

# A Literature Review on Feed Fortification for Egg-laying Hens

**VOLUME II**



**By:**

Lukas Jasiūnas (Healthier Hens)

Kikiopé Oluwarore (Healthier Hens)

Isaac Esparza (Healthier Hens)

**Translation and adaptation:**

Elaine Cristina de Oliveira Sans

(Iniciativa MIRA)

**Review:**

Elsa Helena Barreto (Iniciativa MIRA)

**Design:**

Anaryá Mantovanelli (Carrots Comunicação Animalista)

# SUMMARY

<b>1. Introduction</b>	<b>04</b>
<b>2. The NRC guidelines</b>	<b>05</b>
<b>3. Significance of the housing system and other factors</b>	<b>08</b>
3.1 Keel bone fractures	09
3.2 Fracture specifics	10
3.3 Caged hens	14
<b>4. Key nutrients for bone health and integrity</b>	<b>16</b>
4.1 Calcium	16
4.1.1 Genetic, bone type, and hen age	19
4.2 Phosphorus	20
4.3 Vitamin D	21
4.4 Other nutrients, minerals, and supplements	22
4.4.1 Phytase	22
4.4.2 Omega 3	23
<b>5. Bibliography</b>	<b>24</b>

# 1.INTRODUCTION

Recent studies show that up to 97% of commercially kept laying hens may have at least one fracture of the keel bone, which can take up to eight weeks to heal, indicating that these birds experience pain for a significant period of their lives. This problem can only be resolved and/or alleviated using a combination of interventions regarding housing, nutrition, and genetics.

In particular, the nutrition of laying hens has been constantly studied, but such information is mainly focused on the productivity of birds. You can also see that many recommendations quickly become outdated.

It is important to note that isolating the effects of a nutrient, but not considering the context in which the laying hens are inserted, can lead to inaccurate results, such as changing the amount of calcium offered to the birds without considering the respective rearing system.

Therefore, this new volume analyzes the nutritional requirements of laying hens at different stages of their lives, according to strain, systems, and the interaction between these nutrients, in addition to analyzing specific nutrients that support bone health in birds such as calcium, phosphorus and vitamin D. It is important to note that, although birds kept in cages benefit from better nutrition, with less waste, these improvements do not neutralize the welfare problems caused by the restrictions of this system.

Good reading!

## 2. THE NRC GUIDELINES

The “Nutrient Requirements of Poultry” guidelines by the National Research Council (NRC) were established as the operational arm of the National Academy of Sciences in 1916, EUA, and the first edition of Nutritional Requirements for Poultry was published in 1944 during World War II, setting out nutrients needed to improve poultry nutrition and productivity. The 1994 edition is the most recent.

Feeding the hen can be divided into two main phases: before and after the onset of lay. The feeding regimen is further subdivided in accordance with growth and feed consumption rates typical for the different periods. For instance, the NRC divided the growth period of pullets into four stages: 0 to 6, 6 to 12, 12 to 18, and 18 weeks old to the age of the first egg. However, the researchers suggest that, in the last two decades, mineral and vitamin requirements for both pullets and laying hens have been little studied, with nutrition focusing primarily on nutritional deficiencies or mortality and less on health promotion, such as improving bones and immune function.

Regarding mineral requirements, it is suggested that the age of the birds at which nutrient levels are increased is an important parameter to be considered. Studies have shown that a diet fed to birds at 14 weeks of age with 3.5% calcium had no adverse effect on skeletal integrity or apparent renal function. On the other hand, providing a diet with 3.5% calcium to 7-week-old pullets increased the incidence of urolithiasis, that is, the deposit of crystals in the kidneys from salts and minerals.

Meanwhile, little research is observed regarding phosphorus and vitamin D requirements for pre-lay pullets. An exception is the recommendation to provide diets with excess phosphorus to hens encountering heat stress. The NRC even highlights the differences in nutritional needs between brown and white bird lines. Brown layers consume more feed, so the feed for white hens needs to have a higher concentration of essential nutrients for these birds to absorb the adequate amount.

Still regarding vitamin D, its excessive use can cause intoxication in birds, as well as hypercalcemia and mineralization of soft tissues. This vitamin is still susceptible to destruction by oxidation unless supplemental antioxidants are used. In addition, mycotoxins present in the feed, such as aflatoxin produced by the fungus *Aspergillus*, can interfere with the use of vitamin D. Therefore, oxidation and misuse of vitamin D can cause its deficiency, even if birds receive adequate concentrations.



Calcium, phosphorus, or vitamin D deficiencies result in rickets and other bone problems in birds. Despite a lower risk of toxicity, excess calcium or phosphorus should also be avoided, as they can hinder the intestinal absorption of other minerals.

Inadequate nutrition of laying hens also contributes to the incidence of other bone problems: such as hock enlargement, intensified by niacin and zinc deficiency; perosis caused by imbalance of biotin, choline, vitamin B12, manganese, zinc, and folacin; shortening and thickening of leg bones caused by problems with zinc and manganese metabolism; and curled toes, due to riboflavin deficiency.

This information reflects that the NRC guidelines are still important in the egg industry, but it is necessary to include the changes that laying hens and their rearing systems have undergone over these last few decades.

# 3. SIGNIFICANCE OF THE HOUSING SYSTEM AND OTHER FACTORS

Due to growing public concern about animal welfare, the practice of keeping cage-free laying hens is on the rise in many countries, and it is important to highlight specific issues involving birds housed in this system.

It is a fact that many producers of no-cage systems lack knowledge regarding laying management, nutrition, health, and behavior, but as these collaborators gain experience, the welfare of the chickens tends to increase. The bird mortality rate, for example, is reduced by an average of 0.35% to 0.65% each year that producers gain experience with no cage systems, and in more recent years, there is no difference in mortality between systems. of caged and cage-free poultry, as it opposes the idea that higher mortality is linked only to the production of free-range chickens.

Cage-free hens also have increased activity and behavioral repertoire, making it possible for a reduction in nutrients targeted at egg production to occur. However, eggs from cage-free birds have a more resistant shell, possibly due to increased levels of calcium ingested from foraging in the soil.



## 3.1 Keel bone fractures

Keel bone damage in laying hens is a global welfare concern, as up to 95% of birds at the end of the laying cycle exhibit some damage to this bone. These fractures are caused by a multitude of factors, such as the hen's age, strain, system, way of identifying the fracture, etc.

The perception that cage-free systems increase the prevalence of keel bone fracture may not be supported when the full context of the system is not considered. Laying hens reared in no-cage systems have greater opportunities to exercise, which leads to bone strengthening. Although these may suffer keel fractures and/or deviations caused by the greater risk of collisions, this fact can be corrected when the chicks are given early access to perches or other elevated structures, as in addition to allowing the escape of dominant birds, it will provide physical resistance and behavioral learning necessary to not suffer or considerably reduce the incidence of injuries during landings and takeoffs. Laying hens reared in cages may also suffer not necessarily from fractures, but from deformation of the keel bone. Furthermore, it seems that the lines that lay white eggs are more susceptible to deformations, while fractures are more frequent in hens with high laying rates.

There is some evidence for a positive relationship between adult bird body weight and keel bone mineral density, including heavier hens exhibiting higher bone densities. It was also found that birds with the highest bone mineral densities were the most resistant to keel fractures, indicating that this property plays a key role in determining the breaking strength of the keel bone.

However, conflicting evidence shows that the weight of birds may play a role in increasing the risk of fractures. Therefore, more research is needed to determine the effect of bird weight and bone density on the risk of developing keel deviations and/or fractures.

In addition to its high prevalence, keel bone damage is painful and alters bird behavior. Research on brown and white hens has shown that skeletal health is impaired in all birds, regardless of strain or breeding system. Management strategies such as providing access ramps, perches, and nests can be adopted to reduce the incidence of fractures. The bone strength of birds can still be improved by providing other forms of exercise, evidence that is often observed in cage-free systems. In general, improvements in husbandry and management systems are important to deal with injuries and fractures of the keel bone in laying hens.

## 3.2 Fracture specifics

Although it is believed that keel bone fractures are commonly caused by the impact of birds with structures, a recent study using computed tomography scans and histology in birds suggested that some fractures may result from internal pressures from egg laying.

Regardless of the cause, hens with these fractures experience, in addition to the pain and suffering, reduced production, egg size and quality, increased feed and water consumption, and reduced ability to perch and fly. These birds may even spend time resting and standing on the floor, while those with severe fractures spent more time perching, presumably to avoid descending from perches where they felt safer.

Injured birds will also spend less time inactive, suggesting that they are uncomfortable, leading to restlessness. Studies have even indicated that birds with these fractures experience a negative affective state. All these studies only reinforce that the fracture of the keel bone, in addition to being painful, negatively influences the physical, mental, and behavioral health of hens.

An analysis in a cage-free system in Switzerland found that 99% of hens had at least one keel bone injury and 97% had at least one keel fracture. In 77% of cases, the caudal third (the lower part) of the keel bone was injured. However, we reinforce that this relies on factors such as the experience of both the producer and the hens after being housed in cage-free systems and becoming accustomed to such structures. The researchers recorded that, on average, hens experienced three fractures each during the production cycle, with the majority of fractures occurring between 31 and 33 weeks, just after the peak of lay.

After reaching sexual maturity at around 16-18 weeks, hens stop producing structural bone and this elasticity generally decreases. Although the efficiency of calcium retention improves with age, it does not seem to be sufficient, as the risk of fractures increases. The researchers suggest that the decrease in new fractures after 33 weeks could be partially explained by the additional increase in calcium retention. It was also possible to observe an estimate of the time required for healing of these fractures and according to the injury, it may take up to 36 weeks for the fracture to heal, although most have been completely healed in 7 weeks.

Another study indicated that brown hens are more susceptible to keel bone fractures, as more susceptible hens also tend to produce eggs with less resistance.

---

In general, it appears that underweight hens with a high calcium requirement due to high egg production and without access to natural light are more susceptible to fractures.

Despite having high bone density, hens with high performance showed a significantly higher risk of fracture than lower-performing strains. It was also verified that the prevalence of fracture increased more between 33 and 40 weeks. However, the risk and severity of keel bone deviations were not affected by egg production level and strain, suggesting that these injuries are two independent phenomena caused by different factors. Furthermore, there is no consensus on how deviations affect bird welfare, unlike fractures, where studies point to a significant reduction in bird welfare.

Studies also show that it is possible for hens reared in cages to present continuous loss of bone mass due to restriction of movement. Therefore, a rearing system in which hens have greater locomotor activity seems to allow for greater bone integrity. However, limitations regarding the methodology of this study did not allow the authors to distinguish between the degree of mineralization and the different types of bones. This is important because medullary bone, which forms the primary calcium stored in birds, offers little structural integrity despite being relatively high in radiographic density.

Therefore, radiographic evaluations of bones in laying hens must be accompanied by other measures, such as a morphological examination of bone structure, to more accurately determine the effects of diet and system on bone strength.

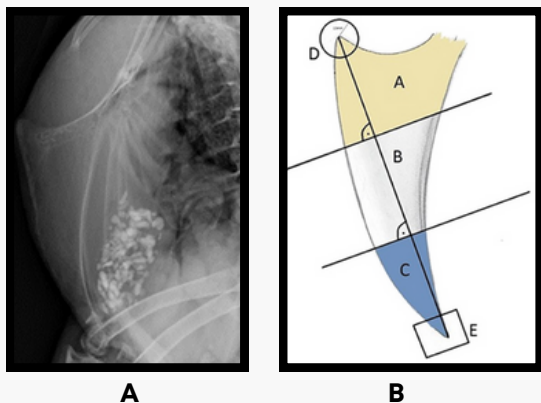


Figure 1. Laterolateral view of (A) the keel bone of the free-range laying 10 years old, without visible fractures or lesions; (B) Laterolateral view of a schematic representation of a keel bone. The keel bone is divided in three parts, each of them measures one third of the base to apex line: Cranial third (A), middle (B), and caudal third (C). The apices are labeled (D) (cranial;  $r = 15\text{mm}$ ) and (E) (caudal  $1/5$  of C). By: Baur *et al.*, 2020.



Figure 2. Examples of fracture and lesion types and localizations. (A) Laterolateral view of a complete transverse keel bone fracture in the caudal third (localization C) with caudodorsal dislocation and angulation. (B) Laterolateral view of two incomplete oblique keel bone fractures in the cranial third (localization A) with a ventral superficial step formation and slight ventral angulation at the caudal fracture. (C) Laterolateral view of a comminuted keel bone fracture in the cranial and middle third (localization AB) with a ventrocranial dislocation and angulation of the caudal main fragment. (D) Laterolateral view of a butterfly keel bone fracture in the middle and the caudal third (localization BC) with ventral dislocation and angulation of the butterfly and caudal main fragment. By: Baur *et al.*, 2020.

## 3.3 Caged hens

A British study identified that the strength and radiographic density of humeri (upper wing bones) of caged hens were 40 to 50% lower than those free cage-free. Hens reared in cages also had tibias (shinbones) that were 25% weaker and 15% less dense. Similarly, according to a Swedish study, broken wings were nearly three times more common in caged birds, and humeri were only half as strong, on average, as in cage-free hens. Even with attempts to increase the metabolic efficiency of the birds by providing them with extra minerals, hens in cages exhibited a physiological limit of absorption of calcium and phosphorus.

Some researchers indicate that it is impossible to prevent osteoporosis by simply improving the birds' diet. However, extra supplementation appears to have a limited effect on reducing structural bone loss during the laying period, as it also stems from severe movement restriction, which is an important welfare issue. Even so, improving the diet can be a start to help birds, especially those housed in cages. You can start by increasing calcium levels that can lead to increased bone strength, such as 4.1% calcium, even though this is a value higher than the NRC recommendation.

In general, hens with bone damage are found in all types of commercial production, with a variable prevalence between systems, countries, and bird age. Still, there is a clear relationship between fractures and indicators of reduced animal welfare.

Additional evidence suggests that other problems in combination can cause poor welfare, such as a relationship between the occurrence of footpad dermatitis and the presence of bone fractures in the keel at the end of the laying period, suggesting that hens with footpad dermatitis may be more prone to slip or fall from perches. Similarly, keel bone damage was associated with poor feather cover. Although the mechanism for this is unknown, flocks of hens with high levels of harmful pecking may be more fearful, resulting in birds that are at greater risk of fractures due to the flight and/or flight process or remaining perched longer.

Therefore, changes in production systems need to include issues around the welfare of laying hens, and they need to be accompanied by greater knowledge and training of professionals, producers, and others, in order to mitigate the risks of fractures and other hens injuries.



# 4. KEY NUTRIENTS FOR BONE HEALTH AND INTEGRITY

There are nutrients that are essential for the growth and bone health of laying hens. Below is information about some of these nutrients.

## 4.1 Calcium

Approximately 10% of the total volume of calcium in a hen's body is used in the daily production of an eggshell and of that percentage, half is acquired from the diet and the other half from medullary bone. The shell of an egg is made up of 90 to 95% calcium carbonate, which also determines the strength of the shell. Today's laying hens lay an egg almost every day, so they require about 4 to 5 g of calcium daily to maintain their reserves.

Appropriate calcium levels depend on both the calcium present in the feed, the daily feed intake, and the calcium to phosphorus (Ca:P) ratio. For growing chicks and pullets, it is recommended that this ratio be 2:1, while for adult hens 10:1 or larger. It is noteworthy that the demand for calcium generally increases with the rate of egg production and the age of the hen.

Hen mortality and egg parameters also depend on the Ca:P ratio, as it affects calcium retention. Temperature can also determine how efficiently the bird utilizes these nutrients.



Early studies show that adding inorganic phosphorus can reduce osteoporosis and can be applied at a higher level than the historically determined ideal in egg production.

Increasing levels of calcium in the diet can increase the strength of the tibia bone and eggshell, with a consensus being observed that at 70 weeks, hens already need higher levels of calcium than the levels required in various patterns.

In general, calcium is very relevant for birds. It was observed that there was an increase in bone strength when calcium was supplied at the level of 5.25% for 80-week-old, as well as an increase in calcium levels from 2.5 to 5.0% at the beginning of the lay period, leading to greater production, bone density and resistance to fractures. There are even recommendations that Hy-Line® W-36 be fed diets containing 4.25 to 4.5% calcium and 5.1% from 32 weeks of age. Even so, researchers point out that 5% may not be enough for hens raised in hot environments.

Regarding bone stiffness, although this feature may reduce the risk of bone damage, bones that are too fragile and not flexible enough can still be injured. However, birds with 72-week-old that received additional calcium in the form of meal or pellets exhibited considerably greater bone stiffness and breaking strength than those without extra calcium. Furthermore, calcium supplementation can still increase egg quality without negatively affecting egg production.

Hens that do not receive an extra source of calcium may try to consume more feed to get more of this mineral. On the other hand, particle size is also important. Benefits of feeding coarse limestone (particle size 1.5 to 4 mm) in the afternoon or evening have been demonstrated, indicating that it is vital that birds have access to a high-quality source of calcium at night, as their response to a hypocalcemic state (low level of calcium in the body) occurs within minutes. In the case of pelletized or crushed feed, the raw material components are mainly ground, making it difficult to add coarse particles, and it is important that this nutrient be added after pelleting to ensure adequate calcium levels for the birds.

The rearing system should also be considered, as it was found that hens housed in aviaries show increased weight and strength in the tibial bone, probably due to greater physical activity, but lower calcium content in this same bone, indicating that higher consumption of calcium in this system may lead to increased excretion of calcium, possibly due to poor absorption. This information indicates that not all parameters can be positively correlated.

Regarding feeding coarse-particle limestone to pullets, there was an improvement in bone mineralization at the beginning of sexual maturity and a reduction in damage to the keel bone during both the pullet and laying phases for brown and white birds. However, the same supplementation was associated with lower egg production in brown hens housed in aviaries.

The researchers suggest that the higher bone mineral density of pullets may have been due to increased bone marrow formation at the onset of sexual maturity. Regardless of system or strain, the use of coarse limestone as a source of calcium has been shown to have the potential to decrease the incidence of keel bone ruptures in 54-week-old hens.

## **4.1.1 Genetic, bone type, and hen age**

Researchers compared the effects of hens' genetics, environment, and nutrition on bone strength and found that improvements in genetics and diet are independent and additive issues, with genetics being more effective. Genetics, for example, improved tibia bone strength by 65%, while ad libitum ingestion of particulate lime improved only 20%. Feeding hens larger limestone particles from 15 weeks onwards also resulted in greater bone strength and eggshell quality. At 25 weeks, the hens' tibiae were slightly thicker, indicating greater bone growth.

Calcium intake should be monitored continuously. Bones can contribute up to 40% of the calcium required for each egg when the dietary calcium concentration is 2%. The removal of calcium from the bones can be even greater when the birds receive reactions with low concentrations of calcium, as well as during the night when no calcium is offered to them, which is when most of the process of calcification of the eggshell occurs. While the amount of calcium in the bones is sufficient to form the eggshell, the extra calcium provided is vital to supplement and restore the birds' reserves.

## 4.2 Phosphorus

The NRC recommends supplying available phosphorus at 2.5 g/kg or 250 mg/bird/day, although noting that levels seen in industry are typically higher. Many variables such as feed composition, rearing system, bird age, and season of the year influence the physiological phosphorus requirement. For example, less phosphorus is needed by the bird if it is given wheat-based feeds, due to the endogenous level of phytase. There are records of a strong correlation between phosphorus demand and egg production stages: less phosphorus is needed after peak laying. In this way, it is verified that layers of 52 to 62 weeks obtain sufficient phosphorus in a concentration of 1.5 g/kg.

The lack of information about the phosphorus requirements for the current strain of laying hens has led the industry to increase the level supplied of this nutrient to the growing birds since the low intake during the first half of the life of the hens results in the increase of prevalence of osteoporosis when they start to lay eggs. However, the use of excess phosphorus raises the issue of sustainability due to the high amounts of this nutrient found in bird excreta.

Current studies show new Ca:P ratios. Hy-Line International, for example, recommends ratios of total calcium to available phosphorus between 8:1 and 13:1 during the laying cycle, while other research suggests ratios of up to 28:1, with no significant negative impact on egg production and eggshell quality.

Although the impact on birds' welfare has not yet been fully evaluated, some studies with caged hens have indicated positive effects of phosphorus supplementation. The researchers identified that the NPP (non-phytate phosphorus) requirement for bone mineralization and tibial bone strength was greater than that for egg production and shell quality. Another study found that egg production was significantly higher on 0.3% than 0.2% or 0.4% NPP diets. It also verified a benefit in the additional NPP (>0.24%) in relation to a potential relief of systemic inflammation in laying hens.

Overall, there is a clear indication that feeding hens a phosphorus-deficient diet during the laying period impairs keel bone quality, possibly due to the onset of osteoporosis. However, phosphorus supplementation without exogenous phytase can lead to reduced productivity, feed intake, or increased bird mortality. Phytase supplementation can still generate additional gains in bone quality and strength in birds. It is possible that increasing phosphorus levels to 0.57% or supplying phytase (1,800 FYT/kg) leads to significant increases in tibial bone strength without loss of bird performance.

## 4.3 Vitamin D

Unlike hens with the possibility of exposure to direct sunlight, a fact that we can observe in poultry rearing systems with access to the external environment and/or aviaries with windows, birds kept in completely closed/air-conditioned aviaries require vitamin D supplementation. This vitamin is essential for proper calcium and phosphorus metabolism and maintenance of skeletal integrity. Its deficiency is apparent within 4 weeks when the eggshell becomes thinner.

Even partial access to open air is sufficient for birds to initiate endogenous vitamin D production. In terms of supplementation, vitamin D<sub>2</sub>, which occurs naturally in plants and fungi in the form of ergocalciferol, is only one-tenth as effective as vitamin D<sub>3</sub> (cholecalciferol). Provitamin D can also be converted to cholecalciferol by the action of ultraviolet B light, especially in the unfeathered areas of the skin of birds. Vitamin D<sub>3</sub> can be absorbed by birds with an efficiency of 60 to 70%.

The benefits of adding vitamin D<sub>3</sub> and its metabolites have also been verified in hens housed in cages. Supplementation of levels below 100,000 IU/kg significantly improved the bone strength of the birds without generating harmful calcium accumulation in their organs. Vitamin D<sub>3</sub> also increased egg production when compared to the production of birds without supplementation and when 2200 IU/kg of vitamin D<sub>3</sub> was used together with 1 $\alpha$ -hydroxyvitamin D<sub>3</sub> (a synthetic metabolite of this vitamin), there was a slight increase in strength of tibia bone.

## 4.4 Other nutrients, minerals, and supplements

### 4.4.1 Phytase

Supplementation of poultry diets with phytase has been shown to increase bone mineral content and bone density in hens, regardless of phosphorus level.

Supplementation can also improve the body weight of birds fed a diet with low phosphorus content and use of calcium in diets deficient in this nutrient. However, many factors affect the effectiveness of phytase such as its source, concentration, and Ca:P ratio, and it will be more efficient when supplied in diets with low phosphorus and sufficient calcium, and its supplementation, in general, does not affect the production of eggs;

## 4.4.2 Omega 3

A lower prevalence of keel bone fractures was observed in cage-free hens fed food enriched with omega-3 (n3). The birds also showed greater load capacity in the keel and tibiae bones and the more flexible humeri. These results support studies that diets supplemented with n3 can reduce fractures, as they increase bone strength without impairing egg production. However, care must be taken with the dosage of omega-3, as excessive amounts or high levels of C20/22 polyunsaturated fatty acids can lead to problems such as increased risk of fractures and reduced productivity. However, there is still little information about the interaction between omega-3 and other nutrients such as calcium, phosphorus, and vitamin D3. When given a lower level of omega-3 to omega-6 ratio (0.767 vs. 1.35), hens maintained egg production levels and decreased mortality, but further research on their effect on bird diet.

# 5. BIBLIOGRAPHY

An, S.H.; Kim, D.W.; An, A.B. 2016. Effects of dietary calcium levels on productive performance, eggshell quality, and overall calcium status in aged laying hens. *Asian-Australasian Journal of Animal Science*, 29(10), p.1477.

Baur, S.; Rufener, C.; Toscano, M.J.; Geissbühler, U. 2020. Radiographic evaluation of keel bone damage in laying hens - morphologic and temporal observations in a longitudinal study. *Frontiers in Veterinary Science*, 7, p.129.

Bryden, W.L.; Li, X., Ruhnke, I.; Zhang, D.; Shini, S. 2021. Nutrition, feeding and laying hen welfare. *Animal Production Science*.

Campbell, D.L.M. 2020. Skeletal health of layers across all housing systems and future research directions for Australia. *Animal Production Science*.

Candelotto, L.; Stratmann, A.; Gebhardt-Henrich, S.G.; Rufener, C.; van de Braak, T.; Toscano, M.J. 2017. Susceptibility to keel bone fractures in laying hens and the role of genetic variation. *Poultry Science*, 96(10), p.3517-3528.

de Matos, R. 2008. Calcium metabolism in birds. *Veterinary Clinics of North America: exotic animal practice*, 11(1), p.59-82.

do Nascimento, G.R.; Murakami, A.E.; Guerra, A.F.Q.M.; Ospinas-Rojas, I.C.; Ferreira, M.F.Z.; Fanhani, J.C. 2014. Effect of different vitamin D sources and calcium levels in the diet of layers in the second laying cycle. *Brazilian Journal of Poultry Science*, 16(2), p.37-42.

Embrapa. Empresa Brasileira de Pesquisa Agropecuária. Manual de boas práticas na produção de galinhas poedeiras. 2020.

Eusebio-Balcazar, P.E.; Purdum, S.; Hanford, K.; Beck, M.M. 2018. Limestone particle size fed to pullets influences subsequent bone integrity of hens. *Poultry Science*, 97(5), p.1471-1483.

Eusemann, B.K.; Baulain, U.; Schrader, L.; Thöne-Reineke, C.; Patt, A.; Petow, S. 2018. Radiographic examination of keel bone damage in living laying hens of different strains kept in two housing systems. *PLoS One*, 13(5), p.e0194974.



- Eusemann, B.K.; Patt, A.; Schrader, L.; Weigend, S.; Thöne-Reineke, C.; Petow, S. 2020. The role of egg production in the etiology of keel bone damage in laying hens. *Frontiers in Veterinary Science*, 7, p.81.
- Fernandez, S.R.; Chárraga, S.; Avila-Gonzalez, E. 2019. Evaluation of a new generation phytase on phytate phosphorus release for egg production and tibia strength in hens fed a corn-soybean meal diet. *Poultry Science*, 98(5), p.2087-2093.
- Fleming, R.H.; McCormack, H.A.; McTeir, L.; Whitehead, C.C. 2006. Relationships between genetic, environmental and nutritional factors influencing osteoporosis in laying hens. *British Poultry Science*, 47(6), p.742-755.
- Frost, T.J.; Roland Sr, D.A.; Untawale, G.G. 1990. Influence of vitamin D3, 1 $\alpha$ -Hydroxyvitamin D3, and 1, 25-Dihydroxyvitamin D3 on eggshell quality, tibia strength, and various production parameters in commercial laying hens. *Poultry Science*, 69(11), p.2008-2016.
- Gebhardt-Henrich, S.G.; Fröhlich, E.K. 2015. Early onset of laying and bumblefoot favor keel bone fractures. *Animals*, 5(4), p.1192-1206.
- Golden, J.B.; Arbona, D.V.; Anderson, K.E. 2012. A comparative examination of rearing parameters and layer production performance for brown egg-type pullets grown for either free-range or cage production. *Journal of Applied Poultry Research*, 21(1), p.95-102.
- Gordon, R.W.; Roland Sr, D.A. 1997. Performance of commercial laying hens fed various phosphorus levels, with and without supplemental phytase. *Poultry Science*, 76(8), p.1172-1177.
- Habig, C.; Henning, M.; Baulain, U.; Jansen, S.; Scholz, A.M.; Weigend, S. 2021. Keel bone damage in laying hens: its relation to bone mineral density, body growth rate and laying performance. *Animals*, 11(6), p.1546.
- Härtel, H., 1990. Evaluation of the dietary interaction of calcium and phosphorus in the high producing laying hen. *British Poultry Science*, 31(3), p.473-494.
- Hans-Heinrich Thiele. Optimal Calcium Supply of Laying Hens, *Poultry News*. 2015, [https://drive.google.com/file/d/12qE0Iu110IM8Iyb9MGRxOC3aPcKyh\\_-d/view?usp=sharing](https://drive.google.com/file/d/12qE0Iu110IM8Iyb9MGRxOC3aPcKyh_-d/view?usp=sharing)
- Hy-line, Technical Update: Understanding the role of the skeleton in egg production. 2016. <https://drive.google.com/file/d/13tPJWxplUriq-hPZW9zTfOt0J8IRmUZF/view?usp=sharing>

Kerschnitzki, M.; Zander, T.; Zaslansky, P.; Fratzl, P.; Shahar, R.; Wagermaier, W. 2014. Rapid alterations of avian medullary bone material during the daily egg-laying cycle. *Bone*, 69, p.109-117.

Keshavarz, K. 1987. Influence of feeding a high calcium diet for various durations in prelaying period on growth and subsequent performance of white leghorn pullets. *Poultry Science*, 66(10), p.1576-1582.

Keshavarz, K. 2003. The effect of different levels of nonphytate phosphorus with and without phytase on the performance of four strains of laying hens. *Poultry Science*, 82(1), p.71-91.

Klasing, K.C. *Nutritional Requirements of Poultry*, MSD Veterinary Manual, 2015, [https://drive.google.com/file/d/10HY1ogp65\\_M66nf70vAoE1PPHLdrHKYk/view?usp=sharing](https://drive.google.com/file/d/10HY1ogp65_M66nf70vAoE1PPHLdrHKYk/view?usp=sharing).

Koutoulis, K.C.; Kyriazakis, I.; Perry, G.C.; Lewis, P.D. 2009. Effect of different calcium sources and calcium intake on shell quality and bone characteristics of laying hens at sexual maturity and end of lay. *Int J Poult Sci*, 8(4), p.342-348.

Kühn, J.; Schutkowski, A.; Kluge, H.; Hirche, F.; Stangl, G.I. 2014. Free-range farming: a natural alternative to produce vitamin D-enriched eggs. *Nutrition*, 30(4), p.481-484.

Leeson, S.; Summers, J.D. 1987. Effect of immature body weight on laying performance. *Poultry Science*, 66(12), p.1924-1928.

Leeson, S. 1986. Nutritional considerations of poultry during heat stress. *World's Poultry Science Journal*, 42(1), p.69-81.

Li, X.; Zhang, D.; Bryden, W.L. 2017. Calcium and phosphorus metabolism and nutrition of poultry: are current diets formulated in excess? *Animal Production Science*, 57(11), p.2304-2310.

Li, X et al. Available phosphorus requirement of laying hens, Final Project Report, Australian Egg Corporation Limited, 2016. [https://drive.google.com/file/d/10Of-yy91qPNTuJ7czLf9UKYx15N1-\\_Bm/view?usp=sharing](https://drive.google.com/file/d/10Of-yy91qPNTuJ7czLf9UKYx15N1-_Bm/view?usp=sharing)

Morrissey, R.; Cohn, R.M.; Empson Jr, R.N.; Greene, H.L.; Taunton, O.D.; Ziporin, Z.Z. 1977. Relative Toxicity and Metabolic Effects of Cholecalciferol and 25-Hydroxycholecalciferol in Chicks. *The Journal of Nutrition*, 107(6), p.1027-1034.

National Research Council – NRC. 1994. Nutrient requirements of poultry. National Academies Press.

Nie, W.; Wang, B.; Gao, J.; Guo, Y.; Wang, Z. 2018. Effects of dietary phosphorus supplementation on laying performance, egg quality, bone health and immune responses of laying hens challenged with *Escherichia coli* lipopolysaccharide. *Journal of Animal Science and Biotechnology*, 9(1), p.1-11.

- Panda, A.K.; Rao, S.V.; Raju, M.V.L.N.; Bhanja, S.K. 2005. Effects of dietary non-phytate phosphorus levels on egg production, shell quality and nutrient retention in white leghorn layers. *Asian-Australasian Journal of Animal Science*, 18(8), p.1171-1175.
- Persia, M.E.; Higgins, M.; Wang, T.; Trample, D.; Bobeck, E.A. 2013. Effects of long-term supplementation of laying hens with high concentrations of cholecalciferol on performance and egg quality. *Poultry Science*, 92(11), p.2930-2937.
- Poletto, R. Galinhas livres x galinhas em gaiolas. *Avinews Brasil*. 2<sup>o</sup> Trimestre de 2022.
- Ratkowski, C.; Fine, N.; Edelstein, S. 1982. Metabolism of cholecalciferol in vitamin D intoxicated chicks. *Isr. J. Med. Sci.* 18:695.
- Riber, A.B.; Casey-Trott, T.M.; Herskin, M.S. 2018. The influence of keel bone damage on welfare of laying hens. *Frontiers in Veterinary Science*, 5, p.6.
- Roland Sr, D.A.; Bryant, M.M.; Rabon, H.W.; Self, J. 1996. Influence of calcium and environmental temperature on performance of first-cycle (phase 1) commercial leghorns. *Poultry Science*, 75(1), p.62-68.
- Rufener, C.; Makagon, M.M. 2020. Keel bone fractures in laying hens: a systematic review of prevalence across age, housing systems, and strains. *Journal of Animal Science*, 98 (Supplement 1), p. S36-S51.
- Schuck-Paim, C.; Negro-Calduch, E.; Alonso, W.J. 2021. Laying hen mortality in different indoor housing systems: a meta-analysis of data from commercial farms in 16 countries. *Scientific Reports*, 11(1), p.1-13.
- Swiatkiewicz, S.; Arczewska-Wlosek, A.; Jozefiak, D. 2015. Bone quality, selected blood variables and mineral retention in laying hens fed with different dietary concentrations and sources of calcium. *Livestock Science*, 181, p.194-199.
- Toscano, M.J.; Booth, F.; Wilkins, L.J.; Avery, N.C.; Brown, S.B.; Richards, G.; Tarlton, J.F. 2015. The effects of long (C20/22) and short (C18) chain omega-3 fatty acids on keel bone fractures, bone biomechanics, behavior, and egg production in free-range laying hens. *Poultry Science*, 94(5), p.823-835.
- Tůmová, E.; Vlčková, J.; Charvátová, V.; Drábek, O.; Tejnecký, V.; Ketta, M.; Chodová, D. 2016. Interactions of genotype, housing and dietary calcium in layer performance, eggshell quality and tibia characteristics. *South African Journal of Animal Science*, 46(3), p.285-293.

Turner J.; Lymbery P. J. Brittle Bones: Osteoporosis and the battery cage. *Compassion In World Farming* (Petersfield, UK, 1999). 21.

Wang, J.; Yue, H.; Wu, S.; Zhang, H.; Qi, G. 2017. Nutritional modulation of health, egg quality and environmental pollution of the layers. *Animal Nutrition*, 3(2), p.91-96.

Wei, H.D.; Chen, Y.J.; Zeng, X.Y.; Bi, Y.J.; Wang, Y.N.; Zhao, S.; Li, J.H.; Li, X.; Zhang, R.X.; Bao, J. 2021. Keel-bone fractures are associated with bone quality differences in laying hens. *Animal Welfare*, 30, p.71-80.

Wideman Jr, R.F.; Closser, J.A.; Roush, W.B.; Cowen, B.S. 1985. Urolithiasis in pullets and laying hens: role of dietary calcium and phosphorus. *Poultry Science*, 64(12), p.2300-2307.

Wilson, S.; Duff, S.R.I.; Whitehead, C.C. 1992. Effects of age, sex and housing on the trabecular bone of laying strain domestic fowl. *Research in Veterinary Science*, 53(1), p.52-58.

