcium levels (hypocalcic

1.8%; recommended calcium 3.6%; hypercalcic 5.4%), found that mineral bone density accompanies negative linear tendency with the calcium reduction in the diet.

Korver et al. (2004) investigated the bone strength of both humeri and femurs of 65-week-old white Leghorn birds at the end of their production cycle and found greater strength in the bones of brown birds, one reason would be fact that they are heavier than white birds.

So, it follows that the environment can aggravate the bone fragility of the animals due to the movement and motion restriction, as well as the diets fed, in particular the calcium and available phosphorus levels, which presented distinct results. Experiments like these need further improvement in order to achieve a satisfactory welfare index for birds associated with production rates.

4. Final considerations

The confinement in cages causes reduction of the physical activity, triggering fragility and bone fracture which increases the incidence of osteoporosis in layers. This, in turn, is a multifactorial metabolic disease that constitutes an economic problem and indicative of well-being. The study of bone mineral density of birds by means of invasive or noninvasive techniques helps in the identification of possible strategies that may contribute to mitigate the severity of osteoporosis.

Thus, by the observation of the results found in this review still being contradictory, the intensification of research on the bone mineralization of birds in their different production stages becomes essential, aiming at the possibility of a better understanding of the physiology of these animals as well as providing subsidies for improvement in the bone condition of birds.

Bone problems are an important indicator of animal welfare and can be a limiting factor in bird productivity, this fact should be indicative in future research in different areas of animal production.

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