Schothorst Feed Research

LIVESTOCK RESEARCH
WAGENINGEN UR

P-requirements in Modern Laying Hens
- a literature review -

Report
no.1326

February 2014

Authors:
W. Lambert
Ir. L. de Lange
Dr.Ir. M.M. Van Krimpen
Dr.Ir. J.D. van der Klis
Dr.Ir. L. Star

Research for development

Research

Ministerie van Economische Zaken,
Landbouw en Innovatie

PRODUCTSCHAP Pluimvee & Eieren

Report: no. 1326

P-requirement in Modern Laying Hens
- a literature review -

(Project PA12-51)

The study is performed on request of the Dutch Ministry of Economic Affairs and subsidised by the Dutch Product Board for Poultry and Eggs

Key words: P-requirement, P-utilisation, laying hen, age, production performance

Authors:
W. Lambert
Ir. L. de Lange
Dr.Ir. M.M. Van Krimpen
Dr.Ir. J.D. van der Klis
Dr.Ir. L. Star

PREFACE

On request of the Dutch Ministry of Economic Affairs, as well as of the Dutch Product Board Animal Feed, this study was performed to investigate the retainable phosphorus requirement and utilisation in laying hens. The modern laying hen has a high egg number and laying persistence, and probably a different P-requirement than the current estimated requirements. Furthermore, laying hens housed in non-cage housing systems have a better bone development. It might be that P supply by feed in such systems can be lowered without negative effects on bone quality and production performance. Determination of the P-requirement, -utilisation, and -excretion is important to support the lump phosphorus excretion and to reduce the phosphorus supply by the feed. The study comprises a literature review and an animal experiment. The results of the review are described in this report. The study is a collaborative project between Schothorst Feed Research and Wageningen Livestock Research.

Marinus van Krimpen and Laura Star
Project leaders

CONTENTS

TABLE OF ACRONYMS

Al: Aluminium
ALP: Alkaline Phosphatase
aP: Available Phosphorus
B: Boron
BMD: Bone Mineral Density
Ca: Calcium
CaB: Cancellous Bone
CA: Citric Acid
CAH: Carbonic Anhydrase
CC: Conventional Cages
CDDGS: Corn Distillers Dried Grains with Solubles
CoB: Cortical Bone
B: Boron
BMD: Bone Mineral Density
EC: Enriched Cages
ECM: Extracellular Matrix
ECPD: Electrochemical Potential Difference
ESG: Eggshell gland
FLS: Fatty Liver Syndrome
FTV: Free Thoracic Vertebra
HAP: High Available Phosphorus
MB: Medullary Bone
Mg: Magnesium
nPP: non-Phytate Phosphorus
OPN: Osteopontin
P: Phosphorus
PG: Prostaglandin
PMCA: Plasma Membrane Calcium ATPase
Pi: inorganic Phosphorus
PP: Phytate-Phosphorus
PTH: Parathyroid Hormone
PTM: Proximal TarsoMetatarsus
SBM: Soybean Meal
SZA: Sodium Zeolite A
TRPV: Transcient Receptor Potential Vanilloid
VDR: Vitamin D receptor

1 Introduction

Phosphorus (P) is a very important nutrient in poultry nutrition and is involved in many functions for laying hens such as bone formation, energy metabolism, cellular structure and egg formation (Ahmadi & Rodehutscord, 2012). However, 50 to 80% of the P present in grain cereal and oilseed is bound to phytic acid, the organic form of P which is called Phytate P (PP; Boling al., 2000; Abudabos, 2012) and this PP is highly unavailable to non-ruminant animals because they do not contain enough endogenous amounts of phytase to hydrolyze the phytic acid complexes (Nelson, 1976). According to Fleming (2008), 5 to 15% of poultry flocks might have inadequate P intake because of variations in P content and availability in diet. Therefore, inorganic P needs to be added to the diet to meet the hens' P requirements (Boling et al., 2000; Ahmadi & Rodehutscord, 2012). In addition, microbial phytase can be supplemented to improve the availability of P (Carlos & Edwards, 1998).

According to Rose (1997), feed represents already 70% of the cost of production in egg farming and P is the third most expensive component after energy and protein (Boling et al., 2000). However, it is generally admitted that thanks to the supplementation in phytase, the cost of P in the feed has come down, especially when all the P has been replaced. The high commercial P supplementation prices are due to the growing scarcity of mined phosphate rock. According to experts, the peak of P extraction is expected to occur before 2040 (Gross, 2010; Neset & Cordell, 2012). To give an example, the commodity price of P rocks raised by 800% in 18 months during the 2008 crisis (Neset & Cordell, 2012).

More than just an issue of cost, P is pointed out to be one of the major causes of environmental problems. The amount of P in excess in the diet is directly excreted via the faeces and the urine (Boling et al., 2000; Abudabos, 2012). The poultry wastes are therefore highly concentrated in P which constitutes the limiting nutrient for algae and other aquatic plant growth and leads to eutrophication (Sharpley, 1999).

As a consequence, reduction of inorganic P needs to be considered to minimize the cost in the feed and the P excretion in poultry manure. Snow et al. (2004) proposed three strategies to solve this issue. First of all, by feeding poultry closer to their P requirement, secondly, by supplementing diets with enzymes or other feed additives to improve the availability of P in plant sources and lastly, by feeding plant ingredients that contain lower levels of PP. All three approaches will be reviewed in this literature study.

The first chapter of this study aims at clarifying the dynamics of Ca and P, the role of Ca and P on bone metabolism and mobilization but also the mechanisms of Ca and P absorption, regulation and excretion. The second part's purpose is to analyze every factor having an influence on hens P requirements. Due to the overall increase of the length of the laying period in the modern hens and the recent change in housing system (from battery cages to furnished cages and non-cage housing systems), the study will also consider the effect of age and housing on P requirement. The last and third chapter provides updated information on P requirements as reported in the literature.

2 Phosphorus and calcium metabolism

2.1 Dynamics in P and Ca during the laying cycle

2.1.1 Egg production

Egg composition

An average egg from egg-laying strain of a domestic fowl weighs 57g and is constituted of 63.8% of albumen, 27.2% of yolk and 9.0% of shell. The main components are water with 74.6% and protein with 12.1%. The shell weight is 5.1g in average and is composed of 98% of $CaCO_3$ and 2% of protein (Rose, 1997). P is most present in the yolk and less than 0.1% of the P requirements is intended for the eggshell (de Vries et al., 2010). P is the first most abundant mineral in the egg (without considering the eggshell) with around 120mg P in a 60g egg compared to 34mg Ca in the same egg (AEB, 2013). The distribution of Ca in the egg is the opposite from that of P. Only the eggshell contains already 2.4g Ca per egg of 60g, so almost 0.1% of the Ca is in the egg without shell.

Egg formation

In modern systems, the majority of the eggs (90%) are laid during the morning (Leeson & Summers, 1978). On average, ovulation occurs 30min after the oviposition and the ovum needs 4h to move from the infundibulum to the uterus, including between 3 and 3.5h in the magnum where the albumen is formed. After, the ovum stays in the uterus during 18 to 20h to accomplish the shell deposition (van de Velde et al., 1984; Keshavarz, 1998). Van de Velde et al. (1984) also precise that the egg calcifaction is only active during 13h out of the 18-20h, it is called the active period in constrast with the inactive period (Figure 1).

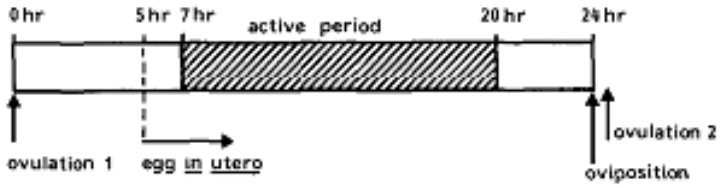

Figure 1. Scheme of the 24h laying cycle of chickens and quails. The period of eggshell formation is shaded (Van de Velde et al., 1984).

As a consequence, the largest part of eggshell formation occurs during the dark hours (afternoon and evening) called the scotophase (de Vries et al., 2010). Kebreab et al. (2009) even go further in the description of the egg shell timing by indicating that egg shell requires 20h to be 100% complete and that the rate of formation is the highest between 7 and 13h after the start of the process (Figures 2 and 3). The period between two ovipositions is close to 24h but slightly longer, especially for younger hens (Keshavarz, 1998).

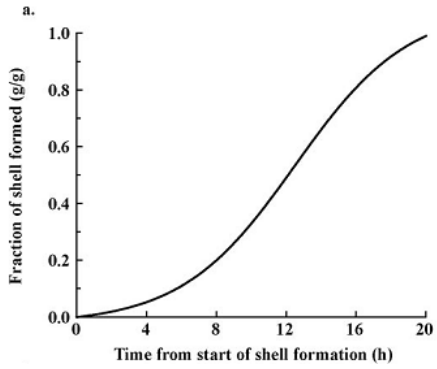

Figure 2. Time course of eggshell formation. Cumulative eggshell formation (Kebreab et al., 2009).

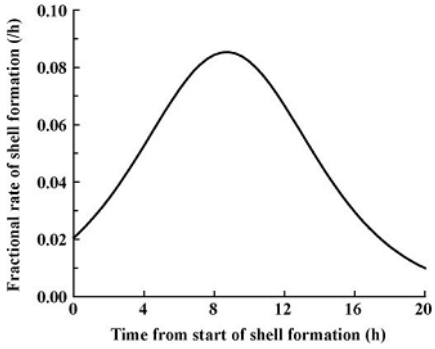

Figure 3. Time course of eggshell formation. Fractional rate of eggshell formation (Kebreab et al., 2009).

2.1.2 Dynamics of P and Ca during the day

Proteins

After considering the process of egg formation, it is logical to think that the deposition of proteins, the main component of albumen and yolk takes place during the morning hours (Keshavarz, 1998).

Calcium

$CaCO_3$ deposition starts only 5h after oviposition and the requirements for Ca are therefore greater during the scotophase (Keshavarz, 1998). De Vries et al. (2010) also indicate that the period of egg shell deposition occurs mostly when Ca intake from feed is low and therefore the supply from the digestive system is also poor. As a consequence, the period when Ca supply is low matches with the period when the needs of Ca to produce the eggshell are great. To compensate this lack, the hen has a special bone mechanism to mobilize Ca from the leg bone reserves. During the active period (11 to 15h), the laying hen transfers 10% of the total bone Ca to the egg (Bar et al., 2002; Bar, 2009). In total, 2 to 3g of Ca is provided to the egg (Bar, 2009). However, when the photo phase starts (moment when the light is switched on), the feed intake starts again and plasma Ca increases which coincides with the moment when egg deposition comes to an end and Ca requirements are reduced.

Phosphorus

P is directly related to the Ca dynamics because Ca is stored in bones mostly as hydroxyapatite ($3Ca_3(PO_4)Ca(OH)_2$) and is bound to phosphate in a constant ratio of 2.1 to 1. Therefore, when Ca is moblized from the bone during the eggshell formation, the plasma P level increases and the P will be immediately excreted by the urine because it cannot be utilized at the moment (de Vries et al., 2010). According to Keshavarz (1998), the voluntary P intake is higher during the morning

than the afternoon. This is due to the need to restore P reserves together with Ca in the bones before eggshell formation (Tolboom & Kwakkel, 1998). The latter is confirmed by Holcombe et al. (1976) who showed that layers ingest more P during the morning, from 06:00 to 14:00 than during the afternoon, from 14:00 to 20:00. To finish, Keshavarz (1998) conducted an experiment on P intake of layers and analyzed the effect of a diet with constant P level (0.4g/kg) along the day compared to a diet with reduced P level (0.2g/kg) in the afternoon. He did not find any difference regarding the hens' performances and concluded that reduced P intake in the afternoon does not impair the production performances, showing that P requirements are lower in the afternoon. This finding shows that current dietary P level is higher than P requirements in the afternoon and that adequate P intake during this period of the day might reduce P excretion.

As it has been demonstrated with Ca, the hen is able to choose his feed according to the P content of the diet (Barkley et al., 2004). In the first phase of the experiment of Barkley et al. (2004), hens were fed a normal P diet (2.2g aP/kg DM) or a low P diet (1.1g aP/kg DM). The low diet was proven to create a deficiency in P. In the second phase, the hens were fed a choice of normal P or low P diets. With a choice of the normal and low P diets, the hens fed the low P diet in phase 1 ate a smaller proportion of the low P feed than the hens fed the normal P diet in phase 1, showing that P deficiency influenced selection for aP. Subsequently, the authors highlighted the presence of a P sensing mechanism as the appetite for P was demonstrated.

According to a study from Cusack et al. (2003), P is deposited at low concentrations in the outside parts of the eggshell. This P concentration gradually increases until the end of the eggshell calcification (Figure 4) which shows that P has only a role in the termination of the calcification.

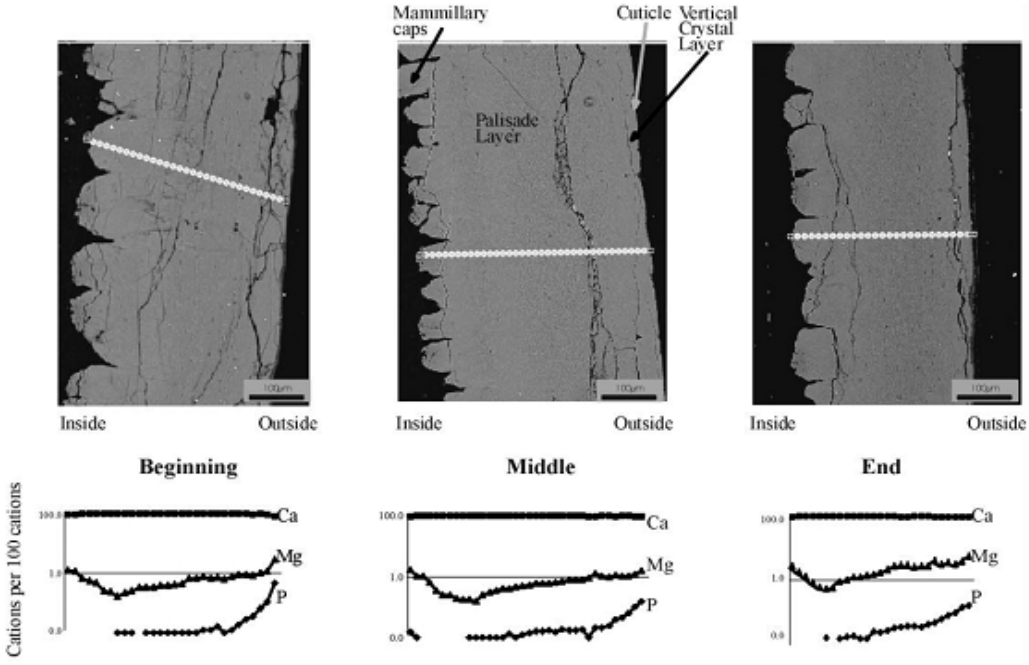

Figure 4. Secondary electron image of polished sections of broiler breeder eggshells from the beginning, middle and end of lay (Cusack et al., 2003).

Modelling

The modern methods of modelling enable researchers to be more accurate. Kebreab et al. (2009) proposed the most updated model in laying hens taking into consideration both Ca and P dynamics (see the paper for the description of the fitted model). In this model, the Ca and P absorption stand for the mineral absorption by the duodenum. Ca and P requirements correspond to the requirements for maintenance and for the egg production (yolk, albumen and shell). Ca and P deposition is the bone mineralization when the values are positive or bone mobilization when the values are negative. The photoperiod is fixed to 16h/d. In this model, the feed intake is assumed to be continuous along the day even though Keshavarz (1998) indicated that the feed intake usually represents 40% in the first 8h of light and 60% in the last 8h of light.

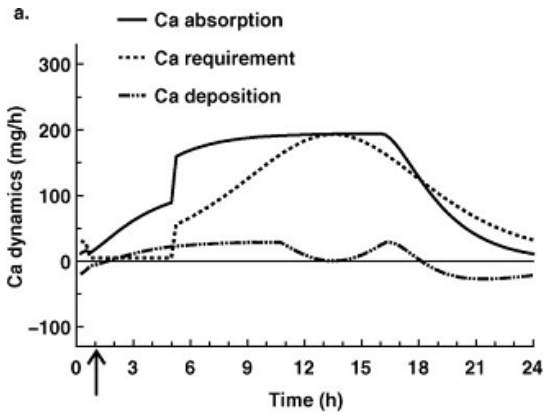

Figure 6. Simulated diurnal dynamics of Ca in a layer at oviposition 1h after light is switched on (Kebreab et al., 2009).

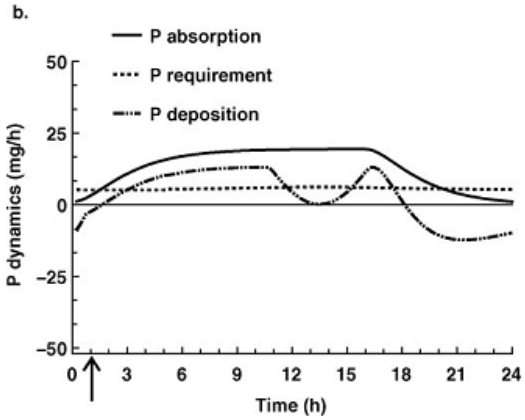

Figure 5. Simulated diurnal dynamics of P in a layer at oviposition 1h after light is switched on (Kebreab et al., 2009).

In the Figures 5 and 6, oviposition occurs 1 hour after the start of the photo phase as indicated by the arrow. At t=0, the light is switched on and the intake of feed starts. The graphs show an increase in Ca and P absorption after this moment. The peaks of P and Ca absorptions occur around the start of the dark period when feed intakes ceases (t=16).

Due to the assumed continuous process of yolk formation and because the majority of P is intended to form the yolk, P requirements are considered constant during the day. However, unlike the eggshell deposition, the yolk needs several days to be formed. As a consequence, the P deposition is maybe less subject to variations than what Kebreab et al. (2009) considered in their model. In the first hour of light, P absorption is smaller than P requirements and therefore mobilization of P and Ca from the bone takes place. In contrast with the rest of the day, in this first hour, P lacking is responsible for bone mobilization. The P is entirely used for maintenance and egg production while Ca cannot be used because the eggshell formation has not started yet, cannot be stored and is therefore excreted in urine.

The trend is totally reversed once the eggshell formation occurs (t=5h). The Ca requirements are the highest after 11 to 17h after oviposition when eggshell rate is the highest (Figure 2, considering t=0 as 5h after oviposition). According to the simulations, the P absorption is

sufficient to meet the P requirements until t=20h. However, excessive P is excreted in the urine because it cannot be stored in the bone. In contrast, the Ca absorption is sufficient to meet the Ca requirements until t=18h and is deposited in the eggshell. From t=18h until the end of the dark hours, the Ca is lacking for eggshell deposition and need to be mobilized from the bone to compensate. P is mobilized as well, cannot be used or stored and is therefore excreted in the urine.

In this simulation, 33% of the available Phosphorus (aP) intake is directly excreted in the urine because of inadequate levels of Ca. In this situation, 22% of P reduction can be considered in the diet because P deposition into the bone is higher than P mobilization.

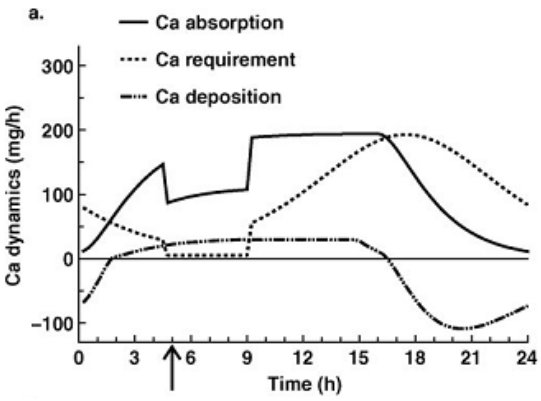

Figure 8. Simulated diurnal dynamics of Ca in a layer at oviposition 5h after light is switched on (Kebreab et al., 2009).

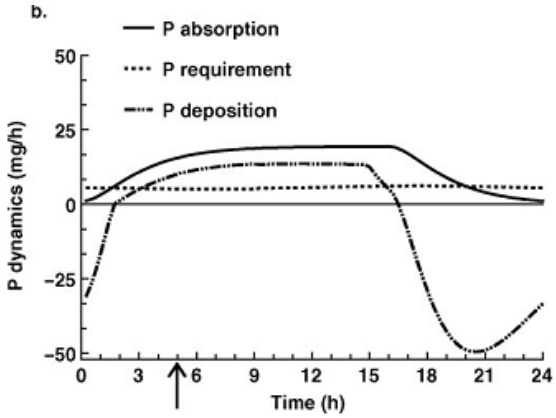

Figure 7. Simulated diurnal dynamics of Ca in a layer at oviposition 5h after light is switched on (Kebreab et al., 2009).

In Figures 7 and 8, oviposition occurs later at (t=5h). The start of the shell forming process is therefore delayed (t=10h) and the peak of calcite deposition takes place after the end of the photoperiod (t=18h), when the absorption of Ca from the gut is small. The Ca requirements are much higher than the Ca absorption, compared to the first situation and strong Ca mobilization from the bone occurs. Large amounts of P are mobilized and excreted in the urine after t=17h. However, from t=2h until t=15h, P is the limiting nutrient for the bone formation and Ca in excess is excreted. In this case, no reduction is possible due to the net P mobilization of bone.

In conclusion, P dynamics are inseparable from the Ca dynamics as Ca and P are bound together to form the mineral matrix of the bones. P metabolism and requirements are highly dependent upon Ca, which justifies reviewing also the Ca metabolism and regulation in later chapters.

As explained by Kebreab et al. (2009), Ca and P have different behaviours over a laying cycle and are often contradictory. For instance, when P is lacking, Ca is in excess and vice et versa. In addition, dietary P is not sufficient to avoid bone resorption but could be reduced when in excess P is excreted. In brief, dietary Ca contributes more to the dynamics of P than the dietary P itself. An increase of Ca intake at the end of the laying cycle to support the eggshell deposition is suspected to decrease P excretion and to decrease dietary P to fill in the bone with P in the

subsequent inactive period. For instance, Mongin & Sauveur (1974) proved that presenting Ca separated from the feed resulted in an increase in Ca intake between 16:00 and 20:00.

However, dietary P is also very important and can be modulated along the day. Before the start of the eggshell formation, in the morning, P requirement is higher because P is used to restore the bone content. In the afternoon, P is excreted from bone mobilization and common dietary P is therefore not adequate and might need to be reduced. Ca and P dynamics in the bone need to be reviewed to understand the mechanisms and consequences of bone mobilization.

2.2 Bone metabolism

2.2.1 Generalities

Bone functions

The skeleton has many functions for the body. It is a support for the muscles, acts as a protector of the organs, produces red and white blood cells and provides storage for Ca and P. In addition, the phosphate and carbonate present in the bone serve as regulators of the acid-base balance in the body (Mackie et al., 2011).

Bone composition

In adult birds, about 65 % of the bone consists of minerals while the rest is build up from organic components, such as fibre and collagen (Brook & Marshall, 2001). However, for young birds, such as chicks, the organic part accounts for 40%. The mineral part of the bones mostly consists of a crystalline hydroxyapatite-like phase. In brief, bone is made up of hydroxyapatite crystals of Ca phosphate deposited on an organic collagen matrix (Whitehead & Fleming, 2000). According to Doyle (1979), Ca and P are the two most abundant minerals in bone with 370 and 170g/kg ash respectively. In laying hens, between 80 to 90% of the total P is stored in the bones and the rest in the nucleotides, nucleic acids and phospholipids (Soares, 1995; Brook & Marshall, 2001). Brook and Marshall (2001) indicated that 99% of the Ca is situated in the bone. It is lower for broilers as 75% of Ca and 54% of P is stored in the bones (Van Krimpen). The two main types of bone providing structural integrity are the compact outer layer of the Cortical Bone (CoB) and the more spongy porous network of the cancellous (or trabecular) bone (CaB; Figure 9), both of them are forms of lamellar bone (Whitehead & Fleming, 2000).

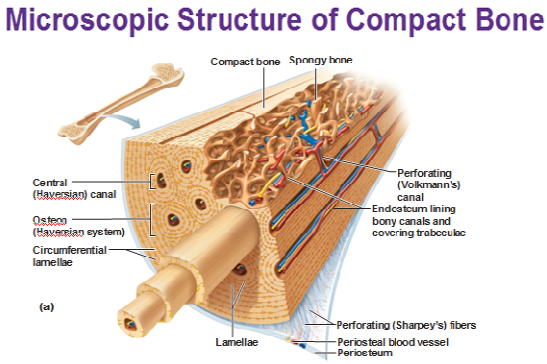

Figure 9. Microscopic structure of compact bone (compact bone = cortical bone; spongy bone = trabecular = cancellous bone) (ProProfs, 2012).

Bone cells

Three main types of cells are active in the formation of bones. First, the chondrocytes are responsible for the cartilage development through a process of proliferation, secretion of cartilage extracellular matrix (ECM) and hypertrophy. When the chondrocytes die, they allow the invasion of other cells such as osteoblasts and osteoclasts. Among this group of cells, osteoclasts are responsible for bone breakdown by removing the ECM while the osteoblasts are responsible for the bone deposition inside the cartilage remnants (Newman & Leeson, 1997; Mackie et al., 2011). After the complete development of the bones, the process of bone deposition and bone resorption takes place to mobilize Ca reserves and maintain Ca balances. If this process becomes unbalanced and osteoclasts are more active than osteoblasts, more bone is mobilized than deposited and the hen can suffer from metabolic bone disorder such as osteoporosis (de Vries et al., 2010).

2.2.2 Bone growth during rearing

The skeleton of the laying hen is fully developed before the sexual maturity (de Vries et al., 2010). The bone growth during rearing takes place with the formation of two structural bone types: the CaB and CoB (Fleming, McCormack, & Whitehead, 1998a). This development occurs through the process of endochondral ossification (Figure 10) which consists of the replacement of a cartilage model by bone tissue (Mackie et al., 2011). This endochondral ossification is based on the epiphyseal growth plate where resting chondrocytes turn into proliferative chondrocytes. They form columns of cells and secrete an ECM constituted of collagen. Then, more matrix is secreted, the chondrocytes start to hypertrophy and the growth takes place in two different regions: the proliferative zone and the hypertrophic zone. The bone formation starts in the lower hypertrophic zone. The hypertrophied chondrocytes secrete Alkaline Phosphatase (ALP) that helps to initiate the formation of hydroxyapatite. The chondrocytes then experience apoptosis and the formation of osteo-cells starts. The coordinated action of osteclastic resorption and osteoblastic bone formation leads to the development of trabecular bone which will resorb to

form the marrow cavity. In brief, the development of the long bones is based on proliferation of the chondrocytes, hypertrophy and finally mineralization (Whitehead, 2004).

Osteoblasts secrete layers of cortical bone in the perichondrium while, in the endosteal surface, the osteoclasts begin to resorb bone. This process creates an expanding ring with bone formation on the outer surface and resorption in the inner. During the early weeks of growth, the ring expands so rapidly that cavities are not filled in with bone before osteoclastic resorption. As the growth slows, the degree of infilling increases (Whitehead, 2004).

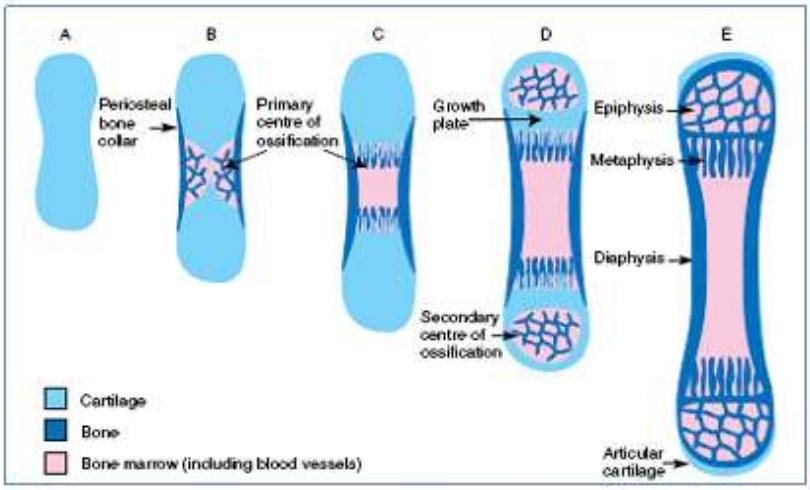

Figure 10. Endochondral ossification in long bones. A is the start of the cartilage mall and E is the mature bone (Mackie et al., 2011).

2.2.3 Bone growth during laying

Medullary Bone (MB) development

Bone growth process is common to most of the domesticated animals. Nevertheless, female birds are unique in the sense that the development of the endosteal cavities in the long bones forms a secondary type of bone, called Medullary Bone (MB; Taylor, 1970; Dacke et al., 1993). The development of the MB has been extensively reviewed in the literature (Taylor & Moore, 1956; Taylor, 1970; Candlish, 1971; Dacke et al., 1993; Whitehead, 2004; Fleming, 2008). At the onset of sexual maturity (16-18 weeks), the follicular maturation coincides with the secretion of oestrogens, especially oestradiol, which will drive the formation of the MB, acting in synergism with androgens. The rise in circulating oestrogens stimulates the osteoblasts to change from a formation of structural bone to a development of soft tissue and to depose the MB on the surfaces of the structural bone (Whitehead, 2004; de Vries et al., 2010). When the MB formation starts, there appear to be little or no further formation of structural bone (Whitehead & Wilson, 1992 cited by Fleming, McCormack & Whitehead (1998)), Fleming, 2008). Taylor & Moore (1958) also brought evidence that this change from structural bone to MB appears 7 to 10 days before a pullet starts to lay. The MB formation occurs rapidly during the early laying period and continues slowly during the rest of the reproductive period (Whitehead, 2004).

MB structure

MB is a special type of woven bone mostly found in the marrow of long bones and the highest content of MB is found in leg bones (especially the femur and tibia; van de Velde et al., 1984; Dacke et al., 1993; Whitehead & Fleming, 2000; Figure 11). MB is not a structured type of bone due to the fast speed of bone turnover which prevents a good orientation of collagen in response to functional loading (Fleming, McCormack, McTeir, et al., 1998). The mineral composition is comparable to CoB or CaB and consists of a hydroxyapatite lattice (Wilson & Duff, 1991; Dacke et al., 1993; Whitehead & Fleming, 2000). However, in CoB, the hydroxyapatite crystals are oriented with respect to the organic matrix while MB has apatite crystals randomly distributed throughout the matrix (Dacke et al., 1993).

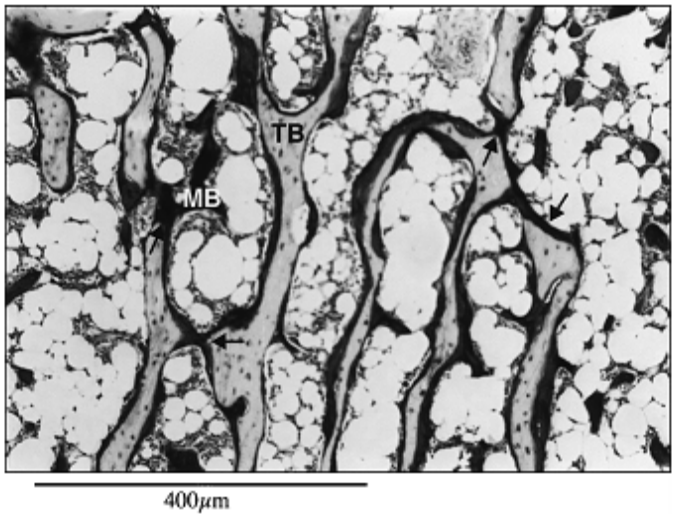

Figure 11. Histological section of bone of a laying hen showing trabecular bone coated with darkly stained MB; Whitehead & Fleming (2000).

Wilson & Duff (1991) found out that in severe situation of Ca, P or vitamin D deficiency, MB is poorly mineralized with low ash content. MB provides a labile source of Ca for shell formation and mobilization of bone minerals (Whitehead & Fleming, 2000), as will be explained later on.

2.2.4 Bone mobilisation during the laying cycle

Proximal TarsoMetatarsus (PTM) and the Free Thoracic Vertebra (FTV)

In order to assess the bone mobilization in the laying cycle, researchers need to measure the relative volumes of the different types of bone (cortical, cancellous and medullary) to define the respective proportion for each type of bone. In the experiments, the most common bones observed are the PTM and the FTV as being respectively representative of the appendicular and axial skeletons (Knott et al., 1995; Rennie et al., 1997; Fleming, McCormack, & Whitehead, 1998a; Whitehead & Fleming, 2000).

Ca and P availability is most crucial during the laying period (de Vries et al., 2010). In the laying hen, approximately 2.4g of Ca is required in 20h to produce a shelled egg of 60g (Etches, 1987; Bar, 2009). As a result, the transfer of Ca from the blood to the eggshell reaches in average 120mg/h and varies from 100 to 200mg/h. In a laying hen, the plasma Ca concentration is around 200mg Ca/L of blood during the period of shell deposition. When, at the end of the day, the supply of Ca from the feed is low, the plasma level will go down to 0 in 7-15min if no alternative source of Ca is available (Etches, 1987). From the amount of Ca needed for eggshell production, only 60–75% can therefore be provided by the feed and the rest is from osseous origin (Van de Velde et al., 1984; Fleming, 2008). When the Ca absorption from the gut decreases at the end of the day because of lower feed intake, the dietary Ca cannot cover the Ca requirements for eggshell deposition anymore and the hen has to mobilize Ca from the bones. However, by using coarse limestone (as Ca grits) that solubilises slowly in the gizzard, mobilization of Ca from the bones can be reduced.

Variations in bone volumes over the reproductive period

According to Fleming, McCormack & Whitehead (1998) the variations in bone volumes is not uniform in all bone types. First of all, there is a marked loss of cancellous bone (first 10 weeks after sexual maturity; Figure 12). When hens get older (after 25wks old), the loss of structural bone continues but at reduced rates. However, they also show a continuous net increase in the total amount of bone. As a result, the total bone volume is the greatest at 70 weeks. The marked loss of CaB in both PTM and FTV suggests that the major development of osteoporosis occurs within the first weeks of egg production. As stated earlier, the onset of maturity determines the end of the structural bone formation and the ostoclastic resorption therefore leads to a net depletion of structural bone. At the onset of sexual maturity, the MB is not formed in the PTM and FTV and the CaB is solicited to supply Ca for the shell deposition. The prevention of early CaB depletion can result in preventing spinal damage. Secondly, in the bones that are the most subject to fractures, such as leg and wing bones, there is a considerable loss of structural bone between 25 and 50 weeks which is mostly CoB in these bones. These observations indicate that loss of structural bone occurs later in the wing and leg bones than in the PTM and FTV (Fleming, McCormack, & Whitehead, 1998b). In brief, structural bone volume declines during the laying period but the accumulation of MB means that total bone volume remain constant or even increase over the laying period (Whitehead, 2004; Figure 13).

Table 3. *Structural and strength characteristics of different bones between 15 and 70 weeks in hens fed on diets containing limestone in powder (C) or particulate (P) form*

		Age (weeks)				Significance of effect		
	Treatment	15	25	50	70	Age	Diet	Interaction
PTM								
Total bone (%)	C	$19{\cdot}6 \pm 0{\cdot}8^{a}$	$^{x}22{\cdot}1 \pm 1{\cdot}0$	$^{x}21{\cdot}5 \pm 1{\cdot}2$	$^{x}23{\cdot}4 \pm 1{\cdot}3^{b}$	***	***	NS
	P	$19{\cdot}4 \pm 0{\cdot}7^{a}$	$^{y}24{\cdot}7 \pm 0{\cdot}9^{b}$	$^{y}25{\cdot}3 \pm 1{\cdot}3^{b}$	$^{y}28{\cdot}6 \pm 2{\cdot}0^{b}$			
Cancellous bone volume (%)	C	$19{\cdot}6 \pm 0{\cdot}8^{a}$	$^{x}10{\cdot}9 \pm 0{\cdot}7^{b}$	$9{\cdot}4 \pm 0{\cdot}9^{bc}$	$6{\cdot}6 \pm 0{\cdot}5^{c}$	***	*	NS
	P	$19{\cdot}4 \pm 0{\cdot}7^{a}$	$^{y}13{\cdot}2 \pm 0{\cdot}5^{b}$	$10{\cdot}4 \pm 0{\cdot}5^{c}$	$7{\cdot}3 \pm 0{\cdot}5^{d}$			
Medullary bone volume (%)	C	0^{a}	$11{\cdot}1 \pm 0{\cdot}9^{b}$	$12{\cdot}1 \pm 1{\cdot}1^{b}$	$16{\cdot}8 \pm 1{\cdot}2^{c}$	***	*	NS
	P	0^{a}	$11{\cdot}5 \pm 0{\cdot}8^{b}$	$14{\cdot}8 \pm 1{\cdot}2^{b}$	$21{\cdot}4 \pm 2{\cdot}1^{c}$			
Free thoracic vertebra								
Cancellous bone volume (%)	C	$15{\cdot}0 \pm 0{\cdot}5^{a}$	$11{\cdot}8 \pm 0{\cdot}5^{b}$	$11{\cdot}0 \pm 1{\cdot}0^{b}$	$10{\cdot}4 \pm 0{\cdot}6^{b}$	***	NS	NS
	P	$14{\cdot}3 \pm 0{\cdot}7^{a}$	$11{\cdot}9 \pm 0{\cdot}7^{ab}$	$9{\cdot}4 \pm 0{\cdot}7^{b}$	$10{\cdot}2 \pm 0{\cdot}5^{b}$			
Radiographic density (mm Al)	C	$2{\cdot}53 \pm 0{\cdot}04^{a}$	$2{\cdot}84 \pm 0{\cdot}04^{b}$	$2{\cdot}62 \pm 0{\cdot}06^{a}$	$2{\cdot}55 \pm 0{\cdot}08^{a}$	***	NS	NS
	P	$2{\cdot}48 \pm 0{\cdot}04^{a}$	$2{\cdot}75 \pm 0{\cdot}04^{b}$	$2{\cdot}54 \pm 0{\cdot}06^{a}$	$2{\cdot}70 \pm 0{\cdot}09^{ab}$			
Tibia								
Radiographic density (mm Al)	C	$1{\cdot}62 \pm 0{\cdot}06^{a}$	$2{\cdot}06 \pm 0{\cdot}08^{b}$	$^{x}1{\cdot}83 \pm 0{\cdot}06^{ab}$	$^{x}1{\cdot}96 \pm 0{\cdot}08^{b}$	***	**	*
	P	$1{\cdot}60 \pm 0{\cdot}03^{a}$	$2{\cdot}09 \pm 0{\cdot}06^{b}$	$^{y}2{\cdot}06 \pm 0{\cdot}04^{b}$	$^{y}2{\cdot}26 \pm 0{\cdot}11^{b}$			
Breaking strength (kg)	C	$26{\cdot}5 \pm 0{\cdot}8^{a}$	$28{\cdot}2 \pm 1{\cdot}4^{a}$	$^{x}18{\cdot}2 \pm 1{\cdot}2^{b}$	$19{\cdot}5 \pm 1{\cdot}0^{b}$	***	*	NS
	P	$26{\cdot}1 \pm 0{\cdot}8^{ab}$	$28{\cdot}2 \pm 0{\cdot}9^{a}$	$^{y}22{\cdot}5 \pm 1{\cdot}0^{b}$	$23{\cdot}6 \pm 2{\cdot}0^{ab}$			
Humerus								
Radiographic density (mm Al)	C	$0{\cdot}72 \pm 0{\cdot}03^{a}$	$0{\cdot}90 \pm 0{\cdot}04^{b}$	$0{\cdot}90 \pm 0{\cdot}05^{b}$	$0{\cdot}73 \pm 0{\cdot}04^{a}$	***	NS	NS
	P	$0{\cdot}65 \pm 0{\cdot}02^{a}$	$0{\cdot}94 \pm 0{\cdot}03^{b}$	$0{\cdot}93 \pm 0{\cdot}03^{b}$	$0{\cdot}78 \pm 0{\cdot}03^{c}$			
Breaking strength (kg)	C	$18{\cdot}3 \pm 0{\cdot}8^{a}$	$19{\cdot}0 \pm 1{\cdot}4^{a}$	$11{\cdot}7 \pm 0{\cdot}7^{b}$	$11{\cdot}8 \pm 0{\cdot}7^{b}$	***	NS	NS
	P	$17{\cdot}6 \pm 0{\cdot}7^{a}$	$18{\cdot}0 \pm 0{\cdot}8^{a}$	$13{\cdot}2 \pm 0{\cdot}7^{b}$	$12{\cdot}1 \pm 0{\cdot}6^{b}$			
Keel								
Radiographic density (mm Al)	C	$0{\cdot}41 \pm 0{\cdot}01^{a}$	$0{\cdot}57 \pm 0{\cdot}02^{b}$	$0{\cdot}45 \pm 0{\cdot}03^{a}$	$0{\cdot}61 \pm 0{\cdot}04^{b}$	***	**	NS
	P	$0{\cdot}43 \pm 0{\cdot}01^{a}$	$0{\cdot}58 \pm 0{\cdot}02^{b}$	$0{\cdot}58 \pm 0{\cdot}03^{b}$	$0{\cdot}68 \pm 0{\cdot}03^{c}$			

Figure 12. Structural and strength characteristic of different hens between 15 and 70 weeks in hens fed on diets containing limestone in powder (C) or particulate form (P; Fleming et al. 1998).

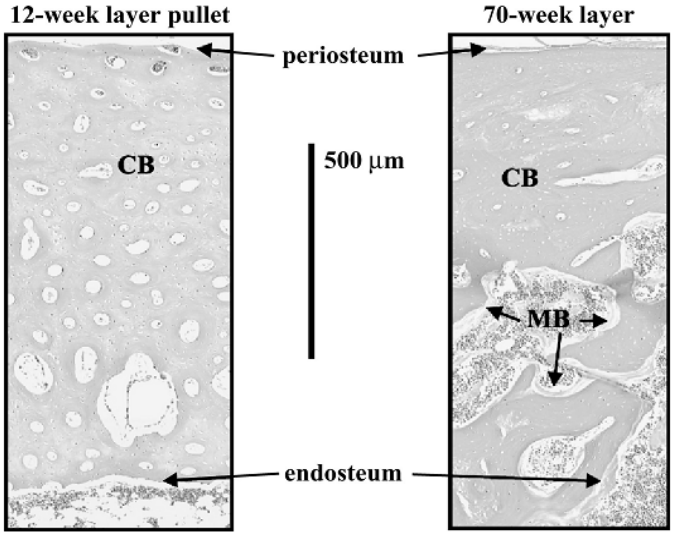

Figure 13. Sections through tibia midshaft of pullets (Whitehead, 2004).

Variations in bone volumes or content over a laying day

Candlish (1971) did not detect any significant change in total volume of the bones during the day. Although the volume seems to vary the very high standard errors make it difficult to demonstrate.

However, he found out that the MB was subjected to significant variations of composition along the day. There is significantly less hydroxyproline and Ca in MB at times 3, 13 and 18h than at 8 h, when the MB is heavily loaded with Ca.

Van de Velde et al. (1985, 1984) confirmed that the total MB volume does not change during a laying day, only the degree of calcification. From 22 to 4h, during the inactive period, the MB matrix is recalcified (van de Velde et al., 1985). This observation was confirmed by Bar (2008).

Ca mobilization from the MB

The MB acts as a temporary depot of Ca for eggshell formation, when requirements are high and absorption from the gut is low (de Vries et al., 2010). During this period, the fully calcified MB is replaced by an organic matrix poor in Ca and therefore Ca is released into the blood to supply the eggshell. When the eggshell is complete, the poorly calcified MB is recalcified during the following inactive period. The osteoblasts replace the osteoclasts and regenerate the medullary bone (Whitehead, 2004). The quantity of Ca in bones is higher during days of no shell formation which indicates that the bone reserves are restored during the inactive period (Schraer & Schraer, 1961; Buss & Guyer, 1984; van de Velde et al., 1984).

The MB is an easy-to-mobilize type of bone

Simkiss (1967) stated that both types of bone (CoB and MB) are different in terms of ease of Ca mobilization. Wilson et al. (1993) showed a very active remodelling of MB (Figure 14). The MB is metabolically very active and in continuous turnover compared to the more inert CoB (Taylor, 1970; Candlish, 1971). Candlish (1971) compared the rate of metabolism of both type of bones (structural and medullary) and concluded that MB can be metabolized 10 to 15 times faster than CoB. MB has a cell cycle, which is about 25% of the cortical bone, and it is therefore a much more active remodelling system (Dacke et al., 1993). Even though the mineral structure is similar, the organic phase is different between both types. According to Kim et al. (2007), the MB has a three times higher mineral to collagen ratio and a higher non collagenous protein content than CoB. The latter could be a reason why the MB can mobilize or restore much faster than a structural type of bone. In addition, Knott & Bailey (2010) explained that this rapid turnover is made possible by the difference in collagen cross-link profile. MB has a lower concentration of mature collagen cross-links and a higher level of immature cross-links.

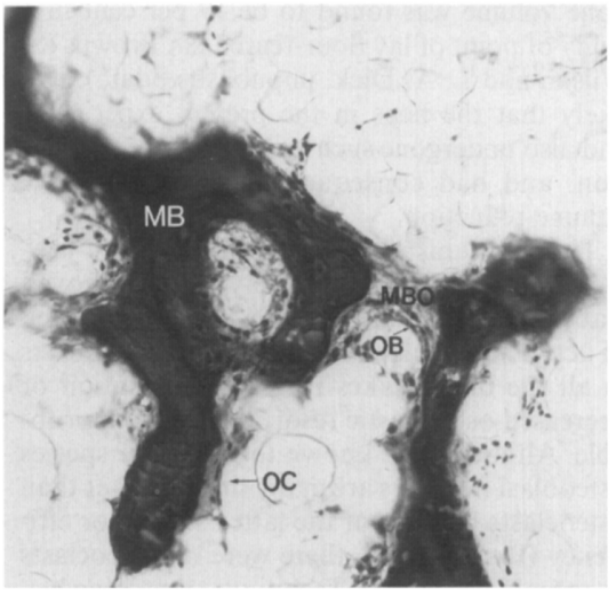

Figure 14. Undecalcified section of proximal tarsometatarsus (Wilson et al., 1993).

Ca mobilization from the CoB and CaB

MB is the favoured reserve of Ca for eggshell formation. However, in the situation of long- term Ca deficiency, the structural bone and especially the CoB can suffer depletion (de Vries et al., 2010). Due to its rich vascular network and its large surface area, the MB is very sensitive to small variations of circulating PTH. However, when PTH concentrations heavily increase, for instance during periods of prolonged Ca deficiency, the MB response to PTH reaches its maximum and PTH has profound effects on CoB (Taylor, 1970). As a consequence, if the hens are fed with highly deficient Ca diets (0.054% Ca) and keep laying, it is the structural bone which suffers attrition (Taylor & Moore, 1958). Taylor & Moore (1954) also indicate that when hens are fed with Ca deficient diets during 7 days, the osteoclastic activity and the volume of MB remained unaffected, only the CoB was depleted. However, during prolonged Ca deficiency, the hen responds by increasing the size of the medullary reservoir at the expense of the less labile CoB. This theory was confirmed by Dacke et al. (1993) and also indicated that MB is restored from structural bone. Once depleted, CoB can most likely not be repaired.

Recent publications also state that even in normal Ca diets, the structural bones are also subject to resorption. The osteoclasts are not specific to MB and the large number of osteoclasts can also resorb Ca from cortical and trabecular types of bone (Whitehead, 2004; Fleming, 2008).

2.2.5 Hormonal regulation of the bone

The bone mass is maintained through a balance between osteoclast and osteoblast activity. The osteoblasts have specific receptors for PTH and 1,25-$(OH)_2D_3$ and both hormones regulate the ALP activity and the transport of Ca^{2+} in and out of the bone.

According to Dacke et al. (1993), the osteoclast and osteoblast activity is mainly regulated by two hormones: the calcitonin and the Parathyroid Hormone (PTH). The main effect of PTH on

bone is undoubtedly to increase resorption and when the Ca plasma level is low, PTH indirectly stimulates osteoclastic bone resorptive activity and reduces osteoblastic activity. PTH, vitamin D_3 metabolites and prostaglandin E_2 (PGE_2) increase osteoclast ruffled borders, clear zones and cell size. The PTH does not effect osteoclasts directly because most researchers report an absence of PTH receptors on osteoclasts and most likely causes the retraction of osteoblasts, allowing the osteoclasts to develop their processes (Dacke et al., 1993). In contrast, according to Dacke et al. (1993), calcitonin acts as an inhibitor of bone resorption by inducing cellular contraction and reducing migration of osteoclasts.

This is showed in terms of osteclasts activity by van de Velde et al. (1984). During the active period, although the total number of osteoclasts remained constant, the percentage of active osteoclasts increases from 9.8 to 63.9%, and the resorption surface per osteoclast increased from 23.6 to 33.4 µm.

2.2.6 Extra information

Bone and eggshell quality

Buss & Guyer (1984) conducted a study to compare the differences in bone parameters in hens from layer breeds producing thick eggshells and from layer breeds producing thin eggshell. They found no difference between the two lines for bone ash and Ca content of bones and therefore concluded that bone metabolism is not a cause of thick or thin eggshell and is not involved in the Ca-related deficiencies in the thin shell line. According to the authors, the hens from thick lines deposited Ca at a faster rate than hens from thin lines. As the bone parameters do not differ between both lines, the hens from thick lines have a more efficient absorption of Ca from the intestinal lumen. Whitehead (2004) also explains that there is no direct relationship between medullary bone volume and eggshell quality. In the early period of lay, the fast rate of trabecular bone resorption is explained by the high mobilization of Ca from structural bone to form the early eggshells. Shell quality is good at the very start of the lay, even when little medullary bone has been formed.

P content in bone

Rennie et al. (1997) indicate that there is no difference in the volumes of structural bone or MB when fed P-deficient diets (4.5g tP/kg) compared to diets with normal P levels (6g tP/kg). Bone was histological normal with no evidence of thickened osteoid seams, indicating that the lower concentration of dietary P did not result in osteomalacia.

Bone and forms of Ca

Later in this study, we will focus on the effect of Ca forms on P requirements. However, it is important to state here that feeding birds with particulate forms of Ca can have a beneficial effect on bone characteristics. Fleming (2008) indicated that the number of osteoclasts is reduced when hens are fed particulate limestone by improving dietary intake with a slow overnight release of

Ca. Fleming, McCormack & Whitehead (1998) also proved that providing a particulate source of Ca results in a decreased loss of CaB and increased accumulation of MB, particularly later in lay. Greater particles enhance the MB formation, decrease the structural bone resorption and therefore decrease the severity of some of the characteristics of osteoporosis. It is confirmed by Guinotte & Nys (1991) who showed that feeding particulate forms of Ca improved the bone breaking strength of egg-laying hens aged from 64 to 77 weeks.

Osteoporosis

As shown earlier, MB is mobilized to supply Ca for the eggshell formation but the osteoclasts can also resorb CoB and CaB (Fleming, 2008). Osteoporosis occurs when there is a net resorption of structural bone (Whitehead & Fleming, 2000). In older hens in particular (>40 weeks of age) osteopenia and the risk of osteoporotic fractures increase (Fleming, 2008). Figure 15 shows the proportional variations of structural bone and MB over the lifetime of a hen. We can observe a gradual loss of CaB replaced by MB in the PTM.

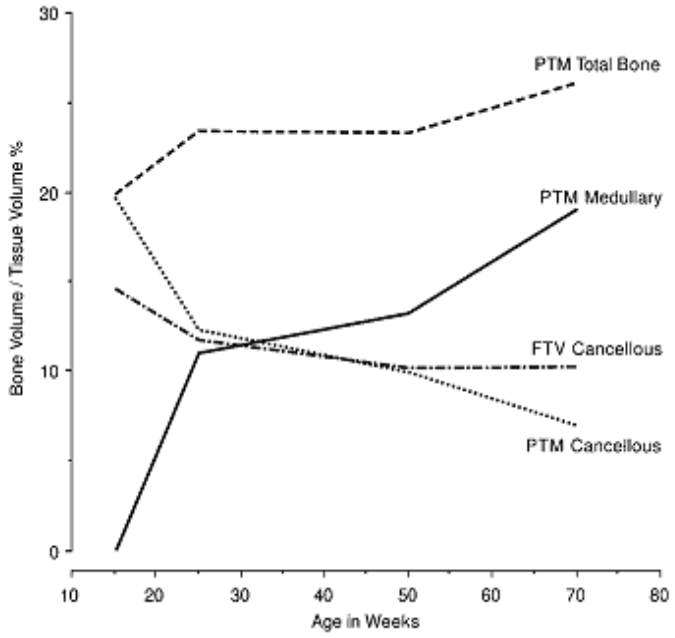

Figure 15. Change with age in the volumes of different types of bone in the proximal tarsometatarsus (PTM) and free thoracic vertebra (FTV) in the caged laying hen (Whitehead & Fleming, 2000).

Over time, the hen presents less structural bone and more MB. Unfortunately, according to Fleming et al. (1998) and Knott et al (1995), the MB contribution to mechanical strength is small compared to CoB and CaB. Figure 16 shows the mean trabecular bone volume and the MB volume in relation with the grade of osteoporosis. For example, the grade 0 which corresponds to "no osteoporosis" is associated with low amount of MB and high volume of structural bone.

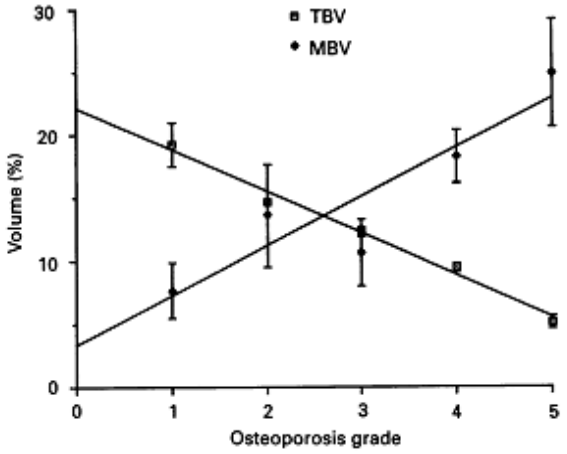

Figure 16. Mean TBV and medullary bone volume (MBV) for each grade of osteoporosis (Knott et al., 1995).

The effect of age on bone is still unclear at this stage and will be discussed later. In brief, the hens dispose of a secondary MB which acts as a labile source of Ca. At the end of the day, when Ca needs become larger than Ca supply from the gut, the MB is favoured to mobilize Ca from its mineral matrix. However, only the degree of calcification is affected, not the volume. In addition, it is well known that MB does not contribute to the bone strength of the birds unlike structural types of bone. The MB is favoured compared to cortical and cancellous bone due to its different collagen organization which makes it easier to resorb and fill in.

However, in case of profound or longer Ca deficiencies, the structural types of bone are favoured to mobilize Ca resulting in a reduction in bone volumes. This complex bone depletion contributes to reduce the breaking strength of the bones and lead to osteoporosis.

How the bone responds to Ca or P deficiency has already been discussed in this chapter but the hen is capable of stimulating also the intestine and the kidney to compensate inadequate supply.

2.3 P and Ca transport and regulation

2.3.1 Concentrations of P and Ca in the blood

According to Maynard et al. (1979), extracellular P varies from 40mg/L to 90mg/L of blood. P absorption increases as well during periods of eggshell formation, although not as strikingly as Ca absorption (Hurwitz and Bar, 1965). Frost & Roland (1990) indicated that the plasma P varies from 44.0 mg/L at oviposition to 52.7 mg/L at 10h post oviposition. The increase of plasma P in the first hours of shell calcification is directly related with the mobilization of Ca form the medullary bone for shell formation. According to the poultry site, the volume of blood in a bird of 2kg is 0,33lbs which corresponds to approximately 150mL of blood volume. As the P content in the blood varies between 40mg/L and 90mg/L, the total pool of P present in the blood can range between 6 and 13,5mg.

The extracellular pool of Ca is higher in egg-laying species (200 to 300mg Ca/L) than in growing birds in order to ensure a high flow of Ca to the eggshell (Maynard et al., 1979; Etches, 1987).

2.3.2 Hormones involved in P and Ca regulation

Ca and P homeostasis are both maintained through a complex mechanism involving modulating hormones. The three major molecules playing a role in P and Ca metabolism are vitamin D, calcitonin and PTH (Bar, 2009; de Vries et al., 2010).

Vitamin D_3

Vitamin D_3, also named cholecalciferol, is a fat-soluble vitamin that is found almost exclusively in animals (Veum, 2010). Vitamin D_3 is necessary to the bird to absorb, transport and utilize Ca and P. According to Frost & Roland (1990), vitamin D_3 is transported to the liver and hydroxylated to form 25-hydroxyvitamin D_3. This metabolite is transported to the kidney and further hydroxylated to form 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$, as shown in Figure 17). This hormone is named calcitriol and is classified as a secosteroid hormone because of its functional roles in Ca and P metabolism (Veum, 2010).

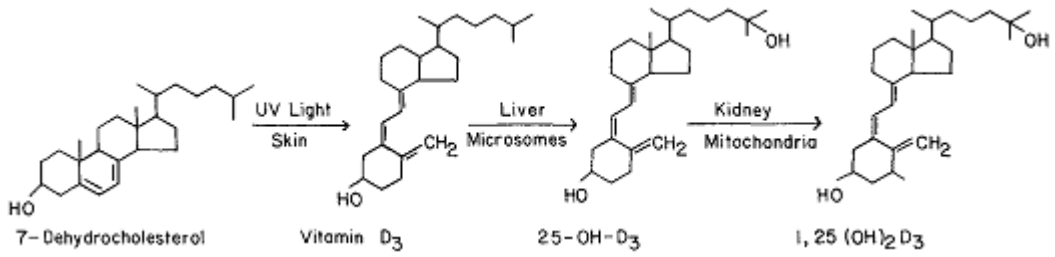

Figure 17. Process of 1,25-dihydroxycholecalciferol production (Soares, 1984).

Parathyroid hormone (PTH)

PTH is a hormone which consists of 84 amino acids with a half-life of 5 minutes. The hormone is produced in the chief cells of the parathyroid gland and his secretion is directly influenced by Ca levels. The Ca binds to the Ca receptor, linked to phospholipase C on the chief cells. This binding reduces the transcription of PTH (Galitzer et al., 2009). In poultry, PTH responds very rapidly on changes in plasma Ca levels, while 1,25-$(OH)_2D_3$ responds much slower. Even though, the understanding of the interrelations between 1,25-$(OH)_2D_3$ is not complete yet. It is likely that 1,25-$(OH)_2D_3$ generally suppresses PTH secretion (Jones et al., 1998; DeLuca, 2008).

Calcitonin

Calcitonin is a hypocalcemic hormone which is produced in the ultimobranchial gland. The breakdown of this hormone occurs in the liver and the kidney (Brook & Marshall, 2001).

Calcitonin directly inhibits osteoclastic activity by reducing the motility of the osteoclasts (Dacke et al., 1993).

2.3.3 Ca transport and regulation

The mechanisms of Ca transport (Bar, 2008; 2009) and regulation (Dacke et al., 1993; Veum, 2010) have been very recently reviewed. The following chapter is mostly based upon those publications with inputs from earlier studies. Ca transport, regulation and deposition are treated in three different parts.

In laying birds, a third pathway via the eggshell gland drains Ca^{2+} and induces a high demand of Ca^{2+}. As stated by Kebreab et al. (2009), the demand of Ca^{2+} from the eggshell gland is the highest when the intestinal lumen becomes completely empty of Ca. Therefore, two mechanisms are enhanced to compensate for the lack of Ca. First, the absorption of Ca from the intestine is enhanced. Secondly, as already discussed in the bone chapter, an active bone resorption takes place (Bar, 2008).

Ca absorption

In terms of absorption efficiency, an increase of dietary Ca reduces relative the rate of absorption but increases the absolute volume of absorption (Bar, 2009). The Ca^{2+} absorption in the laying hen is more intense during the period of shell calcification than during the inactive period. The Ca transport during the active period (shell formation) accounts for net absorption of about 80% of the dietary Ca^{2+} intake in hens while much less (36%) is absorbed during the period of inactivity (Bar, 2008, 2009).

The apparent absorption of Ca takes mostly place in the duodenum and jejunum of the small intestine (Hurwitz & Bar, 1965; Hurwitz, 1996). It is confirmed by Bar (2009) who states that the proximal intestine appears to have greater capability than the distal intestine to absorb Ca^{2+}. Most of the Ca is absorbed before it reaches the lower ileum, most likely as a result of the high efficiency of the proximal intestine in absorbing Ca^{2+}.

Ca absorption occurs both by diffusion (passive transport) and active transport (energy-dependent transport; Hurwitz, 1996). In poultry, up to 50% of the dietary Ca^{2+} is absorbed by passive diffusion. Proportion of active transport is greater in situation of longer and larger plasma Ca^{2+} deficiency (Veum, 2010). According to Bar (2009), the transcellular mechanism (active transport) acts predominantly at the proximal intestinal segments, the duodenum and jejunum, whereas the paracellular transport (passive transport) occurs along the whole length of the intestine, driven by the electrochemical potential difference (ECPD). The paracellular transport is more important in animals fed adequate Ca^{2+}.

Ca transport

Vitamin D appears to be the most important factor modulating Ca^{2+} transport. Its metabolite form, 1,25-$(OH)_2D_3$, binds to vitamin D receptors (VDRs) and then stimulates the synthesis of

RNAs coding for several vitamin D-dependent proteins such as calbindin D_{28k}, Plasma Membrane Ca^{2+}ATPase (PMCA) and epithelial Ca channels (TRPVs). In addition, other proteins appear to be vitamin D dependent: osteocalcin, osteopontin (OPN), collagen type I and carbonic anhydrase II (CAH; Bar, 2008). Another group of proteins is involved in Ca regulation, the Na^{+}/Ca^{2+} exchangers. All the aforementioned proteins are involved in transcellular transport but a last group is involved in paracellular transport, the tight junction proteins (Bar, 2009).

Vitamin D receptors (VDRs)

The stimulation of Ca-transport proteins by 1,25-$(OH)_2D_3$ is operated through the binding to VDRs. Following the binding of 1,25-$(OH)_2D_3$ to a VDR, the receptor is phosphorylated and thereby simulates the RNAs coding for osteocalcin, osteopontin, calbindins, 24-hydroxylase, TRPVs and Carbonic Anhydrase (CA). The VDRs level is markedly increased during maturation, prior to the onset of egg production in order to allow the development of the Ca transport mechanisms. In the laying hen, the EggShell Gland (ESG) VDRs concentration represents 33% to 20% of the intestinal VDR content which indicates a lower dependency of the ESG on the vitamin D_3 metabolites compared to the intestine (Bar, 2008; 2009).

Calbindins

In the bird, high levels of calbindins, previously named Ca-binding proteins, are found in the intestine, the ESG and the kidney. The calbindins are considered to facilitate the movement of Ca^{2+} inside the epithelial cells of the Ca-transporting organs (intestine, kidney and ESG). Moreover, calbindins may act as a buffer, by maintaining a low Ca^{2+} concentration in close proximity to the TRPVs pores, and thereby ensuring the “downhill” movement of Ca^{2+} into the cell. However, differences exist among the three organs regarding calbindin metabolism. Whereas the calbindin contents in the kidney and in the ESG are correlated with the mass of Ca transported (weight unit), the intestinal calbindin is correlated with Ca transport capability (% of absorption). In addition, whereas intestinal calbindin reflects the change in intestinal change of 1,25$(OH)_2D_3$ in the intestinal cell, the ESG calbindin is not a vitamin D dependent protein.

Plasma Membrane Ca ATPase (PMCA)

PMCA uses the energy stored in ATP to extrude Ca^{2+} out of the cell against the electrochemical gradient. It is has been clearly demonstrated that intestinal and renal PCMA are modulated by vitamin D. Nevertheless, as for ESG calbindin, ESG PMCA is not dependent on 1,25-$(OH)_2D_3$.

Epithelial Ca channels (TRPVs)

Among the Transcient Receptor Potential Vanilloid family, TRPV5 and TRPV6 are known to facilitate the entry of Ca^{2+} into the epithelial cells of the Ca-transporting organs. Most findings support the idea that TRPVs are vitamin D dependent.

Carbonic Anhydrase

CAH is involved in bone resorption, bone calcification and Ca transport.

Na^{+}/Ca^{2+} exchange

The Na+/Ca2+ exchange mechanism is another transporting system involved in the “uphill” extrusion of Ca^{2+} (Bar, 2009).

Osteopontin (OPN)

OPN is a glycosylated, highly phosphorylated protein expressed in bone and ESG. OPN appears to affect migration and maturation of osteoclasts precursors, osteoclast activity and is also involved in eggshell formation even though its role is not clearly defined (Bar, 2009).

The transcellular transport consists of three major steps. First, the entry of Ca2+ through the brush border is ensured by TRPV6 and, to a lesser extent, TRPV5. Secondly, the diffusion or movement to the basal membrane is facilitated by intestinal calbindins. Finally, the energy-dependent extrusion through the basal membrane results from the low cellular Ca2+ concentration and the higher plasma Ca2+ concentration. This step is facilitated by PMCA. The entire process is dependent on vitamin D because every step involves vitamin D dependent proteins.

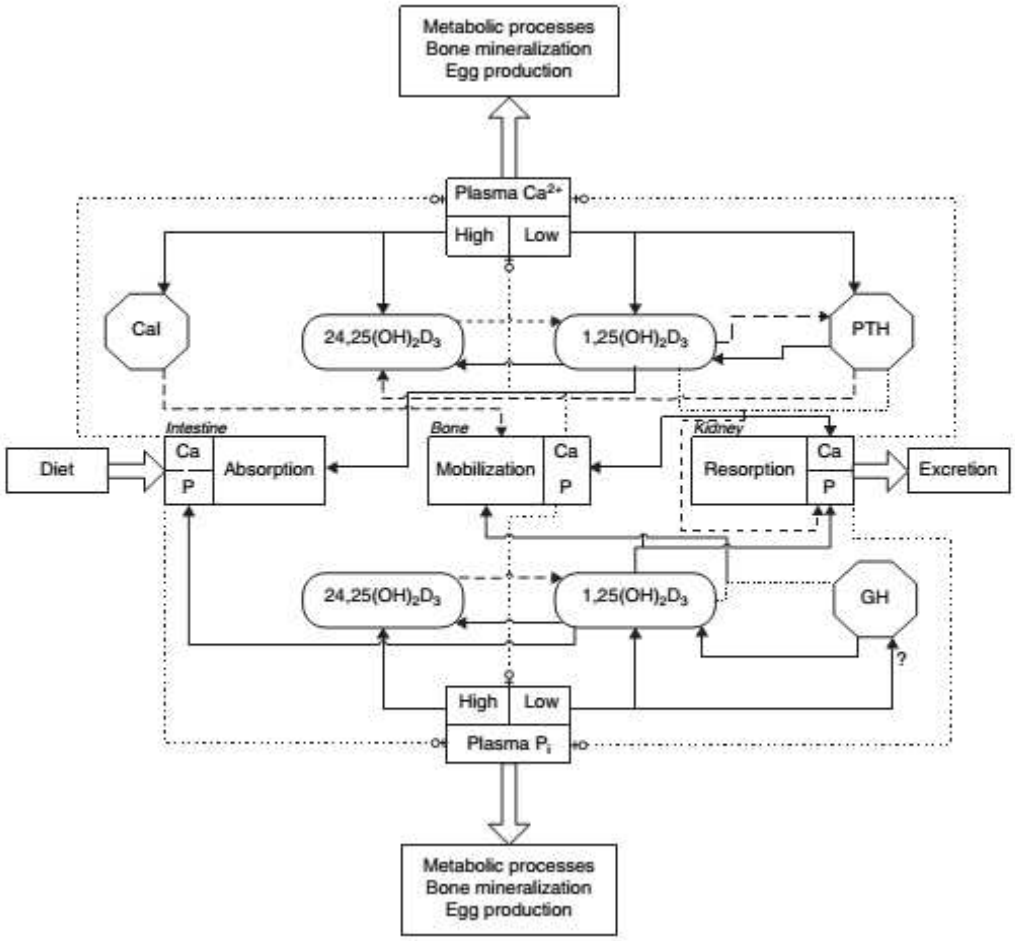

Figure 18. Ca and P homeostasis in the laying hen (De Vries, 2010).

Ca regulation

Ca changes in plasma levels are detected by Ca^{2+}-sensing receptors (Bar, 2008). The upper part of Figure 18 reports the different mechanisms of Ca regulation in case of low or high plasma Ca.

Hypocalcaemia

Hypocalcaemia occurs when body Ca losses exceed absorption, for example, in case of Ca deposition into the eggshell (DeLuca, 2008). In case of low plasma Ca, the parathyroid gland is stimulated to secrete PTH, the peptide hormone that responds to short term perturbations (Bar, 2008; Veum, 2010). In case of longer perturbations of Ca concentrations, PTH stimulates the hydroxylation of 25-OHD_3 to 1,25-$(OH)_2D_3$ in the kidney, in association with 17-β-oestradiol, prolactin and GH (de Vries et al., 2010; Veum, 2010). This hydroxylation is also stimulated directly by lower plasma Ca. 1,25-$(OH)_2D_3$ has three main roles on the Ca metabolism. In the intestine, its secretion increases the efficiency of absorption of Ca. In the bone, 1,25-$(OH)_2D_3$ stimulates, in association with the action of PTH, the resorption of the medullary bone to release Ca. Thirdly, 1,25-$(OH)_2D_3$, together with PTH, stimulates the absorption of Ca in the kidney, consequently reducing Ca excretion (de Vries et al., 2010; Veum, 2010). In the kidney, about 96

to 99% of the Ca is reabsorbed during filtration, half by transcellular and half by paracellular diffusion. Most of the reabsorption occurs in the proximal tubule but the regulation takes place in the distal tubule where 10% of the reabsorption takes place. PTH and 1,25-$(OH)_2D_3$ stimulate absorption of Ca^{2+} by the kidney (Veum, 2010). The conversion of 25-hydroxyvitamin D3 (25-$(OH)D_3$) into 1,25-$(OH)_2D_3$ is homeostatically regulated by plasma $Ca^{2+,}$ the secretion of PTH (Soares, 1984). However, when the levels of Ca are adequate to support eggshell deposition, 1,25-$(OH)_2D_3$ stimulates bone mineralization (de Vries et al., 2010).

Hypercalcaemia

On the contrary, high dietary levels of Ca supplied to the hens result in an increase in plasma Ca^{2+} which induces the release of calcitonin from the ultimobranchial gland. In case of hypercalcaemia, calcitonin blocks the resorption of the Ca from the bone in order to reduce the concentration of Ca^{2+} in the blood (de Vries et al., 2010; Veum, 2010). Calcitonin is secreted in laying birds when the plasma level of ionic Ca exceeds 1.5mmol/l (Baimbridge & Taylor, 1980). Ca excretion is also increased whereas the formation of 1,25-$(OH)_2D_3$ is inhibited. The reduction of 1,25-$(OH)_2D_3$ metabolism leads to a reduced efficiency of Ca intestinal absorption (Shafey, 1993). High Ca^{2+} levels also induce the production of 24,25-$(OH)_2D_3$ from 25-$(OH)_2D_3$ and from 1,25-$(OH)_2D_3$ (24-hydroxylation; Veum, 2010; de Vries et al., 2010).

Prostaglandins (PGs)

As PGs, and in particular PGE_2, are drastically more elevated during eggshell formation and falls after oviposition, it is likely that they are involved in the process of Ca mobilization in avian species. The understanding of the role of PGE_2 on bone resorption is not complete yet (Dacke et al., 1993).

<u>Ca deposition into the eggshell</u>

The eggshell is mostly composed of Ca^{2+} and HCO_3^- bound together to form a solid matrix of Ca carbonate. Their transport into the eggshell by the ESG is fluctuating over the egg-formation cycle. The peak of transport occurs 11 to 12h after ovulation and ends 22 to 24h after ovulation. Most of the ESG proteins, egg membrane and shell matrix are formed in the ESG. Some of the constituents are structural proteins (collagen) and others have more functional properties (CA, calbindins, PCMA, VDR). A significant correlation appears between uterine calbindin and shell Ca because hens laying shell-less eggs have a lower uterine calbindin content (Bar et al., 1984; Nys & de Laage, 1984) and also show a lower ATPase activity (Castaldo & Maurice, 1990).

Eggshell calcifaction takes place by transcellular or paracellular transport of Ca^{2+}.Two of the three proteins involved in transcellular transport are present un high concentrations in the ESG (calbindin, PMCA). It indicates an “uphill” transport and the presence of an active transport. Balnave et al. (1992) indicate that Ca transport across the shell gland is an active process that utilizes adenosine triphosphate as the energy source. It is confirmed by Castaldo & Maurice

(1990) who found that the ATPase activity in the shell gland of hens producing eggs with strong shells was greater than that of hens producing eggs with weak shells.

Despite the high concentrations of vitamin-D-dependent proteins such as CA, calbindin, PCMA, VDR or OPN, the vitamin D dependency of ESG transport of Ca^{2+} is not certain. Even though most workers found no interaction, Corradino et al. (1968), on the basis that hens fed a vitamin D-deficient diet, showed lower ESG calbindin, suggesting a possible dependency. The non-dependency of the Ca transport proteins on vitamin D shows that the ESG capacity to transport Ca^{2+} is not modulated by vitamin D metabolism or Ca^{2+} homeostasis. In addition, most workers tend to show that CA is the driving force for Ca^{2+} deposition.

2.3.4 P transport and regulation

P absorption

As well as Ca, P absorption mostly takes place in the duodenum and jejunum (Hurwitz & Bar, 1965; Veum, 2010). Moreover, as indicated by Wasserman & Taylor (1973), the absorption of P occurs more rapidly in all parts of the small intestine than Ca. They also state that P absorption is higher in the duodenum than in the jejunum (Figure 19) and that the rate of P absorption in the jejunum is linearly correlated with the P concentration in the duodenum.

Segment[1]	Vit. D_3	Absorption	In gut tissue	Transferred to body
		%	%	%
Duodenum	−	79 ± 2[2]	59 ± 1	20 ± 1
Duodenum	+	84 ± 2	63 ± 1	26 ± 2
		(NS)	(NS)	($P < 0.025$)
Jejunum	−	76 ± 4	63 ± 3	13 ± 2
Jejunum	+	91 ± 4	61 ± 2	31 ± 2
		($P < 0.01$)	(NS)	($P < 0.001$)
Ileum	−	47 ± 7	34 ± 4	13 ± 5
Ileum	+	75 ± 4	49 ± 1	26 ± 3
		($P < 0.01$)	($P < 0.005$)	($P < 0.05$)

[1] Mean gut lengths: duodenum = 9.1 cm.; jejunum = 22.7 cm.; ileum = 14.0 cm. [2] Values represent mean ±SEM of six chicks.

Figure 19. Phosphate absorption from different intestinal segments (Wasserman & Taylor, 1973).

P is absorbed in the small intestine by two different pathways: paracellular phosphate transport (passive diffusion) and active transport through the sodium-dependent phosphate co-transporters. The paracellular phosphate transport depends on electrochemical gradients across the epithelial layer. This paracellular movement occurs through tight junctions consisting of proteins including claudins and occludins. Beside this passive transport, a sodium-dependent active transport takes place and contributes to 30 to 80% of total transport. The ratio between active and passive transport is highly dependent on the phosphate concentration. The major phosphate transporter found in the intestine is the Npt2b from the type II sodium dependent family. According to various studies, Npt2b accounts for between 45 to 90% of the total phosphate transport (Sabbagh et al., 2011).

Diets containing low P content result low absolute plasma P and therefore higher relative P absorption rate (Bar & Hurwitz, 1984; Rao et al., 1991; Frost et al., 1991). The study from Frost et al. (1991) showed that lower dietary P induced lower plasma P (mg/dL; Figure 20). It is confirmed by the study of Bar & Hurwitz (1984) in which hens fed a low P diets showed a lower plasma P (mg/dL). They also demonstrated that the low plasma P levels resulted in a higher P absorption (% of intake; Figure 21). As a consequence, P absorption from the intestine increases at low levels of P supply which might partly compensate for the reduced P intake.

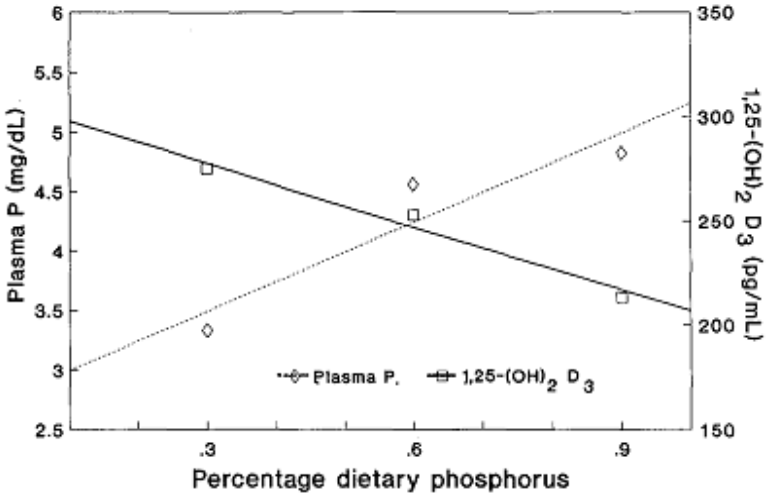

Figure 20. The effect of dietary P on plasma P and $1,25(OH)_2D_3$ (Frost et al., 1991).

	3.58% Ca, .67% P	3.58% Ca, .22% P
Feed intake, g/day	103	69
Egg production, %[2]	78.1 ± 4.3[a]	60.0 ± 4.3[b]
Egg weight, g[2]	62.9 ± .9[a]	60.5 ± .8[b]
Shell weight, mg[3]	5300 ± 139[a]	4344 ± 174[b]
Shell density, mg/cm^3 [2]	71.6 ± 2.0[a]	60.4 ± 2.0[b]
Plasma Ca, mg/dl[3]	26.6 ± 1.2[a]	24.3 ± 1.8[a]
Plasma P_i, mg/dl[3]	4.7 ± .4[a]	1.6 ± .7[b]
Intestinal CaBP, mg/g[3]	2.02 ± .25[a]	2.88 ± .10[b]
Ca Absorption, % of intake[3]	28.0 ± 4.3[a]	55.7 ± 4.2[b]
P Absorption, % of intake[2]	29.6 ± 2.8[a]	52.4 ± 4.5[b]

[a,b]Means designated by different superscripts are significantly different (P<.05).

[1] Hens were fed the experimental diets for 39 days. They were bled and killed during a period of ESG inactivity.

[2] Mean ± SEM of 18 hens.

[3] Mean ± SEM of 6 hens that laid at least 5 eggs during the 7-day period prior to the assays. P_i = Inorganic phosphorus.

Figure 21. The effect of dietary P restriction on egg production, egg shell quality and on Ca and P absorption in laying hens (Bar & Hurwitz, 1984).

P regulation

P, as well as Ca homeostasis, is maintained through a complex regulatory system although according to Veum (2010), there is less regulation of P than for Ca. The effects of PTH and vitamin D might be secondary to those on Ca. An improvement of Ca absorption reduces the opportunity of Ca to form unabsorbable phytates in the gut and therefore reduces the P availability.

Three major organs are concerned by P regulation. Firstly, in the intestine, when plasma P is low the concentration of $1,25(OH)_2D_3$ increases in the blood (Figure 20). $1,25(OH)_2D_3$ improves the efficiency of P absorption via up regulation of the Ntp2b protein when plasma P levels are low. Npt2b is also found to be stimulated by a low dietary P level through a post- transcriptional system. However, the Npt2b protein is not sensitive to PTH and this might explain why PTH does not have any influence in the P metabolism in the intestine (Sabbagh et al., 2011).

Secondly, together with Growth Hormone, $1,25(OH)_2D_3$ stimulates the bone resorption of Ca and P in case of low plasma P (de Vries et al., 2010).

Thirdly, in case of low plasma Ca, the $1,25(OH)_2D_3$, together with PTH, has a suppressing effect on renal P resorption. As a consequence, $1,25(OH)_2D_3$, associated with PTH increases P excretion in urine and reduces plasma P level. However, in the situation of low plasma P, there is a secretion of $1,25(OH)_2D_3$but not of PTH (Deluca, 1988; Rao et al., 1991). The findings indicate that, in the absence of PTH, $1,25(OH)_2D_3$stimulates renal resorption and therefore reduces P excretion. The mechanisms behind this paradox are still unknown (de Vries et al., 2010).

About 85-90% of the P is reabsorbed during glomerular filtration in the kidney with about half by paracellular and half by transcellular diffusion. When plasma P is elevated, vitamin D induces the excretion of P in the kidney to correct the hyperphosphatemia (Edwards, 1973; Veum, 2010).

As indicated by Bar & Hurwitz (1984) and reviewed by Bar (2008), low plasma P do not increase the renal activity of 25-hydroxyvitamin D3-l-hydroxylase enzyme, responsible for the synthesis of $1,25(OH)_2D_3$ (Figure 22). However, most findings indicate that in the situation of low P levels, there is an increase of $1,25(OH)_2D_3$ in the plasma and target tissues (Newman & Leeson, 1997; Bar, 2008). As a consequence, the mechanism inducing stimulation of $1,25(OH)_2D_3$ by low plasma P levels is still uncertain (de Vries et al., 2010). This could be due to the fact that low plasma P levels induce 1-hydroxylation of $25\text{-}(OH)D_3$ not in the kidney but in the intestine or the bone (de Vries et al., 2010).

	3.58% Ca, .67% P	1.70% Ca, .67% P	3.68% Ca, .33% P	
	24[2]	24[2]	24[2]	34[2]
Egg production, %[3]	97.8 ± 2.8	85.7 ± 4.3	90.0 ± 2.8	82.9 ± 7.1
Plasma Ca, mg/dl	26.1 ± 1.6	18.1 ± .8	24.1 ± .8	21.6 ± .7
Plasma P_i, mg/dl	5.2 ± .3[a]	4.0 ± .6[ab]	2.8 ± .5[b]	1.6 ± .8[b]
CaBP, mg/g:				
Intestinal	1.94 ± .08[a]	2.74 ± .18[b]	1.77 ± .2[a]	2.85 ± .43[b]
ESG	1.38 ± .12	1.55 ± .06	1.43 ± .02	1.37 ± .01
Kidney	.29 ± .02[a]	.27 ± .02[a]	.56 ± .06[b]	.46 ± .04[b]
Kidney 1-hydroxylase, pmole/g/15 min	20.9 ± 5.0[a]	51.9 ± 8.9[b]	15.1 ± 4.6[a]	19.8 ± 4.7[a]
Tibia ash, mg/bone				
Structural	2997 ± 89	2745 ± 142	1748 ± 127[a]	2805 ± 60[a]
Medullary	165 ± 16[a]	173 ± 14[a]	84 ± 8[b]	106 ± 12[b]

[a,b]Means designated by different superscripts are significantly different (P<.01).

[1] Means ± SE of eight hens.

[2] Day of treatment.

[3] The hens selected for this trial laid at least five eggs during the 7-day period prior to the assays. Therefore, any practical comparison between these means should not be made.

Figure 22. Effect of dietary Ca and P restriction on bone ash, egg shell gland, intestinal and kidney Ca-binding protein, and on kidney hydroxylase activity of laying hens (Bar & Hurwitz, 1984).

Unlike Ca regulation, low plasma P does not induce a secretion of PTH but GH to increase plasma P levels. The maintenance of P homeostasis only involves $1,25(OH)_2D_3$ which acts less rapidly than PTH. We can conclude that the response time of the P mechanism is high compared to Ca^{2+} mechanism (de Vries et al., 2010).

Both high plasma Ca^{2+} and P induce the production of another derivate of vitamin D, the $24,25(OH)_2D_3$ from $25(OH)_2D_3$ but also from $1,25(OH)_2D_3$ (24-hydroxylation). The effects of this molecule on P and Ca^{2+} are not well understood but it is clear that this metabolite can affect vitamin D regulation (Jones et al., 1998).

Many other molecules have not been discussed here for several reasons: 1) Some have not been specifically discussed in egg-laying birds in the literature: thyroid hormone, PTHR1, cytokine KB ligand, norepinephrine, insulin-like growth factor 1; 2) Some might not have a clear effect on P and Ca metabolism: Indian hedgehog protein, fibroblast growth hormone, bone morphogenetic proteins, epidermial growth factor, glucocorticoids, matrix extracellular phosphoglycoprotein, leptin.

It is clear that P regulation has been much less subject of study than Ca regulation. This can be explained by the strong dependency of P metabolism on Ca. In addition, P regulation is known to be less efficient than Ca regulation. For instance, P is only regulated by the slow-process vitamin D, and not by the PTH. In case of high levels of P, only a stimulation of P excretion can reduce the plasma P.

3 Factors influencing P requirements

3.1 Dietary effects

3.1.1 Phosphorus

It is generally admitted that a great majority of the P present in cereal grains and seeds is bound to phytic acid as Phytate P (PP; Eeckhout & De Paepe, 1994). However, PP has a low availability in monogastric animals, unless their feed contains phytase enzyme to hydrolyze it (Nahm, 2008). Approximately 60 to 75% of P in commonly used feedstuffs are not available for digestion by the hen (NRC, 1994). The available P content of a diet is dependent on three parameters: the PP content, the non-PP content and the phytase content (Eeckhout & De Paepe, 1994).

PP content

Generally, maize-soybean diets in poultry contain about 2.5g/kg phytate-P or 8.9g/kg phytic acid (Selle and Ravindran, 2007; cited by Lei et al. (2007)). According to Eeckhout & De Paepe (1994) and as presented in Figures 24 and 25, the PP content (%) of cereals is as follow: triticale (0.25), wheat (0.22), rye (0.22), barley (0.22), oats (0.21), sorghum (0.19) and maize (0.19). Wheat by-products have the highest PP content: wheat fine bran (0.72), wheat fine brand pelleted (0.78) and wheat midllings (0.53). Those data are similar to the findings of Cromwell (1992; cited by Nahm (2008)). However, Even though all the authors used the same method (Haug and Lantzsch method (1983) some feedstuffs have controversial amount of PP. For example, PP/tP ratio in rapeseed meal is estimated to 17% (Hopkins et at., 1989; cited by Eeckhout & De Paepe (1994)), 36% (Eeckhout & De Paepe, 1994) or 67% (Lantzsch, 1989; cited by Eeckhout & De Paepe (1994)). The corresponding values for sunflower meal are 32%, 44% and 75%, while for soybean meal the respective values are 43%, 53% and 56%. PP content can be predictably determined in function of total P content as the two parameters are significantly correlated, but only in wheat, wheat by-products, maize and maize by-products (Figure 23). However, phytase content cannot be predicted from total P content in any of the available feedstuffs (Eeckhout & De Paepe, 1994).

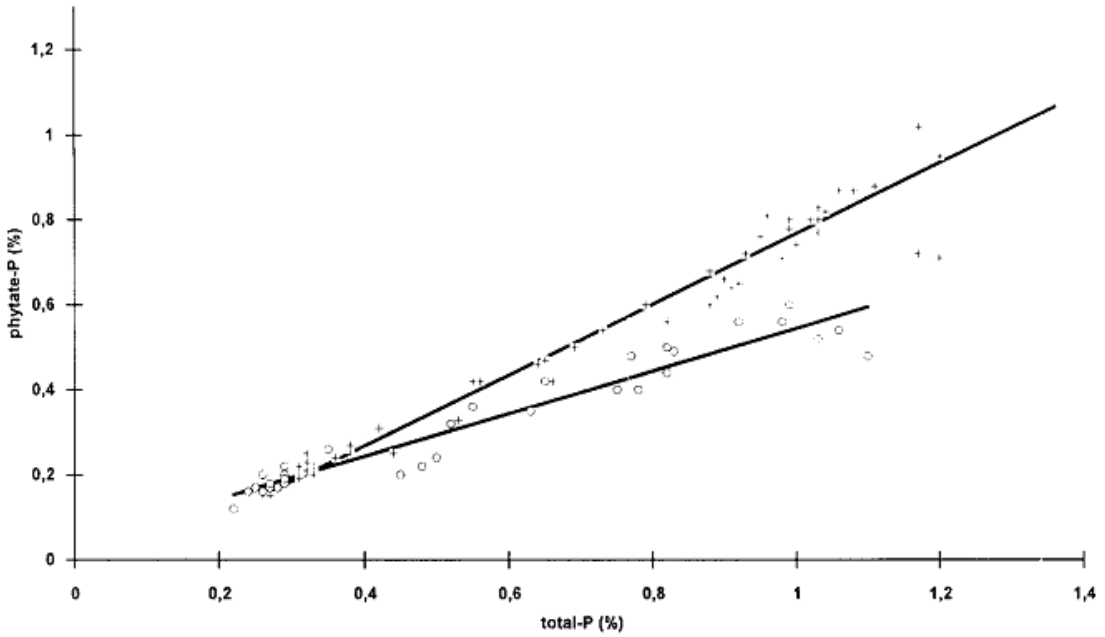

Figure 23. Linear relationship between PP (%) and tP (%) for wheat and wheat by-product (+) and maize and maize by-products (Eeckhout & de Pape, 1994).

	n	Total P (%) mean ± SD (range)	Phytate-P (%) mean ± SD (range)	(Phytate-P/ total P) × 100 mean ± SD (range)	Phytase (units kg^{-1}) mean ± SD (range)
Seeds					
Rye	2	0.36 (0.35–0.36)	0.22 (0.20–0.23)	61 (56–66)	5130 (4132–6127)
Triticale	6	0.37 ± 0.02 (0.35–0.40)	0.25 ± 0.02 (0.22–0.28)	67 ± 3.7 (61–70)	1688 ± 227 (1475–2039)
Wheat	13	0.33 ± 0.02 (0.31–0.38)	0.22 ± 0.02 (0.19–0.27)	67 ± 4.8 (61–78)	1193 ± 223 (915–1581)
Barley	9	0.37 ± 0.02 (0.34–0.39)	0.22 ± 0.01 (0.20–0.24)	60 ± 2.4 (55–62)	582 ± 178 (408–882)
Peas	11	0.38 ± 0.02 (0.36–0.40)	0.17 ± 0.03 (0.13–0.21)	45 ± 6.2 (36–53)	116 ± 54 (36–183)
By-products					
Wheat fine bran	6	0.95 ± 0.06 (0.88–1.03)	0.72 ± 0.08 (0.60–0.81)	76 ± 5.6 (68–84)	4601 ± 860 (3485–5345)
Wheat fine bran (pellets)	15	1.01 ± 0.08 (0.88–1.17)	0.78 ± 0.08 (0.62–0.88)	77 ± 5.6 (62–82)	2573 ± 0.59 (1206–4230)
Wheat middlings	5	0.80 ± 0.25 (0.53–1.20)	0.53 ± 0.14 (0.33–0.71)	66 ± 6.9 (59–76)	4381 ± 956 (2825–5042)
Wheat feed flour	11	0.56 ± 0.20 (0.26–0.91)	0.39 ± 0.16 (0.15–0.64)	70 ± 7.6 (56–76)	3350 ± 1244 (1007–4708)
Wheat bran	5	1.16 ± 0.14 (1.03–1.36)	0.97 ± 0.20 (0.77–1.27)	84 ± 7.3 (75–93)	2957 ± 1556 (1180–5208)
Malt sprouts (pellets)	4	0.60 ± 0.09 (0.52–0.73)	0.01 ± 0.03 (0–0.05)	2 ± 3.4 (0–7)	877 ± 242 (605–1174)
Corn distillers	3	0.90 (0.86–0.96)	0.19 (0.17–0.21)	21 (20–24)	385 (141–850)
Rice bran	2	1.71 (1.37–1.74)	1.10 (1.08–1.11)	64 (62–66)	122 (108–135)

Figure 24. Total P, phytate-P and phytase activity of feedstuffs with phytase activity of more than 100 units/kg (Eeckhout & De Pape, 1994).

Phytase activity

Eeckhout & De Paepe (1994) indicated that cereals such as rye, triticale, wheat and barley have higher phytase activity than others. It is also remarkable how wheat by-products present considerably higher phytase activity than any other feedstuffs (Figure 24). Maize, oats and sorghum have no sufficient phytase activity to hydrolyze phytic acid in appreciable quantity (Figure 25).

Total P, phytate-P and phytase activity of feedstuffs with phytase activity of less than 100 units kg^{-1}

	n	Total P (%) mean ± SD (range)	Phytate-P (%) mean ± SD (range)	(Phytate-P/ total P) × 100 mean ± SD (range)	Phytase (units kg^{-1}) mean ± SD (range)
Cereals					
Maize	11	0.28 ± 0.03 (0.25–0.35)	0.19 ± 0.03 (0.16–0.26)	68 ± 5.9 (61–77)	15 ± 18 (0–46)
Oats	6	0.36 ± 0.03 (0.33–0.40)	0.21 ± 0.04 (0.16–0.28)	59 ± 11 (48–78)	42 ± 50 (0–108)
Sorghum	5	0.27 ± 0.05 (0.20–0.33)	0.19 ± 0.04 (0.14–0.24)	70 ± 6.2 (61–76)	24 ± 32 (0–76)
Maize (moist ensiled)[1]	7	0.30 ± 0.05 (0.24–0.38)	0.13 ± 0.02 (0.11–0.18)	43 ± 5.3 (35–49)	12 ± 11 (0–30)
Cereal by-products					
Maize gluten feed	9	0.87 ± 0.16 (0.63–1.10)	0.47 ± 0.06 (0.35–0.54)	54 ± 6.2 (44–62)	48 ± 68 (0–177)
Maize gluten feed (pellets)	5	0.89 ± 0.10 (0.75–0.99)	0.52 ± 0.08 (0.40–0.60)	58 ± 3.1 (53–61)	5 ± 7 (0–15)
Maize germs (extracted)	1	0.65	0.42	65	16
Maize feed flour	2	0.23 (0.22–0.24)	0.14 (0.12–0.16)	61 (55–67)	5 (3–6)
Maize feed flour (USA)	5	0.50 ± 0.04 (0.45–0.55)	0.27 ± 0.07 (0.20–0.36)	54 ± 9.7 (44–65)	37 ± 30 (0–78)
Rice feed flour	1	0.32	0.23	72	0
Rice bran (extracted)	4	1.89 ± 0.27 (1.57–2.21)	0.79 ± 0.19 (0.69–1.07)	42 ± 9.6 (31–54)	45 ± 67 (0–145)
Wheat gluten feed	6	0.78 ± 0.06 (0.71–0.87)	0.56 ± 0.10 (0.44–0.69)	71 ± 11.0 (39–90)	25 ± 61 (0–150)
Oil meals					
Peanut (extracted) (pellets)	3	0.68 (0.65–0.70)	0.32 (0.30–0.34)	47 (46–49)	3 (0–8)
Coconut (expeller)	4	0.53 ± 0.05 (0.47–0.58)	0.18 ± 0.03 (0.14–0.20)	34 ± 4.0 (30–39)	24 ± 37 (0–80)
Linseed (expeller)	4	0.75 ± 0.02 (0.73–0.78)	0.42 ± 0.02 (0.39–0.43)	55 ± 2.9 (52–58)	5 ± 6 (0–12)
Linseed (extracted)	1	0.82	0.47	57	41
Rapeseed (extracted)	5	1.12 ± 0.04 (1.07–1.17)	0.40 ± 0.05 (0.34–0.48)	36 ± 3.4 (32–41)	16 ± 16 (0–36)
Palm-kernel (expeller)	6	0.59 ± 0.03 (0.55–0.62)	0.39 ± 0.03 (0.33–0.41)	66 ± 3.9 (60–71)	37 ± 31 (0–91)
Sunflower (extracted) (pellets)	11	1.00 ± 0.11 (0.86–1.28)	0.44 ± 0.05 (0.32–0.51)	44 ± 3.9 (35–47)	62 ± 53 (0–185)
Soyabean 44 (extracted)	15	0.66 ± 0.03 (0.61–0.71)	0.35 ± 0.02 (0.33–0.39)	53 ± 2.5 (46–57)	40 ± 45 (0–120)
Soyabean 48 (extracted)	5	0.61 ± 0.01 (0.59–0.62)	0.32 ± 0.02 (0.28–0.33)	52 ± 3.7 (46–56)	8 ± 8 (0–20)
Soyabean 50 (extracted)	9	0.71 ± 0.02 (0.67–0.73)	0.38 ± 0.01 (0.37–0.40)	54 ± 2.2 (51–56)	31 ± 50 (0–149)

Figure 25. Total P, phytate-P and phytase activity of feedstuffs with phytase activity of less than 100 units/kg (from Eeckhout & De Pape, 1994).

A remarkable finding is the reduced phytase activity in pelleted wheat bran compared to non-pelleted one. This might be due to heat treatment during pelleting (Eeckhout & De Paepe, 1994). Plant phytase is heat-labile and may be eliminated when diets are steam- pelleted at high temperatures in excess of 85°C (Selle et al., 2009).

P availability

Kiarie & Nyachoti (2010) investigated the scientific literature to determine the P availability of most feedstuffs in swine, poultry and ruminants. Their findings regarding poultry production are presented in Figure 26.

P in maize is for 21% available to poultry. However, when maize is presented as DDGS, the availability is about 76%. Eeckhout & De Paepe (1994) predicted a higher bioavailability of maize when it is moist ensiled because it induces a reduction of the PP content. (Nahm, 2008) also noticed the considerably higher P availability of corn gluten feed, because of the presence of moisture in the steeping process prior to removal of the starch.

Wheat, wheat bran and wheat middlings have a higher P bioavailability than maize, respectively 39%, 41% and 30%, due to high intrinsic phytase activity. Nahm (2008), based on Cromwell (1989), found a lower availability of P in maize with an average of 14% and a higher availability in wheat (50%).

The bioavailability of P in soybean meal (SBM), the most common protein source in non-ruminant diets, is low at around 20% for poultry. Interestingly, soybean hulls have a high P availability for poultry (119%), although the relevance of this value is not large, because of the low P content of soybean hulls. In the CVB tables, soybean hulls is associated with a digestibility close to 75%.

Rice is the cereal which presents the lowest P availability. This is mostly of interest for poultry farmers in Eastern Asia.

Animal protein sources present considerably higher P availabilities compared to plant stuffs. For example, as indicated in the CVB tables, fishmeal presents a P content of 22-25g P and a digestibility of 73%. The two main inorganic phosphate sources, dicalcium phosphate and mono-calcium phosphate, present P bioavailability close to 100% for poultry.

Genetically modified low-phytate feedstuffs such as maize (Spencer et al., 2000) and soybean meal (Sands et al., 2003) have higher P bioavailability and true digestibility values than the conventional feedstuffs.

Feedstuff	Mean	Min.	Max.
Cereal grains and by-products			
Barley	38	32	50
Barley, brewers' dried grain	32	–	–
Maize, dry	21	12	33
Maize, DDGS	76	69	102
Maize, gluten feed	95	–	–
Maize, hominy feed	34	–	–
Sorghum, dry	26	18	36
Sorghum, high moisture	47	–	–
Oats	42	28	47
Oats, groats	4	–	–
Oats, bran	60	–	–
Rice	0	–	–
Rice bran	10	2	18
Triticale	31	–	–
Wheat	39	28	58
Wheat, middlings	41	–	–
Wheat, bran	30	23	36
Oilseed meals			
Canola meal	45	–	–
Cottonseed meal	42	–	–
Soybean meal, hulled	16	11	40
Soybean meal, low phytate	57	–	–
Soybean meal, hulled	60	–	–
Soybean meal, low phytate	77	–	–
Soybean meal, dehulled	24	16	33
Soyabean meal, hulls	119	–	–
Sunflower meal	23	–	–
Pulses			
Field peas	28	–	–
Pinto beans	40	–	–
Animal-based feedstuffs			
Bonemeal	90	89	94
Meat and bonemeal	76	52	99
Fishmeal	103	–	–
Casein	48	–	–
Inorganic			
Curaçao Island phosphate	81	55	100
Dicalcium phosphate	91	71	123
Monophosphates, Na and Ca	96	93	101
Rock phosphate, raw	75	67	91
Rock phosphate, defluorinated	89	82	103
Rock phosphate, soft	47	25	76

Figure 26. P availability of different feed stuffs (%; Kiarie & Nyachoti (2010)).

Supplementation of inorganic forms of P

Shastak et al. (2012) compared two different sources of P in broilers: Anhydrous monosodium phosphate and the anhydrous dibasic Ca phosphate. P retention and prececal P digestibility were higher for monosodium phosphate than dibasic Ca phosphate.

Keshavarz (1994) compared monobasic and dibasic phosphate. Supplemental levels of dibasic phosphate did not have an adverse effect on performance and did not affect shell quality whereas performance was seriously impaired by a supplemental level of monobasic phosphate. However, the levels used in the study were considerably greater than commonly used levels of P.

The common maize-soybean meal diet is known to present a low availability in P for poultry. The various levels of P availability for feedstuffs show that poultry diets can be modulated to include feedstuffs with a high P-availability to poultry (wheat by-products, genetically modified corn, ethanol by-products). The incorporation of such products could reduce the P supplementation levels and will be discussed later.

3.1.2 Influence of Ca source, particle size and dietary level

Egg producers mostly use two sources of Ca, limestone and oyster shell (Soares, 1995). Both forms provide Ca as Ca carbonate and contain about 38% of Ca (Saunders-Blades et al., 2009).

Effect of origin of Ca source

Most of the studies agree on the absence of effect of Ca origin (seashell, oyster shell or limestone, regardless of particle size and Ca level) on body weight, egg-day production or egg weight (Miller & Sunde, 1975; Guinotte & Nys, 1991; Grizzle et al., 1992; Saunders-Blades et al., 2009). Moreover, bone parameters such as bone weight, bone-breaking strength and bone percentage ash do not vary between the Ca sources (Cheng & Coon, 1990; Guinotte & Nys, 1991; Saunders-Blades et al., 2009). However, the effect of Ca source on eggshell quality is controversial, even though most studies have reported no difference between particulate limestone and oyster shell (reviewed by Roland (1986); Saunders et al. (2009)).

Effect of particle size of Ca source

As for origin of Ca, particle size does not affect egg production nor body weight (Miller & Sunde, 1975; Cheng & Coon, 1990; Guinotte & Nys, 1991; Grizzle et al., 1992; Keshavarz & Nakajima, 1993; Saunders-Blades et al., 2009). However, most studies demonstrated a positive effect of greater particle size on bone quality such as total or trabecular bone mineral density, cortical area, total or cortical bone mineral content, bone weight or bone-breaking strength (Guinotte & Nys, 1991; Rennie et al., 1997; Fleming et al., 1998). As for the origin of Ca source, the effect of particle size on eggshell quality is controversial in the literature. Although most studies suggest a positive effect of greater particle size of Ca in eggshell quality (Cheng & Coon, 1990; Keshavarz & Nakajima, 1993), Saunders-Blades et al. (2009) reported no difference with regards to the particle size. This difference might be explained by the degree of oyster shell or particulate limestone in exchange of the ground limestone diet. Saunders-Blades et al. (2009) replaced only one third of the ground limestone by particulate Ca while the other studies replaced two thirds to 100%. Cheng & Coon (1990) investigated the levels of daily intake of Ca (2.0, 2.5, 3.0, 3.5, 4.0 and 4.5g) and six different sizes (average United States Screen Number 6, 8, 12, 18, 35, and 100 corresponding to sieve diameters of 3.36, 2.38, 1.68, 1.02, .50, and .1 5mm, respectively). Any of the treatment affected the egg production and egg weight. However, the parameters related to shell quality were depressed by fine Ca source (Figure 27). Regarding the bone parameters, the particle size does not affect the bone ash content of the bones but fine Ca sources depressed the bone-breaking force and bone ash and organic matter concentrations (Figure 28).

Variable	SWUSA[1]	Shell weight	Specific gravity	Shell thickness
	(mg/cm^2)	(g)		(mm)
Source				
1	73.7^{a}	5.13^{a}	1.0825^{a}	.2845^{b}
2	75.5^{a}	5.16^{a}	1.0824^{a}	.2981^{a}
Size, average screen number[2]				
6	75.6^{a}	5.27^{a}	1.0837^{ab}	.3015^{a}
8	74.3^{a}	5.21^{a}	1.0839^{a}	.2897^{abc}
12	74.0^{a}	5.23^{a}	1.0828^{ab}	.2959^{ab}
18	73.7^{a}	5.16^{ab}	1.0825^{ab}	.2942^{abc}
35	73.0^{b}	5.05^{bc}	1.0821^{b}	.2861^{bc}
100	70.9^{b}	4.97^{c}	1.0802^{c}	.2804^{c}
Calcium intake level, g/day				
2.0	69.0^{b}	4.83^{c}	1.0782^{c}	.2716^{b}
2.5	73.2^{a}	5.06^{b}	1.0821^{b}	.2772^{b}
3.0	75.8^{a}	5.20^{ab}	1.0832^{ab}	.2988^{a}
3.5	74.6^{a}	5.20^{ab}	1.0839^{a}	.2960^{a}
4.0	75.4^{a}	5.31^{a}	1.0833^{ab}	.2984^{a}
4.5	75.6^{a}	5.29^{a}	1.0838^{a}	.3058^{a}
Interaction	. . .	S × Ca	S × Ca	. . .

$^{a-c}$Means within each column and variable with no common superscripts are significantly different (P<.05).

[1]SWUSA = shell weight per unit surface area.

[2]Average screen number = screen number where 50% of limestone passed through and 50% was retained by the screen. Screen Number 6, 8, 12, 18, 35, and 100 correspond to sieve diameters 3.36, 2.38, 1.68, 1.02, .50, and .15 mm, respectively.

Figure 27. Effects of source(s), size and Ca intake level (ca) on various shell quality traits (Cheng & Coon, 1990).

Variable	Bone ash concentration	Bone organic matter concentration	Bone ash	Bone-breaking force
	(mg/mL)		(% fat-free dry weight)	(kg)
Source				
1	353^{a}	327^{b}	56.45^{a}	12.07^{a}
2	353^{a}	347^{a}	56.05^{a}	12.70^{a}
Size, average screen number[1]				
6	348^{b}	332^{bc}	55.72^{ab}	12.89^{ab}
8	388^{a}	333^{bc}	56.43^{ab}	13.28^{a}
12	371^{ab}	349^{ab}	57.46^{a}	13.18^{a}
18	360^{ab}	375^{a}	56.79^{ab}	13.05^{a}
35	340^{b}	318^{c}	55.09^{b}	11.21^{bc}
100	334^{b}	316^{c}	56.01^{ab}	10.70^{c}
Calcium intake level, g/day				
2.0	316^{c}	297^{c}	55.17^{b}	9.84^{c}
2.5	330^{bc}	320^{bc}	55.24^{b}	11.12^{bc}
3.0	340^{bc}	342^{b}	56.09^{b}	12.71^{ab}
3.5	352^{b}	341^{b}	56.34^{b}	13.05^{a}
4.0	379^{a}	349^{ab}	55.95^{b}	13.44^{a}
4.5	403^{a}	375^{a}	58.70^{a}	14.15^{a}

$^{a-c}$Means within each column and variable with no common superscripts were significantly different (P<.05).

[1]Average screen number = screen number where 50% of limestone passed through and 50% was retained by the screen. Screen numbers 6, 8, 12, 18, 35, and 100 correspond to sieve diameters 3.36, 2.38, 1.68, 1.02, .50, and .15 mm, respectively.

Figure 28. Effects of treatments on various femur parameters (Cheng & Coon, 1990).

In the experiment from Rennie et al. (1997), the use of the diet oyster shell (50% of the limestone replaced by crushed oyster shell) did not induce a different amount of trabecular bone but induced a higher amount of MB in the PTV and in the FTV. It also increased the level of ALP (Figure 29).

		Proximal tarsometatarsus				Free thoracic vertebra				
		TB		MB			TB		MB	
Treatment	*n*	% +	logit (sed^{++})	%	logit (sed)	*n*	%	logit (sed)	%	logit (sed)
Control	14	12·98	−1·926	11·85	−2·241	13	12·15	−2·032	0·49	−5·639
Oystershell	18	12·38	−1·987 (0·139)	19·85**	−1·466 (0·242)	14	11·40	−2·105 (0·098)	0·70*	−5·078 (0·302)
Fluoride	17	12·87	−1·958 (0·141)	17·53*	−1·617 (0·246)	10	11·92	−2·028 (0·106)	0·54	−5·492 (0·324)
Ascorbic acid	15	12·05	−2·015 (0·144)	13·39	−2·063 (0·254)	7	12·01	−1·930 (0·105)	0·40	−5·286 (0·319)
1,25-DHCC	13	13·47	−1·945 (0·148)	15·12	−1·860 (0·264)	5	11·14	−2·119 (0·109)	0·44	−5·279 (0·334)
Low CP, high vitamin K	17	12·58	−1·940 (0·142)	12·45	−2·068 (0·245)	13	13·19	−1·942 (0·099)	0·84	−5·224 (0·304)
Low phosphorus	16	12·60	−1·940 (0·142)	13·54	−1·957 (0·249)	9	10·00	−2·219 (0·108)	0·63	−5·204 (0·330)
J-line	9	18·14*	−1·535 (0·162)	2·38**	−4·234 (0·294)	3	16·43	−1·824 (0·157)	1·13	−4·797 (0·477)

+ mean proportion of trabecular or medullary bone bone.
++ standard error of the difference between logit means for the control group and each treatment group.
*$P < 0·05$, **$P < 0·01$ (significantly different from control on basis of differences on logit scale).
1,25-DHCC, 1,25-dihydrocholecalciferol; CP, crude protein.

Figure 29. Proportions of trabecular bone and MB expressed as mean percentages and logit transformations of the percentages, in the PTM and FTV at 68 weeks of age given different dietary treatments (Rennie et al. 1997).

In the study from Skřivan et al. (2010), two diets were used: a diet containing Ca carbonate in fine limestone particles (diet with limestone particles <0.5mm), and a diet containing Ca carbonate in coarse limestone particles (diet with 91% of particles larger than 0.8mm and smaller than 2.0mm). The Ca content was constant with 38g/kg of feed. In this study, feeding the diet containing coarse limestone particles improved shell weight by 0.2g, the shell thickness by 4µm and the shell Ca content by 2mg/g DM. Skřivan et al. (2010) therefore determined that a larger particle size of 0.8 to 2mm should be considered rather than fine ground limestone.

Saunders-Blades et al. (2009) compared four Ca sources (A, B, C limestone and oyster shell) and 2 particle size combinations (ground= 100% ground or mix =66% ground and 33% large particle). They also tested *in vitro* solubility and stated that oyster shell has the highest in vitro solubility. In this study, feed consumption, body weight, egg production, egg weight and egg specific gravity did not differ among treatments (Figure 30). However, hens fed the mixed Ca particle treatments had greater feed consumption compared to those fed 100% ground Ca source. The mixed Ca treatments also had positive effects on tibia quality (trabecular density, trabecular and cortical volumes, cortical mineral content, bone weight and breaking strength).

In conclusion, particulate size of Ca should be provided to the poultry diets to enhance bone quality and probably eggshell quality.

Large particles of Ca have a lower solubility (Saunders-Blades et al., 2009) and therefore the gizzard empties more slowly at night, resulting in better meeting of the Ca requirement in the dark hours and a greater utilization of Ca from the Ca source (Scott et al., 1971; Mongin & Sauveur, 1979). It is confirmed by Cheng & Coon (1990) who measured the solubility *in vitro* of the Ca sources and found out that the shell quality traits and bone parameters related to solubility provide a better fitted line than when related to particle size (Figure 31).

Item	Density[1] (mg/cm³)			Cross-sectional area[1] (mm²)			Bone mineral content[1] (mg/mm)			Bone weight,[2] g	Length, cm	Breaking strength, kg	Ca, %	Ash, %
	Total	Cortical	Trabecular	Total	Cortical	Trabecular	Total	Cortical	Trabecular					
Source														
A[3] (n = 31)	691.89	997.59	197.56[a]	35.37	20.96	12.41	21.71	18.24	2.39	8.06	12.01[a]	17.69	27.95	56.06
B[4] (n = 29)	643.59	1,060.31	155.69[b]	35.13	18.95	14.43	24.38	20.67	2.27	7.93	12.01[a]	17.57	28.08	57.64
C[5] (n = 32)	665.73	1,032.85	181.96[ab]	35.00	19.74	13.38	22.59	19.94	2.06	7.70	11.75[b]	17.61	26.98	55.96
Control[6] (n = 30)	626.34	1,033.59	159.78[b]	34.72	17.58	15.39	23.28	20.36	2.39	7.64	11.93[ab]	15.53	27.02	56.95
SEM	20.60	27.50	11.74	0.70	0.90	0.89	0.83	1.01	0.19	0.18	0.08	1.00	1.99	0.50
Particle size														
Ground[7] (n = 62)	618.76[b]	1,033.54	160.99[b]	34.98	17.92[b]	15.32[a]	21.56[b]	18.39[b]	2.33	7.56[b]	11.91	16.18 b	27.07	56.35
Mixed[8] (n = 60)	695.0[a]	1,028.55	186.50[a]	35.14	20.70[a]	12.49[b]	24.42[a]	21.22[a]	2.22	8.10[a]	11.94	18.02 a	27.94	56.96
SEM	13.72	18.32	7.82	0.46	0.59	0.58	0.55	0.92	0.13	0.12	0.05	0.66	1.32	0.33
ANOVA *P*-value														
Source (S)	0.1141	0.4136	0.0313	0.9180	0.0510	0.0805	0.1254	0.2867	0.4923	0.2975	0.0428	0.2831	0.9578	0.0426
Particle size (P)	0.0001	0.8470	0.0226	0.7979	0.0013	0.0009	0.0004	0.0035	0.5590	0.0023	0.6713	0.0508	0.6435	0.1903
S × P	0.8023	0.5288	0.1709	0.9387	0.7601	0.3966	0.9392	0.8427	0.8688	0.6932	0.5547	0.3803	0.7150	0.6291

[ab]Means within the same column and main effect (Ca source and particle size) with no common superscripts differ significantly ($P \leq 0.05$).

[1]The right tibia was scanned at the midpoint after bone were cleaned of all adhering flesh and dried. Bone mineral density was measured for cortical = measurements taken on the area define as >500 mg/cm³ and the outer part of the bone; trabecular = measurements taken in the inner part of the bone in the trabecular space; and total = the total for the entire bone; bone mineral content was calculated as BMD multiplied by the cross-sectional area and is the amount of bone mineral contained in a 1-mm linear section of the scanned region of the bone.

[2]Fat-free, moisture-free bone weight.

[3]Hens fed a diet consisting of test limestone source A, a white limestone, from 19 to 74 wk of age.

[4]Hens fed a diet consisting of test limestone source B, a brown limestone, from 19 to 74 wk of age.

[5]Hens fed a diet consisting of test limestone source C, a gray limestone, from 19 to 74 wk of age.

[6]Hens fed a diet consisting of a commercially used ground limestone (CGL) source in the 100% ground control treatment, and a mixture of the CGL and oyster shell in the mixed treatment, from 19 to 74 wk of age.

[7]Ground Ca source diet.

[8]Mixed particle size Ca source diet (1/3 particulate + 2/3 ground).

Figure 30. The effects of Ca source and particle size on laying hen tibia quality at 74 wk of age (Saunders-Blades et al., 2009).

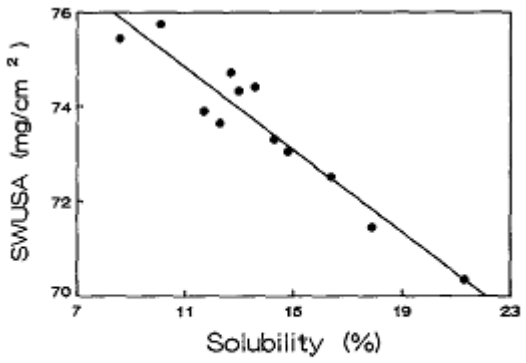

FIGURE 2. Plot of shell weight per unit of surface area (SWUSA) on limestone solubility. The fitted line was Y = 79.63 –.04X; R^2 = .91.

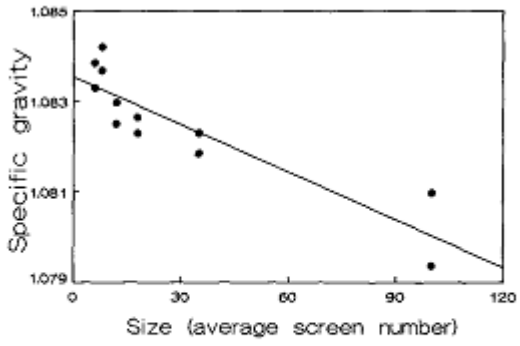

FIGURE 5. Plot of specific gravity of egg on limestone particle size. The fitted line was Y = 1.08 – .00004X; R^2 = .79.

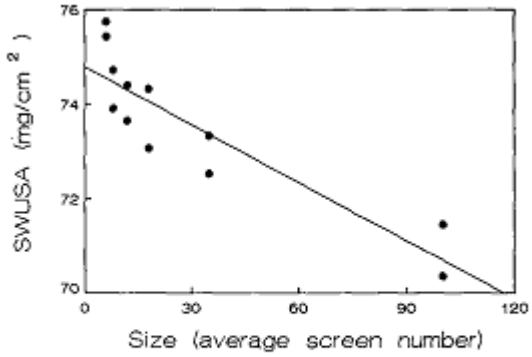

FIGURE 3. Plot of shell weight per unit of surface area (SWUSA) on limestone particle size. The fitted line was Y = 74.89 –.04X; R^2 = .80.

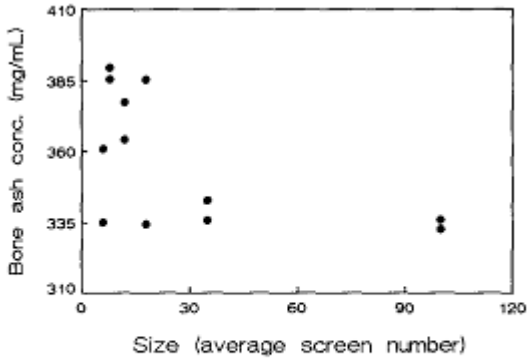

FIGURE 6. Plot of bone ash concentration (conc.) on limestone particle size. A fitted line was Y = 352.11 + 2.74X – .01X^2 + .0009X^3; R^2 = .22.

Figure 31. Cheng & Coon, 1990

Moreover, coarse particle of Ca maintain a higher blood level of Ca which reduces the necessity of bone resorption to deposit Ca into the eggshell (Guinotte & Nys, 1991). In addition, Fleming (2008) proved that the number of active osteoclasts is reduced when hens are fed particulate limestone, reducing overall bone resorption. As a consequence, greater particle size has a positive effect on bone-breaking strength and bone percentage ash (Guinotte & Nys, 1991).

Dietary Ca levels

As stated by Taylor (1965; cited by Selle et al. (2009)), PP can chelate to cations such as Ca and form mineral-phytate complexes. However, the Ca-phytate complexes are insoluble and therefore resistant to hydrolysis by phytase. This is supported also by Driver et al. (2005), who stated that Ca and P are antagonists to each other in the gut of broiler chickens. When high concentrations of Ca are provided, the two minerals tend to form non-soluble complexes resulting in lower P absorption (Hurwitz & Bar, 1965; Rama Rao et al., 2006). The formation of mineral complexes between Ca and P and their effect on phytase efficiency has been extensively reviewed by Härtel (1990).

The role of Ca is contrasted since it has a deleterious digestive effect on P utilization by monogastrics (Létourneau-Montminy et al., 2010) and high levels of dietary Ca are known to decrease the availability of PP (Damron & Harms, 1965; Ballam et al., 1984; Soares, 1995; Lim et al., 2003; Rama Rao et al., 2006; Selle & Ravindran, 2008; Nahm, 2008; Selle et al., 2009). To give an example, Van der Klis et al. (1997) showed that increasing dietary Ca levels from 30 to 40 $g.kg^{-1}$ in non-supplemented P diets reduced ileal phytate degradation from approximately 33% to 9% in laying hens. Six studies are reviewed below to determine the ideal dietary Ca level for laying hens (Cheng & Coon, 1990; Clunies et al., 1992; Keshavarz & Nakajima, 1993; Chandramoni et al., 1998; Lim et al., 2003; Fleming, 2008). Most of the studies consider that 3.0% Ca is too low to ensure best eggshell quality and advice at least 3.5% Ca (Table 1).

Table 1. Ca recommendations from six publications

Author(s)	**Ca levels (%)**	**Ca recommendations**
Cheng & Coon (1990)	2.0, 2.5, 3.0, 3.5, 4.0, 4.5	3.0% is enough to ensure best shell weight, shell specific gravity, shell thickness and bone breaking strength
Clunies et al. (1992)	2.5, 3.5, 4.5	3.5% is recommended for the lowest egg deformation and highest shell Ca (g)
Keshavarz & Nakajima (1993)	3.5, 4.0, 4.5, 5.0, 5.5	Levels of Ca above 3.5% do not bring any beneficial effects on eggshell quality and egg production
Chandramoni et al. (1998)	2.6, 2.9, 3.2, 3.6, 3.9	The optimal hen performances are obtained with 3.6%
Lim et al. (2003)	3.0, 4.0	3.0% Ca decreases egg specific gravity, eggshell strength, and eggshell thickness.
Fleming (2008)	/	The Ca level should be 3.5% diet to benefit best eggshell quality

In brief, even though origin of Ca (oyster shell or limestone) has no effect on production, a larger particle size of Ca source has positive effects because it releases Ca more slowly over night. It is therefore expected to reduce P excretion due to a lower mobilization of bone Ca despite the lack of studies to prove it. The dietary Ca level is very delicate to determine because it should ensure a good eggshell quality, but it is also known that high levels reduce P availability. If we consider the feeding of low P-diets to laying hens, the common dietary Ca level might induce lower degradation of PP. The addition of phytase brings a new perspective because high dietary Ca levels impair the efficacy of phytase. According to Selle & Ravindran (2007), the Ca level should be kept to the strict minimum in phytase supplemented broilers diets. This statement can be transposed in laying hens diets even though the level of Ca needs is much higher.

3.1.3 Magnesium

In broilers, Magnesium (Mg) supplementation has been shown to reduce the body weight and bone ash (Damron & Harms, 1965; Figure 32). However, as compared to high levels of dietary Ca, Mg does not produce such a great effect on production performance. Mg reduces membrane permeability but is not accumulated by mitochondria, as with Ca. According to McCuaig et al. (1972), the depressive effects induced by high levels of Mg are due to the decrease in phytase intestinal activity in chicks by binding to P.

Supplemental phosphorus source	Total phosphorus[1] (%)	Total calcium[1] (%)	Tibia ash[2] (%)		Body weight[2] (gms.)	
			+Mg	−Mg	+Mg	−Mg
Soft Phosphate	0.37	0.42	32.3^{ab}	35.1^{cdef}	259^{ab}	266^{abc}
		0.51	33.7^{abc}	35.9^{defg}	246^{a}	274^{bcd}
		0.65	33.9^{bc}	35.9^{defg}	253^{a}	266^{abc}
	0.44	0.52	35.4^{cdef}	38.1^{hij}	274^{bcd}	294^{efgh}
		0.61	35.8^{defg}	37.6^{hi}	271^{bcd}	300^{fghi}
		0.75	34.2^{cd}	36.3^{efg}	261^{ab}	275^{bcde}
Monosodium Phosphate	0.35	0.32	33.1^{ab}	32.1^{a}	280^{cde}	257^{ab}
		0.47	34.9^{cde}	36.8^{fgh}	283^{cdef}	309^{ghij}
		0.62	35.1^{cdef}	35.6^{def}	279^{cde}	324^{ij}
	0.40	0.34	34.4^{cd}	35.7^{def}	289^{def}	294^{efgh}
		0.50	39.0^{ij}	39.4^{j}	320^{j}	335^{jk}
		0.66	39.8^{j}	38.8^{ij}	316^{ij}	312^{hij}

[1] Basal diet contained 0.30% phosphorus and 0.26% calcium.
[2] Means with different superscripts are significantly different according to Duncan's multiple range test (1955).

Figure 32. Tibia ash and body weight of chicks fed diets containing two levels of P from two sources with and without supplemental Mg (2,000 ppm; Damron & Harms, 1965).

According to Kim et al. (2013), Mg is a very important nutrient for eggshell quality and is the second most present mineral in the eggshell ($MgCO_3$), ranging from 0.44 to 1.88% of the weight of the eggshell (Hossain & G. Bertechini, 1998). In laying hens, no effect of dietary Mg (1.7 to 7.7g/kg) was found on egg production, Mg content in eggshell or bone and egg weight (Atteh & Leeson, 1983; Hossain & Bertechini, 1998). Hossain & Bertechini (1998) only found a significant interaction between levels of Mg and aP for feed consumption and egg weight. In aged laying hens (19 month old), bones present lower Mg concentration but similar Ca concentration compared to younger hens (Roland et al., 1977; Figure 33). It is therefore suspected that aged hens have higher Mg requirements than those suggested by NRC (1994) of 0.4g/kg. Kim et al. (2013) proved that supplementation of Mg (2.3, 2.6 or 3.0g/kg Mg) on aged laying hens of 72 weeks has beneficial effects on eggshell strength.

Hen age	Calcium	Mineral (%)[e] Calcium	Sodium	Potassium	Magnesium	Manganese[f]
Young	3.00	40.20^{ab}	1.03^{b}	.54^{b}	.67^{a}	2.00^{ab}
	1.75	41.45^{a}	.96^{c}	.50^{cd}	.70^{a}	2.00^{ab}
	1.00	40.75^{ab}	1.14^{a}	.65^{a}	.69^{a}	1.89^{b}
Old	3.00	40.14^{ab}	.90^{c}	.38^{d}	.56^{b}	1.93^{ab}
	1.75	40.09^{ab}	.93^{c}	.47^{c}	.57^{b}	2.00^{ab}
	1.00	39.62^{b}	1.02^{b}	.63^{a}	.61^{b}	2.11^{a}

^{a,b,c,d}Values followed by different letters in the same column are significantly different (P<.05).

^{e}Expressed as percentage of ash.

^{f}Value × 10^{-3}.

Figure 33. Relationship of hen age and induced Ca stress on tibia mineral content (Roland et al., 1977).

3.1.4 Isoflavones

Isoflavones are one type of phyto-oestrogens and possess an oestrogen-like activity. They act by binding to oestrogen receptors and are known to have positive effects on cancer, osteoporosis and plasma cholesterol. However, in hens, the effects of supplementary soy isoflavones are not well documented. In the study of Sahin et al. (2007), soy isoflavones improved egg production, egg quality and bone mineralization. However, this study was conducted on aged quails and under a depressive environment (34°C). Among isoflavones, daidzei becomes increasingly popular as a dietary supplement. A study from Shi et al. (2013) proved that total P in the blood was affected by supplementation of daidzein and that it increased the P retention in the blood. However, in the study of Ni et al. (2007), serum P was not affected by dietary daidzein unlike serum Ca.

3.1.5 Aluminium

Nelson et al. (1968) already indicated that levels of aluminium (Al) have a great influence on P bioavailability. Elliot & Edwards (1991b) confirmed this hypothesis in broilers explaining that Al can bind with P in the intestinal tract and form insoluble AlP. This results in the reduction of retention of P and PP. Therefore, increased levels of Al significantly reduce weight gain, feed efficiency, and percentage bone ash.

In laying hens, egg production and feed intake are significantly lower when P not bound by Al (PNB) is very low (0.03% and 0.18%). Egg production even ceased before the end of the experiment in 0.03 % PNB-diet. Plasma P was also lowered when dietary Al diet was increased. However, all the adverse effects were reversed by increasing level of P in the diet (Rossi et al., 1990). Addition of supplemental Al of 0.3% has adverse effects on egg production and P metabolism (Hussein et al., 1987; 1989)

3.1.6 Zeolite

Zeolite has been proven to affect tibia bone parameters such as reduction of ash rate or P content (Leach et al., 1990; Elliot & Edwards, 1991b; Abas et al., 2011) and to have a positive effect on eggshell quality (Roland et al., 1985; Frost et al., 1992). Edwards (1987) also stated that addition of 1% zeolite lowers the broilers performance when P-deficient diets are fed. Chung & Baker (1990) reported a reduction of bone weight.

The effect of Sodium Zeolite A (SZA), the most common form of dietary Zeolite, on plasma P is controversial. Watkins & Southern (1989) reported a depressive effect of dietary SZA on plasma P whereas Frost et al. (1992) did not notice any effect. However, it is commonly agreed that high dietary SZA impairs P utilization and affects P metabolism (Edwards, 1987; Chung & Baker, 1990; Leach et al., 1990; Elliot & Edwards, 1991b; Watkins & Southern, 1992). Zeolite, however, increases Ca utilization (Chung & Baker, 1990; Elliot & Edwards, 1991b; Frost et al., 1992), which could explain the improvement found in eggshell quality.

The large effect that zeolite has on P metabolism results from the binding of phosphate ions with Al ions released from dietary SZA (Edwards, 1987; Chung & Baker, 1990; Frost et al., 1992; Abas et al., 2011). Frost et al. (1992) indicated that the beneficial effect of zeolite on eggshell quality and increased Ca absorption does not result from the increased production of 1,25-$(OH)_2D_3$ as suspected.

However, surprisingly, in the study of Eleroğlu et al. (2011), plasma P, tibia weight, tibia volume and tibia ash P were not influenced by the presence of zeolite. They concluded that there were no synergetic or antagonist effects of zeolite with P.

3.1.7 Boron

Boron (B) is essential for bone and mineral metabolism and B deficiency influences ALP activity (Hunt & Nielsen, 1981; cited by Dupre et al. (1994); Nielsen & Shuler, 1992) and plasma P levels (Hunt, 1989; Hegsted et al., 1991; Nielsen & Shuler, 1992; Dupre et al., 1994). In addition, diet supplemented with B increases the serum inorganic P levels (Hunt, 1989; Eren, 2004). However, the cause of this increase is not well known. It could be attributed to an improvement of the absorption or a reduction of the excretion.

Moreover, live weight, egg production and feed consumption are depressed with a high supplementation level of B (400mg/kg; Eren, 2004). Egg specific gravity and feed conversion are not influenced by dietary B, whereas egg shell thickness increases with higher B supplementation (10 and 200mg/kg; Eren, 2004)

3.1.8 Organic acids

In order to reduce expensive supplemental inorganic phosphate, organic acids (OA), and especially citric acid (CA) can be added to the diet to improve P utilization in chicks (Boling, et al., 2000; Boling-Frankenbach et al., 2001; Brenes et al., 2003), although it has deleterious effects on weight gain and tibia ash (Boling-Frankenbach et al., 2001; Brenes et al., 2003). Boling-Frankenbach et al. (2001) also indicated that supplemented CA reduces the aP requirement by 0.1% of the broiler diet.

In laying hens, addition of CA does not improve egg production or egg weight (Boling, Douglas, Snow, et al., 2000; Sari et al., 2012) and reduces feed efficiency (Boling et al., 2000; Nezhad et al., 2007). Unlike in broilers and unlike phytase supplementation, additional CA does not improve P utilization (Boling et al., 2000; Nezhad et al., 2007; Sari et al., 2012). Nezhad et al. (2007) suggested that the absence of effect of CA on P utilization might result from the much higher dietary Ca levels (3.8%) of laying hens, compared to chicks. According to Erdman (1979), phytate binds to Ca and CA, a strong chelator of Ca, removes Ca or decreases Ca binding to the phytate molecule making it less stable and more susceptible to endogenous phytase (Boling et al., 2000; Boling, Douglas, Snow, et al., 2000). The hypothesis is that the high dietary Ca level in the laying hen diet (3,8% compared to 1% in chicks) results in the binding of the CA non-phytate Ca. As a result, there is still ample Ca available for binding to phytate, and the CA is not available to bind to the Ca in the Ca-phytate complex (Boling, Douglas, Snow, et al., 2000).

Addition of CA is sometimes considered as a solution to lower the pH in the gizzard and therefore facilitate the absorption of Ca and P because microbial phytase is more active at low pH (2.5 or 5.5; Brenes et al., 2003). When CA is supplemented in association with phytase, the response to phytase should be, in theory, enhanced. However, in the experiment from Brenes et al. (2003), growth response to phytase was negatively affected by CA. Moreover, according to (Boling, Webel, et al., 2000), as CA is an organic acid metabolized in the body, it is not expected to greatly affect gut pH. They also state that the findings concerning the effect of dietary CA on gastrointestinal tract pH are inconsistent. The only positive effect of CA on hen performance is the additional increase of egg production when hens are fed a phytase-supplemented diet combined with CA (Sari et al., 2012).

3.1.9 High Available Phosphorus corn

High Available Phosphorus (HAP) mutation on corn was developed by USDA scientists. This mutation, in turn, was bred into a hybrid by Pioneer, using the low phytic acid 1-1 (1pal-1) allele of the corn LPA1 gene (Snow et al., 2003).

The HAP corn has low levels of PP (38%) compared to normal corn (68%; Eeckhout & De Paepe, 1994). Therefore, using HAP corn could help nutritionists to reduce the addition of expensive inorganic P.

Ceylan et al. (2003) measured egg production, feed consumption, eggshell percentage and mineral retention and excretion of hens fed two different diets (HAP corn and not HAP corn with five levels of nPP: (0.40, 0.35, 0.30, 0.25, 0.20% + phytase). Hens fed HAP corn show the same performances than hens fed normal corn and higher levels of PP. Use of HAP reduced Ca utilization but this was overcome by phytase utilization. It is probably because HAP components can bind to Ca and reduce its digestibility. Waldroup et al. (2000) showed advantages in growth performance, tibia ash, P retention and P excretion for HAP corn in chicks, benefiting from the higher content of available P. Huff et al. (1998) demonstrated that diCa phosphate supplementation can be reduced by at least 24% in broiler diets when HAP corn is used without affecting chick performance or health. Finally, Snow et al. (2003) fed hens with diets containing HAP corn, normal yellow dent corn (0.1% P) and a positive control (0.45% P). The results show that the HAP-diet shows similar egg production and egg mass than the positive control although body weight and feed intake were impaired. They concluded that hens can be fed HAP corn-soybean meal diets containing little P supplementation without adversely affecting the production performance.

3.1.10 Corn Distiller's Dried Grain with Solubles

Distiller's dried grains with soluble is a by-product of ethanol production where DDGS is obtained from the dry milling process of maize, using fermentation with the yeast Saccharomyces cerevisiae (Deniz, Gezen, et al., 2013).

CDDGS contain low levels of PP and appreciable amounts of phytase activity, 21% and 385 FTU/kg, respectively, compared to 68% and 15 FTU/kg, for normal corn. The fermentation process that distiller's grains undergo seems to increase the P availability probably as a result of partial phytate hydrolysis (Eeckhout & De Paepe, 1994). The bioavailability of DDGS is 54% (NRC, 1994) but Amezcua et al. (2003) found the bioavailability to be ranging from 69 to 102% in chicks. Although most of the studies demonstrate a reduction of P digestibility when feed is heat treated (Jongbloed & Kemme, 1990; Schlemmer et al., 2001; Blaabjerg et al., 2010), Amezcua et al. (2003) report higher P bioavailability of DDGS when they are autoclaved compared to when they are not, 87 and 75%, respectively.

Deniz et al. (2013) fed hens with diets containing five inclusion levels of CDDGS as 0 (basal diet), 5%, 10%, 15% or 20%. Feeding until 15% of CDDGS does not affect performance parameters (egg-day production, egg weight or feed intake) or egg quality parameters (thickness and shell strength). As inclusion rate of CDDGS increased, addition of diCa phosphate decreased, leading to an overall reduction of diet cost. The inclusion rate of 15% without comprising performance was also confirmed by Roberson et al. (2005). Wamsley et al. (2013) reported that an increasing inclusion rate of 16% in chicks did not negatively affect production performance. It was also found that the use of CDDGS decreases the total P present in the poultry manure (Deniz, Gezen, et al., 2013). However, it is not relevant to suggest standard inclusion rate of CDDGS in a diet because its digestibility is dependent on the way of processing

and on the variability of protein digestibility. The use of CDDGS is still controversial and a precise recommendation cannot be provided.

3.1.11 Vitamin D_3

Most of the work done on effects of inclusion of vitamin D_3 metabolites has been with broiler chickens, where it has been proven to have beneficial effects on PP retention and feed efficiency (Roberson & Edwards, 1994), body weight and bone ash (Mitchell & Edwards, 1996) and tibial dyschondroplasia (Edwards et al., 1992; Roberson & Edwards, 1994). Mohammed et al. (1991) also highlighted that high cholecalciferol levels can have an influence on the utilization and retention of PP in broilers. See also Nahm (2008) for further explanations on broilers.

As explained in the part about P regulation, vitamin D_3, and especially its active metabolite form the 1,25-$(OH)_2D_3$, has a major influence on P metabolism. In brief, 1,25-$(OH)_2D_3$ can make PP more available to poultry. However, in laying hens, the use of 1,25-$(OH)_2D_3$ does not result in such positive effects compared to broilers. In the study from Koksal et al. (2012), the addition of 0.06% 1,25-$(OH)_2D_3$ into the diet did not result in a significant difference in production performance. Bilal et al. (2010) found no difference in serum Ca, serum inorganic P and ALP activity between hens fed phytase supplemented diets and hens fed phytase and 1,25-$(OH)_2D_3$ (400U/kg) supplemented diets. Carlos & Edwards (1998) showed that the addition of 5µg/kg of vitamin D_3 numerically improved the body weight, the bone ash and the PP retention of hens, but without significant difference. Also no effect on egg weight or egg production was noticed in this study. In contrast to these findings, in their first experiment, Frost & Roland (1990) showed that inclusion of any of the vitamin D_3 metabolites used (lα-(OH) D_3 or 1,25-$(OH)_2D_3$) at any of the five dietary levels (0, 0.75, 1.50, 3.00 and 4.50 µg/kg feed) did affect eggshell quality or production criteria.

In their second experiment, Frost & Roland (1990) showed that 1,25-$(OH)_2D_3$ increased percentage of shell, shell weight, egg breaking strength, egg production, feed consumption, and egg weight when 0 ICU D3/kg of vitamin D_3 was fed, but it had no effect at higher levels of vitamin D3. Tibia weight and tibia breaking strength were also increased by adding 1,25-$(OH)_2D_3$ to the diet. They concluded that laying hens metabolize enough 1,25-$(OH)_2D_3$ from dietary vitamin D_3 to sustain shell quality but not to maintain bone quality. Finally, Bolukbasi et al. (2005) discovered that supplementation of vitamin D_3 (3000 U/kg) significantly increased the level of Ca in the eggshell, proving that vitamin D_3 improves Ca deposition by increasing Ca absorption. It also increases P deposition in the eggshell but only in case of low-Ca diets (0 and 1%).

Keshavarz (2003) compared the effect of two sources of vitamin D_3 (1,25-$(OH)_2D_3$ and 25-OH-D_3), which were provided at a level of 2,760IU/kg. the substitution of 1,25-$(OH)_2D_3$ by 25-OH-D_3did not produce any effect on egg shell quality or production performance.

It can be concluded that among the different components of poultry diets discussed above, OA, daidzein, Vitamin D_3 and B do not have significant effects on P metabolism, except for B on P

retention. It is surprising that dietary supplementation of vitamin D_3 does not improve production performances or P retention, but this finding can be explained by a sufficient endogenous amount of vitamin D_3. Mg, SZA and Al show significant depressive effects as a result of a binding to P, which renders it less available. However, dietary Mg is found to be low compared to the real needs in aged hens. Finally, only HAP corn and CDDGS present positive results on P and can be used to reduce supplemental inorganic P without adversely affecting production performances. Phytase supplementation will be discussed later in this study.

3.2 Effect of age

Effects of dietary P on laying hens performance have been extensively studied over the last decades (Keshavarz & Nakajima, 1993; Gordon & Roland, 1997; Carlos & Edwards, 1998; Boling, Douglas, Johnson, et al., 2000; Keshavarz, 2000; Boorman & Gunaratne, 2001; Bar et al., 2002; Sohail & Roland, 2002; Marounek et al., 2008; Skřivan, Englmaierová, et al., 2010; Lei et al., 2011; Ahmadi & Rodehutscord, 2012). Most of these papers deal with hens in first cycle production (21-70 wk of age), however, only a few study the effects of age on P metabolism and P requirements (Carlos & Edwards, 1998; Boling, Douglas, Johnson, et al., 2000; Bar et al., 2002; Sohail & Roland, 2002; Snow et al., 2004) and only two focus on P requirements of hens older than 70wk (Bar et al., 2002; Snow et al., 2004).

3.2.1 Physical aspects as affected by age

Number of eggs laid per day is significantly lower in aged hens than in young hens (Garlich et al., 1982; Izat et al., 1984; Bar et al., 1988, 1999). Bar et al. (1988) indicated a greater decline along a clutch in the aged than in the young hens regarding shell density (30/39 compared to 82/91wk old hens). It is well documented that egg weight and body weight increase along the reproduction period (Garlich et al., 1982; Bar & Hurwitz, 1987; Bar et al., 1999; Kim et al., 2005). However, shell quality as defined by shell thickness, percentage shell or shell density among the studies is significantly altered by age (Abe et al., 1982; Garlich et al., 1982; Izat et al., 1984; Bar & Hurwitz, 1987; Bar et al., 1988, 1999). Reduced shell density generally results in an increase in broken eggs along with age (Bar & Hurwitz, 1987; Bar et al., 1988). Kim et al. (2005) found no influence of age on bone parameters such as bone volume, fresh weight, ash concentration or bone breaking strength in neither tibia nor femur.

3.2.2 Physiological aspects as affected by age

Abe et al. (1976) suggested that the reduction of shell density along the production period might be due to a defect in vitamin D metabolism. However, Bar & Hurwitz (1987) showed that the production of $1,25(OH)_2D_3$ by the kidney and its concentration in the plasma were similar in young hens (30wk) and aged hens (82wk) in normal Ca diet (3.6% Ca). They also indicate that

intestine and ESG calbindin, a vitamin D dependent protein, are similar in both groups. These results are confirmed by Yosefi et al. (2003) and extended to 117 wk-old hens. They concluded that vitamin D metabolism is constant among layers regardless of age when hens are fed with normal Ca diets (3.6% Ca).

The only physiological difference regarding age found in the study from Bar & Hurwitz (1987) is the adaptive capacity to respond to Ca deficiency (1.9% and 1.4% Ca). As stated earlier, $1,25(OH)_2D_3$ regulatory mechanism is responsible for the increase in Ca absorption in response to Ca deficiency. Bar & Hurwitz (1987) observed a change in $1,25(OH)_2D_3$ production in response to a Ca deficiency in young hens but not in older ones (Figure 34). As a consequence, although young and old hens have similar vitamin D metabolism, old hens lose their capacity to respond properly to Ca deficiency. As during a production cycle, hens are often subjected to Ca deficiency, such as at the end of the day, it results in reduced Ca deposition in shell and impaired eggshell quality, especially in hens older than 70wk.

	7 mo		19 mo	
Measure	Normal diet	Low Ca diet	Normal diet	Low Ca diet
Plasma Ca, *mg/dl*	25.2 ± 1.6[ab]	22.50 ± 0.6[a]	28.7 ± 0.8[b]	21.7 ± 1.0[a]
Plasma $1,25(OH)_2D_3$, *nM*	0.77 ± 0.08[a]	1.25 ± 0.03[b]	0.83 ± 0.10[a]	0.80 ± 0.10[a]
1-Hydroxylase, *mM/min*	4.0 ± 0.1[a]	5.3 ± 0.2[b]	4.1 ± 0.1[a]	4.3 ± 0.1[a]
Tibia ash, *mg/bone*	2830 ± 85[a]	2250 ± 120[b]	2859 ± 77[a]	2484 ± 140[b]

[1]Mean ± SEM of seven or eight birds fed for 16 d diets containing either 3.6 or 1.9% Ca. The hens laid at least five eggs during the seven terminal days and were bled and killed during a period of late shell calcification (20–22 h postoviposition). Means designated by different superscript letters are significantly different ($P < 0.01$).

Figure 34. Plasma Ca, kidney 1-hydroxylase, plasma $1,25(OH)_2D_3$ and bone ash as influenced by age and dietary Ca (Bar & Hurwitz, 1987).

Shell thickness decreases in old hens, in spite of their greater bone reservoir of Ca (Garlich et al., 1984). In addition, Bar et al. (1999) indicated that when young hens face severe Ca deficiency, it results in the production of eggs with reduced shell density. Older hens respond by stopping to lay or, for some, by correcting partially and for a short period shell thickness. This Ca homeostatic mechanism is not vitamin D dependent, but most likely PTH dependent.

3.2.3 P requirements and phytase supplementation as affected by age

Boling et al. (2000) evaluated the effects of dietary P and supplemental phytase in a long term experiment (50 wk) in young hens (starting at 20 wk of age) and in old hens (70 to 76 wk of age). Young and old hens were fed various dietary P (0.1, 0.15, 0.2, 0.25, 0.45% aP) during the entire. The young hens showed signs of impaired egg production from the 8th week when fed unsupplemented diet (0.1% aP), whereas old hens demonstrated a reduced egg production already from the 3rd week when fed unsupplemented diet (0.1% aP). These results show that older hens are more sensitive to dietary P deficiency than hens early in their production cycle. This lower adaptive capacity to P deficient diets in old hens was confirmed by Sohail and Roland (2003). In their study, they fed 21-wk-old hens and 47-wk-old hens with four levels of dietary P

(0.1, 0.2, 0.3, 0.4% nPP). The effect of a P-deficient diet (0.1% nPP) on the rate of egg production in older hens was much faster compared with egg production in younger hens. Sohail & Roland (2003) proposed two explanations to this phenomenon. First, the ability of hens to utilize PP might decline with age. Secondly, aged hens might have more depleted P bone content which reduces the bone capacity to respond by resorption to P deficiency.

The first hypothesis had already been tested by Scheideler and Sell (1987; cited by Carlos & Edwards (1998)), Ravindran et al. (1995; cited by Marounek et al. (2008)), Carlos & Edwards (1998) and Marounek et al. (2008). Ravindran et al. (1995) and Marounek et al. (2008) concluded that older birds hydrolyse PP more efficiently than younger ones because more endogenous phytase is present in the gastrointestinal tract of older birds. However, Scheideler & Sell (1987) and Carlos & Edwards (1998) indicated that older hens have a reduced utilization of PP. The controversy of this subject does not allow any certain conclusion and further investigation needs to be considered.

However, the second hypothesis has been denied by several studies (Gordon & Roland, 1997; Keshavarz, 2000; Snow et al., 2004; Kim et al., 2005) who indicated that mineral bone content is not altered by P-deficient diets. Snow et al. (2004) concluded that P depletion is not ocurring as a result of aP treatments or at increasing age using a fixed Ca level.

A third hypothesis could be a less efficient Ca absorption in aged than in young hens inducing an increased binding of Ca to P and, as a result, a reduced P availability. Unfortunately, there is not enough available information to deny or confirm this hypothesis.

Carlos & Edwards (1998) investigated the effects of supplemental $1,25(OH)_2D_3$ and phytase on 24 wk old hens and 56 wk old hens. Both young and old laying hens are able to utilize large amounts of PP when 600 FTU/kg phytase and, to a lesser extent, 5ug/kg $1,25(OH)_2D_3$ are added. The only difference between both groups of hens was the PP retention. In young hens, PP retention increased from 43 to 63 or 76.6 when phytase or a combination of phytase with $1,25(OH)_2D_3$ was added. However, in the old hens, the magnitude of increase was higher (0.70 to 64.8). The PP retention of hens fed a diet without supplemental phytase or $1,25(OH)_2D_3$ is much higher in young hens than in older hens. The reason of this result is still unknown but it was also confirmed by Scheideler & Sell (1995) and by Marounek et al. (2008).

Bar et al. (2002) investigated the Ca and P requirements of aged laying hens of 57, 66, 80 and 92 weeks of age. They found out that the Ca requirements of aged hens are slightly higher than the NRC recommendations of 3.25g/day. They recommend more than 3.6g/d after 57 weeks of age to sustain normal hen performances. They also state that a better eggshell quality can be obtained when aged hens are fed 5.5g/d during a period of 10 to 12 weeks. They agree with previous findings that 2.0 to 2.3g/kg aP (1.0g/kg of added Pi) is sufficient for young hens to maintain hen performance when dietary Ca varies between 32.5 to 40g/kg. However, they advise to increase to 4.5g/kg aP (1.0g/kg added Pi) for hens older than 57 weeks. As they recommend dietary Ca to be higher than the normal range applied in poultry, they also state that the dietary P must be increased by 0.6g/d to compensate for it. But this increase can be avoided by use of supplemental phytase.

Snow et al. (2004) studied the effect of 7 dietary treatments (0.10, 0.12, 0.14, 0.16, 0.18, 0.20, and 0.45% aP) on hens aged from 21 to 108wk of age. In this study, at 64wk of age, hens were subjected to a 10d-fast and then fed two different molt-recovering diets. The nutritionally-molt recovery diet contained 16% CP, 2% Ca, and 0.45% aP while the corn molt recovery diet contained 8.5% CP, 0.02% Ca, and 0.08% aP. After the feeding of the molt recovery diets, the hens were returned to the same aP level that had been fed from 21 to 63 wk of age, and they remained on these dietary treatments until 108 wk of age. The results of the study are graphically presented in Figure 35.

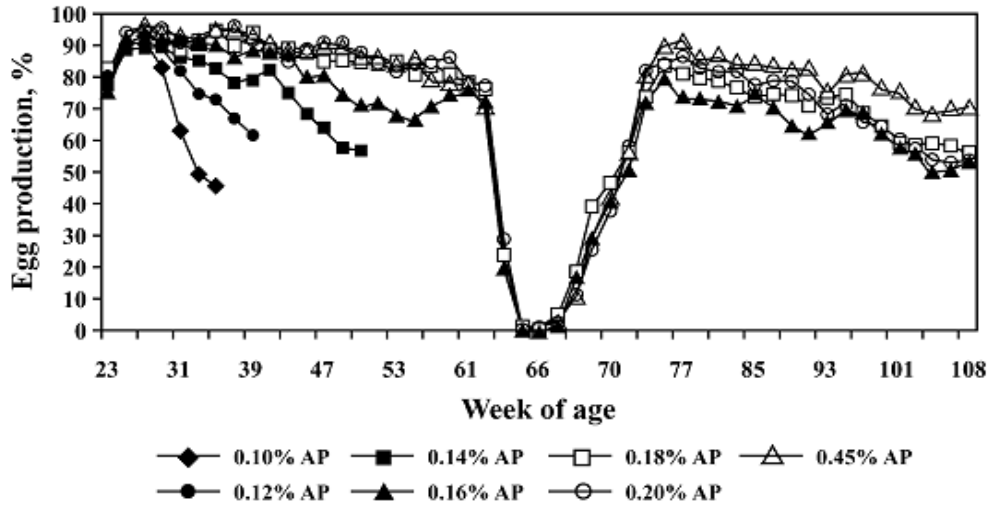

Figure 35. Hen-day egg production from hens fed different aP levels from 21 to 108 wk of age (Snow et al., 2004).

In the first cycle, from 21 to 64wk, only hens fed 0.18 or 0.20% aP had similar egg production than 0.45% treatment from 21 to 63 wk of age. However, P excretion was significantly lower than the 0.45% treatment. The authors therefore recommend approximately 0.18% aP or 198mg aP/hen per day in this laying period. In the second cycle, from 77 to 108wk of age, significant reductions in hen-day egg production, egg mass, and feed efficiency occurred when hens were fed all aP treatments compared with 0.45% aP. It can therefore been concluded that laying hens can be fed low aP diets (0.18% AP) during the first-cycle period without compromising hen performance and survivability during and immediately following the induced molt period. In the second cycle, although 0.18% and 0.20% AP diets could maintain similar egg production than 0.45% aP until the last 12 weeks, all the P treatments failed to sustain egg production until the end of the production cycle. As a conclusion, aP requirement was higher in the second cycle than in the first cycle and the second cycle requirement was in excess of the 0.20% aP treatment. Snow et al. (2004) therefore recommend further research to determine precise P requirements of post-molted hens. (Pelicia et al., 2009) indicated that 0.25% aP is sufficient to sustain egg performance and quality of post-molted hens. Very recently, Khalaji et al. (2013) investigated the aP requirements of post-molted hens by using the broken-line. The hens were kept from 85wk-old to 102wk old. In their study a 0.21% aP diet could not support egg production during the second cycle but hens fed 0.3% aP diet had similar production performances than hens fed

0.47% aP diet. In their study, the P requirement of aged hens varies according to the performance criteria chosen. As a result, the minimum aP requirement for aged laying hens ranges from 1.7 to 2.4g/kg of diet and aP requirement for maximizing egg production and egg mass is 2.8g/kg of feed.

Even though its main topic was the comparison of two different housing systems and not the effect of age, the work from (Neijat et al., 2011) can be used to study the age effect. In their study, Ca and P dynamics were analyzed from 19 to 63 week of age. The results concerning the P balance over the laying period are available in Figure 36. The graph shows a lower P balance as the age advances.

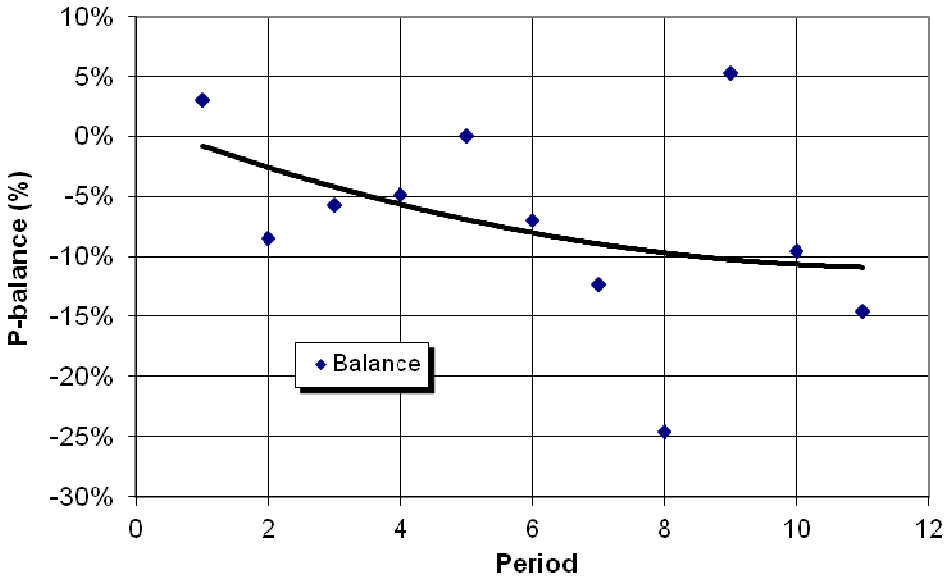

Figure 36. Effect of age (period) on P-balance (%) in laying hens (Neijat et al., 2011).

Table 2. Review of studies comparing hens of different ages

Author(s)	Age hens (weeks)	Main conclusions
Bar et al., 2002	57, 66, 80 and 92	For aged hens, Ca requirement for best shell quality are higher than NRC recommendations (3.25g/d). Dietary P content needs to be at least 4.5g/kg to maintain hen performance. However, increased dietary Ca induces higher needs regarding dietary P (use of phytase to compensate).
Boling et al., 2001	18 compared to 76	Aged hens are more sensitive to P deficiency as they show signs of impaired performances earlier than young hens (diet with 0.1% P). However, this can be compensated by a diet with 0.45% aP or by supplemental phytase.
Carlos and Edwards, 1998	24 compared to 56	In both experiments, phytase had a positive effect on BW and increased plasma dialyzable P, tibia bone ash, and PP retention. PP retention increased from 43% to 63 or 77% when phytase or combination phytase with 1,25-(OH)2D3 was added. With the older hens, the magnitude of increase was higher (0.70 to 65%). Phytase, and to a lesser extent 1,25-(OH)2D3, can be used to increase the utilization of PP by laying hens.
Khalaji et al., 2013	From 85 to 102	The minimum aP requirement for aged laying hens ranges from 1.7 to 2.4g/kg of diet and aP requirement for maximizing egg production and egg mass is 2.8g/kg of feed.
Marounek et al., 2008	20 compared to 47	Older hens hydrolyse phytate more efficiently than young hens. The P retention was significantly higher in younger hens, presumably due to a higher P requirement for bone mineralization in young birds.
Pelicia et al., 2009	90 to 108 weeks	The lowest avP level fed (0.25%) is sufficient to maintain the performance and the egg quality of semi-heavy commercial layers after molting
Sohail & Roland, 2000	21 compared to 47	The effect of a P-deficient diet (0.1% nPP) on the rate of egg production in older hens was much faster compared with egg production in younger hens.
Snow et al., 2004	From 21 to 108	The authors therefore recommend approximately 0.18% AP or 198mg aP/hen per day in this laying period. aP requirement was higher in the second cycle than in the first cycle and the second cycle requirement was in excess of the 0.20% aP treatment.

Molting

Molting is sometimes used to extend laying hen performance. Molting induced by fasting is the most applied method although it has depressive consequences on bone strength and indirectly animal welfare. Two other molting methods have been reviewed by Berry & Brake (1984) who compared fasting with high zinc diet and low sodium diet. Fasting and high zinc treatments produced a cessation of egg production within five days after the initiation of treatment. Egg production was reduced but did not cease in the low sodium treatment. The fasting treatment

resulted in the greatest degree of body and organ weight loss, while these effects were the least in the low sodium treatment.

In general, duodenal Ca uptake, shell weight, shell thickness and egg production increase immediately after an induced molt before declining during the subsequent period (Garlich et al., 1982; Al-batshan et al., 1994). However, bone characteristics, such as bone mineral content, need more time to come back to its previous level (Al-batshan et al., 1994). In the study of Mazzuco & Hester (2005), induced molt was detrimental to skeletal integrity with a depressing effect on the BMD. Hen humerus never recovered after the molt and tibia BMD recovered late, only when the hen showed reduced rate of egg production (Figure 37).

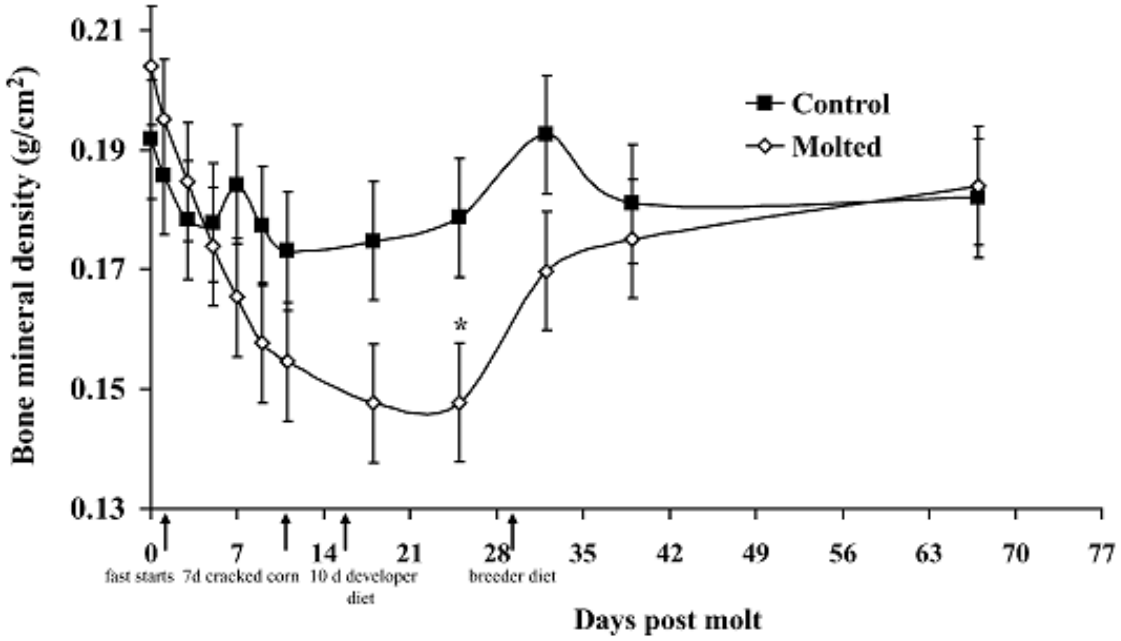

Figure 37. BMD measured in live hens subjected to a molt between 76 and 80 wk of age as compared with non-molted control hens (Mazzuco & Hester, 2005).

These results confirmed the findings of Garlich et al. (1984) who also showed that feed-removal molt decreased femur weight and density in laying hens. Bone density and relation with molt was studied in the paper of Kim et al. (2007). They investigated the effects of different molting treatments: pre-trial control (PC), fully fed (FF), Fed Withdrawal, 90% alfalfa:10% layer ration (A90), 80% alfalfa:20% layer ration (A80), and 70% alfalfa:30% layer ration (A70) in 80wk-old hens. Obviously, the hens fed the 100% layer ration (FF) had significant higher feed, energy, protein, Ca, and P intakes compared with the other groups (Figure 38).

Treatment[1]	Feed (g/bird)	Energy (kcal/bird)	Protein (g/bird)	Ca (g/bird)	P (g/bird)
FF	997.0[a]	2863.4[a]	149.6[a]	32.4[a]	13.46[a]
FW	—	—	—	—	—
A90	136.5[c]	186.6[b]	22.9[c]	2.2[b]	0.46[b]
A80	121.5[c]	186.4[b]	20.2[c]	2.2[b]	0.50[b]
A70	263.2[b]	447.9[b]	43.2[b]	5.2[b]	1.32[b]
Pooled SE	30.1	66.2	4.7	0.7	0.26

[a–c]Means within a column with different superscripts differ ($P < 0.05$).

[1]FF = fully fed (100% layer ration); FW = feed withdrawal; A90 = 90% alfalfa:10% layer ration; A80 = 80% alfalfa:20% layer ration; A70 = 70% alfalfa:30% layer ration.

Figure 38. Feed, energy, total protein, Ca and P intakes of hens fed different diets during a 9-d molt period (Kim et al., 2007).

Gregory & Wilkins (1996) reported no broken bones in hens younger than 25 weeks, but with increasing age, they found a gradual rise of fresh broken bones and old breaks. The latter can be explained by observations of Wilson et al. (1992), who indicated that trabecular bone volume decreased between 25 and 60 weeks old hens.

Aged hens and in particular post-molted hens show depressed production performances (egg production, egg quality and bone quality) compared to young hens. The latter can be explained by the fact that older hens lose their capacity to respond properly to mineral deficiencies. In conclusion, the P requirements of aged hens (>70wk) are higher than first cycle hens and range from 0.20 to 0.25 % aP. P requirements of young hens will be discussed in chapter 3.

3.3 Effect of housing

The effects of different housing systems, and especially outdoor systems, have been extensively studied on hen performance (Hughes et al., 1985; Mench et al., 1986; Abrahamsson & Tauson, 1995; Vits et al., 2005), on egg quality (Hughes et al., 1985; Mench et al., 1986; Duncan et al., 1992; Wall et al., 2002; Van Den Brand et al., 2004), on skeletal integrity (Nørgaard-Nielsen, 1990; Duncan et al., 1992; Hughes et al., 1993; Abrahamsson & Tauson, 1995; Jendral et al., 2008) and on behavioural aspects (Mench et al., 1986; Nørgaard-Nielsen, 1990; Duncan et al., 1992).

According to Vits et al. (2005), furnished cages, in order to meet the demand of the EU directive 1999/74/EG, need to provide 750 cm2 of floor space per hen, perches, a nest box, a dust bath and devices to shorten the nails. However, all studies do not compare conventional cages as defined in the EU regulation. Some might deal with only aspects of it (presence of perches, higher cage density) or completely different systems (deep litter, outdoor free range system). The results are therefore to be taken into consideration very carefully.

3.3.1 Effect on production performance

Egg production

Egg production was found to be comparable in aviary housing systems (tiered wire floors and litter) and battery cages (Abrahamsson & Tauson, 1995). However, Hughes et al. (1985) indicated that hens placed in free-range groups (38 birds per cage) presented higher egg production than birds in conventional cages. This result is confirmed by Mench et al. (1986), who found higher egg production in hens housed in floor pens (25 birds per cage, same density than single-bird cages) or single-bird cages (1/1394cm²) than in low density cages (2/2788cm²) or high density cages (2/1394cm²). Feed efficiency or egg weight did not vary among systems.

Van Den Brand et al. (2004) compared hens housed in outdoor free range system and battery cages. From hatching until 11 weeks of age, all birds were housed in two-deck breeder cages. At 11 weeks of age, hens were randomly assigned to one of two housing systems. As outdoor-housed birds matured later than the other group, they had delayed first oviposition (21wk compared to 18wk). Egg weight and production were therefore lower at the start of the laying period in free range hens but they reached the same levels at the end of the reproductive period. Although free-range birds presented a faster increase in egg production as age was advancing, there was finally no difference between both groups in terms of egg production.

Eggshell quality

Duncan et al. (1992) reported a higher proportion of cracked eggs from hens housed in cages with perches. Hens were observed laying on top of the perches. The "perch-effect" was also reported by Vits et al. (2005) and Wall et al. (2002). Nevertheless, in other studies, hens housed in conventional cages presented more cracked eggs than in a free-range group (Hughes et al., 1985). It is supported by the lower eggshell strength found in eggs from cage-group hens compared to range-group hens (Mench et al., 1986; Hughes et al., 1993). The latter is in accordance with the findings of Van Den Brand et al. (2004), who found that in conventional cages, hens show a declining eggshell thickness over the reproductive period whereas, in outdoor systems, the eggshell quality remains constant.

Bone parameters

Hens in aviary systems showed stronger tibia and humerus compared to battery cages (Abrahamsson & Tauson, 1995). According to Duncan et al. (1992), perches improved the tibia-breaking strength, whereas Hughes et al. (1993) did not find any difference in bone strength. However, Hughes et al. (1993) reported a more severe osteoporosis development in hens without access to perches and found a correlation between trabecular bone volume and time spent on the perches. Humerus and tibia strength were lower in battery cages than in deep-litter hens (Nørgaard-Nielsen, 1990).

Behavioural parameters

Perches reduce feather damage (Duncan et al., 1992). Increased cage density has no effect on fear-related response. Activity such as head movement or tonic immobility is not affected by cage density (Lee & Moss, 1995). The latter is not confirmed by Mench et al. (1986) who suggests that birds in pens have more locomotion and show more activity by using nest boxes.

3.3.2 Comparison between conventional cages (CC) and enriched cages (EC)

Only three studies compared current furnished cages to former conventional cages (Wall et al., 2002; Vits et al., 2005; Jendral et al., 2008). In the study from Wall et al. (2002), hens housed in conventional cages presented a lower percentage of broken eggs than eggs from furnished cages. As stated earlier, this difference comes from the design itself of the cages. Vits et al. (2005) reported stronger eggshells from hens housed in furnished cages than in conventional cages. These results indicate that increase in broken eggs in furnished cages results from the presence of perches.

Moreover, bone strength, bone mineral density, bone mass, cortical bone mass and area were significantly greater in furnished cages than in conventional cages -same diets were fed to hens in both systems- (Vits et al., 2005; Jendral et al., 2008). In addition, Vits et al. (2005) indicated that the high standards of conventional cages for production and egg quality were met in furnished cages.

3.3.3 P metabolism as affected by housing system

Already in 1961, Singsen et al. suggested an alteration of P requirements according to the type of housing. However, since that time, P requirements as affected by housing have only been studied by Sohail et al. (2001) and Neijat et al. (2011).

Sohail et al. (2001) investigated the effect of cage density on P requirements of laying hens. Hens were housed at three cage densities (300, 400 and 600cm² per hen) corresponding to four, three and two hens per cage and fed four nPP levels (0.15, 0.25, 0.35 and 0.40%). Cage density had a linear and quadratic effect on feed consumption. Reduction of hens from four to three hens per cage resulted in an increase of 4g/hen/d of feed consumption. Further reduction of cage density did not affect feed consumption. The most substantial effect in this study is the egg production. The nPP levels and cage density have significant interactions. Increase of cage density from two to four hens per cage had a much greater adverse effect on egg production at 0.15 and 0.25% NPP than at 0.35 and 0.40% nPP which means that a low dietary P might be sufficient to sustain egg production at low density but not at high bird density (Figure 39). Cage density had no influence on egg weight and almost no effect on egg specific gravity. The authors concluded that cage density influences the P requirement (g/kg) of modern hens.

Table 3. nPP levels, cage density, feed intake, mg nPP/day and hen day production of laying hens (Sohail et al., 2001).

nPP level (%)	Cage density	FI (average)	mg nPP/d (average)	Hen day production (average) (%)
0.15	2/cage	99	148.5	75.1
0.15	3/cage	101	151.5	73.4
0.15	4/cage	97	145.5	65.5
0.25	2/cage	99	247.5	80.1
0.25	3/cage	101	252.5	78.0
0.25	4/cage	97	242.5	74.7
0.35	2/cage	99	346.5	81.7
0.35	3/cage	101	353.5	79.6
0.35	4/cage	97	339.5	78.4
0.4	2/cage	99	396	80.3
0.4	3/cage	101	404.0	78.5
0.4	4/cage	97	388.0	75.7

On average over the experiment period, the feed intake does not vary linearly as cage density increases (Table 3). Even with very similar daily nPP intake among the cage density treatments, there is a strong variation of the hen day production. The reduction of hen day production is more important in hens fed 0.15% nPP than in hens fed higher amounts of nPP. As a conclusion, the hens housed in the 2/cage system have a better P utilization than hens housed in 4/cage systems.

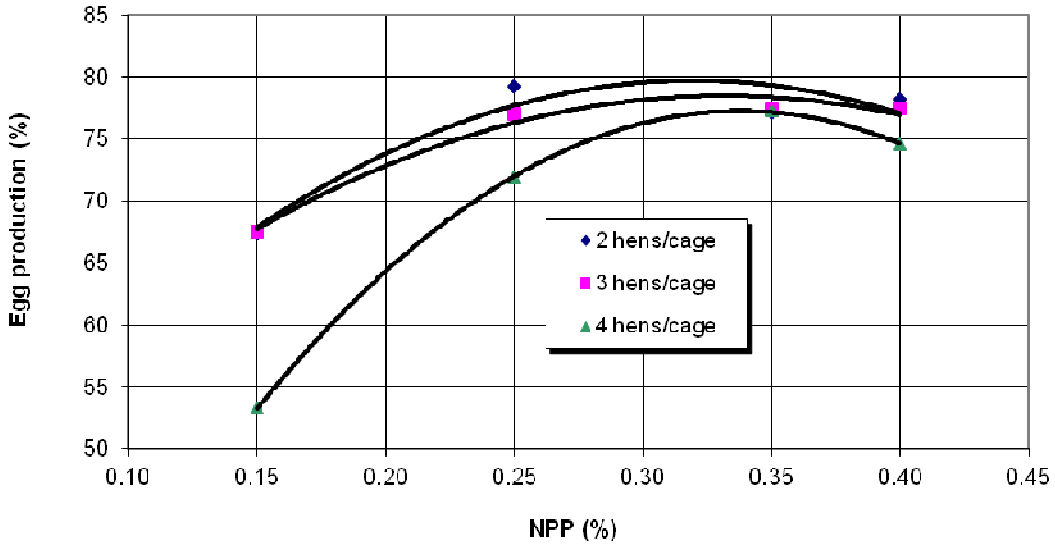

Figure 39. Egg production in function of the percentage of nPP in the diet and the cage density (Sohail et al., 2001).

Neijat et al. (2011) compared the Ca and P dynamics in hens housed in enriched or conventional cages. The hens were fed Ca levels of 4.2, 4.3 and 4.4% and available P levels of 0.45, 0.43 and 0.41%, respectively for phase 1 (19-42wk), phase 2 (43-54wk) and phase 3 (55-63wk). Egg production and egg weight did not differ between the two systems. Hens housed in conventional cages deposit more Ca into the eggshell and into the manure which results in higher Ca balance. Birds kept in conventional cages tended to excrete larger amounts of Ca, mobilized more Ca from bone resorption and therefore P as well which leaded to a decrease in P retention. The reduction in P excretion in EC-housed hens corroborates this finding. However, the overall mean P balance is similar between the two groups. The higher Ca balance and the reduction of Ca and P in the manure indicated improved BMD and bone strength for hens housed in enriched cages. The latter agrees with the findings of Vits et al. (2005) and Jendral et al. (2008). Neijat et al. (2011) found out that the Ca intake was higher in CC, which also agreed with Mench et al. (1986). In fact, as expressed in % of Ca intake, both systems are similar which indicates higher efficiency of Ca utilization in EC. As a conclusion, the authors suggested a better utilization of Ca and P in hens housed in non-cage housing systems.

In brief, it is widely accepted that new housing systems have improved hen welfare. It is comforting that most studies show comparable or even higher performances with animal welfare-friendly systems (egg production, eggshell quality). Regarding P, cage density is known to increase P requirements of hens. Finally, enriched cages show a better utilization of Ca and P through lower Ca and P excretion and higher Ca balance.

3.4 Heat stress

In terms of climate effect on P requirements, P retention by birds exposed to heat stress is reduced coupled with increased urinary P excretion (Belay et al., 1992). Usayran et al. (2001) demonstrated that high temperature depressed the plasma inorganic phosphate levels. During heat stress, the hen will mobilize more Ca from its medullary leg bone which means more P in circulation in the blood and an adverse effect on the shell formation (Leeson & Summers, 1991). However, according to Persia et al. (2003), heat stress did not adversely affect performance levels except in the case of low-P diets. Sahin et al. (2007) also stated that heat stressed hens showed a reduction in feed intake and resulting in a reduced egg production and egg quality.

3.5 Health status

3.5.1 Fatty liver syndrome

Harms et al. (1985) reported that plasma P was significantly higher for old hens with FLS compared to the normal young hens. The authors also found that the old non-laying hens have higher plasma P levels than those that were not laying. The results are in accordance with those from Miles et al. (1982) who indicated that high levels of plasma P found in their study (between 5.98 and 7.56mg/100mL) compared to the literature are the result of the FLS of the hens. However, the mechanisms behind this increase of plasma P and the effect on the P requirements are not explained yet.

3.5.2 Osteoporosis

Osteoporosis in laying hens is defined as a decrease in the amount of fully mineralized structural bone, leading to increased fragility and susceptibility to fracture. It contrasts with another cause of bone mineral loss, osteomalacia, in which defective mineralization of bone tissue occurs, with thick seams of a poorly mineralized organic matrix. Both conditions will lead to poor quality bone, but osteomalacia is primarily associated with nutritional deficiencies of Ca, P, or vitamin D, whereas osteoporosis is a more complex problem (Whitehead & Fleming, 2000).

4 P requirements

4.1 Expression of P availability

As the papers of this review come from all over the world, there is no consensus on how to express P availability. Even though it is not recommended to use nPP and aP levels inter-changeably, we will consider in this review that nPP and aP are basically the same. In this review, all the P levels extracted from the scientific publications are confronted to the feed intake level and therefore expressed in mg aP/day/hen in the summarizing tables.

Available P (**aP**) is that part of dietary total P that, at marginal level of P supply, can be utilized to cover the P requirement of the animal. Availability describes the potential of a diet or a raw material. This definition of aP is different from previous interpretations that were linked to the binding form of P or to a certain approach of determination. The NRC (1984, 1994) for example considered only non-phytate P available to poultry, and used the terms available P and non-phytate P interchangeably. The system used in Germany also ignores the fact that phytate P is available to a certain extent and implies that non-phytate P has an availability of 70 %, irrespective of its origin In the French system, the term aP refers to the proportion of P retained in the animal's body compared to a highly available reference source, assumed to have a biological value of 100 (monoCa phosphate).Bone mineralisation parameters (% tibia or toe ash) were used as response criteria in the French system. The system that is in use in The Netherlands is based on quantitative measurements of retainable P.

Total P (**tP**) comprises all P contained in a feed as chemically analysed, irrespective of the binding form.

Phytate P (**PP**) is all the P contained as phytic acid (myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate, **$InsP_6$**) and its salts.

Non-phytate P (**nPP**) is the difference between analysed tP and analysed PP. The nPP fraction is composed of different organic and inorganic compounds. The composition of the nPP fraction varies between feedstuffs. This implies that the availability of nPP for animals is not constant. For this reason, and because a variable proportion of PP also can be utilised by birds, it is not appropriate to use the terms aP and nPP interchangeably.

Retainable P (**rP**) is that proportion of dietary total P that is deposited in the body of an animal. The determination of rP needs animal studies with quantitative determination of P intake and P in excreta (faeces plus urine). Alternatively, rP can be determined by using an indigestible marker.

Relative bioavailability of P uses responses in bone data (ash content, breaking strength, etc.) or other biological data (e.g. body weight gain, blood inorganic P concentration). Responses to a certain P source are compared with the response to a standard reference P source (review from (Rodehutscord, 2012).

4.2 P requirements of first-cycle hens

4.2.1 P recommendations (NRC, industry)

The 1994 NRC recommendation of P intake for laying hens was (250mg nPP/day) already considerably lower than 10 years before (350mg/day; NRC, 1984; NRC, 1994). In addition, Ceylan et al. (2003) also stated that recommendations from breeding companies are even higher. For instance, Hy-Line International recommends 380mg P/day per hen. However, recent studies reported that P requirements of modern hens are lower than what is usually recommended (Punna & Roland, 1999; Keshavarz, 2000; Snow et al., 2004; Skřivan, Englmaierová, et al., 2010; Lei et al., 2011; Ahmadi & Rodehutscord, 2012). As nutritional requirements depend upon animal genetics and system design, only recent studies (2000s) are considered in this part.

4.2.2 P recommendations (literature)

Although the exact requirements of P are not clear yet, because of interfering parameters such as oviposition time (Kebreab et al., 2009), levels of Ca (Van der Klis et al., 1997; Rama Rao et al., 2006), age (Bar et al., 2002; Snow et al., 2004; Pelicia et al., 2009) or housing (Sohail et al., 2001; Neijat et al., 2011), many recent studies have been trying to precisely determine the P needs of laying hens.

First of all, nutritionists have to be aware that the P requirement of laying hens varies for different performance criteria. For instance, Sohail & Roland (2002) stated that 0.3% aP diet was not sufficient to maintain eggshell specific gravity, although it was sufficient to maintain bone quality compared to the 0.4% aP diet. Also, feeding 0.15% aP diet did not adversely affect egg production or egg weight but it was not sufficient to maximize the bone quality of hens compared to 0.3% aP diet.

In some studies, the use of low-P diets did not induce depressive effects on production performance (0.175% aP; (Musapuor et al., 2006), 0.15%; (Boling, Douglas, Johnson, et al., 2000), 0.16% (Boorman & Gunaratne, 2001)). However, Lei et al. (2011) showed that a reduction from 0.26% aP diet to 0.14% aP diet resulted in decreased feed intake, egg production, bone quality and increased mortality although no change in egg shell quality was noticed. Liu et al. (2007) showed that a reduction from 0.28 to 0.15% aP in a hen diet significantly depressed egg production and eggshell quality. Punna & Roland (1999) reported reduced feed intake, egg production, bone mineral density and increased mortality in hens fed 0.1% aP diet. However, it is commonly recognized that low-P diets have several advantages. First, Snow et al. (2004) and Rama Rao et al. (2006) observed a reduced P excretion by feeding diets lower in P, respectively from 0.45% aP to 0.16% aP and from 0.45% aP to 0.3% aP. More surprisingly, Lim et al. (2003) showed that reducing nPP levels from 0.25% to 0.15% resulted in an improvement of egg specific gravity and eggshell thickness.

Table 4. nPP or aP (%), mg aP/kg feed, feed intake (g/day) and mg aP/day of 10 studies

Author(s)	nPP or aP (%)	mg aP/kg feed	Feed intake (g/day)	mg aP/day
Sohail & Roland (2002)	0.3	3	84	252
	0.4	4	82	328
Musapuor et al. (2006)	0.175	1.75	96.3	168.5
Boling et al. (2000)	0.15	1,5	114	171
Boorman & Gunaratne (2001)	0.16	1,6	105	168
Lei et al. (2007)	0.26	2.6	117.7	306
	0.14	1.4	1112.4	157
Liu et al. (2007)	0.28	2.8	97.4	272.7
	0.15	1.5	94.1	141.1
Punna & Roland (1999)	0.1	1.0	76.2	76.2
Snow et al. (2004)	0.45	4.5	109	490
	0.16	1.6	106	170
Rama Rao et al. (2006)	0.45	4.5	63.2	284
	0.3	3.0	55.8	167
Lim et al. (2003)	0.25	2.5	111	277
	0.15	1.5	110	165

The most recent study specifically dedicated to precisely determine the P requirements of modern laying hens by dose-response was performed by Snow et al. (2004). Their results indicate that first-cycle hens require approximately 0.18% or 198mg aP/hen per day.

Finally, Ahmadi & Rodehutscord (2012) confronted by linear modelling the results of 12 equivalent studies in terms of diet (90% corn + SBM, 3,6 to 3.7% of Ca) and age (first cycle-hens, aged from 36 to 72 weeks; Table 5). This study constitutes the most updated and reliable assessment of the P requirements in modern laying hens. Analysis of the model revealed that diets containing 0.22% nPP resulted in high performance with regards to egg production, egg mass, and feed efficiency.

Table 5: Description of data used in the meta-analysis (Ahmadi & Rodehutscord, 2012)

Table 1. Description of data used for the meta-analysis

Trial	Strain	No. of hens	No. of treatments	Main diet ingredients	P supplementation sources[1]	Ca level (%)	Nonphytate P level (%)	Phytase[2] (FTU/kg)	Age range (wk)	Reference
1	Hy-Line W36	400	6	Corn-soybean meal	DCP	4	0.22, 0.32, 0.42	0, 300	37–48	Punna and Roland, 1999
2	ISA-White	360	12	Corn-soybean meal	MDCP	3.7, 4	0.22, 0.42	0, 250, 500	36–51	Scott et al., 1999
3	ISA-White	360	12	Corn-soybean meal	MDCP	3.7, 4	0.14, 0.24	0, 250, 500	55–67	Scott et al., 1999
4	BabcockB300	1,200	24	Corn-soybean meal	MDCP	3.8	0.1, 0.15, 0.20, 0.25, 0.30, 0.35	0, 300	42–54, 54–56	Keshavarz, 2000
5	Hy-Line W36	400	10	Corn-soybean meal	DCP	3.82	0.10, 0.15, 0.25, 0.35	0, 250, 300	40–60	Jalal and Scheideler, 2001
6	ISAbrown	60	2	Wheat-soybean meal	DCP	3.5	0.16, 0.31	0	49–61	Boorman and Gunaratne, 2001
7	Hy-Line W36	960	8	Corn-soybean meal	DCP	4	0.1, 0.2, 0.3, 0.4	0	45–53	Sohail and Roland, 2002
8	Babcock B300, DeKalb Delta White, Hy-Line W36, ISA-White	2,100	46	Corn-soybean meal	MDCP	3.8	0.1, 0.15, 0.2, 0.45	0, 150, 300	36–51, 52–63	Keshavarz, 2003a
9	Babcock B300	300	12	Corn-soybean meal	MDCP	3.8	0.11, 0.21, 0.41	0, 300	50–66	Keshavarz, 2003b
10	Babcock B300	1,280	14	Corn-soybean meal, Corn-barley-soybean meal	MDCP	3.5	0.2,0.35,.40	0, 300	36–49	Keshavarz and Austic, 2004
11	DeKalb Delta White	432	5	Corn-soybean meal	DCP	3.8	0.14, 0.15, 0.16,0.17,0.47	0	40–56	Snow et al., 2004
12	ISA-Brown	240	6	Corn-soybean meal, Wheat-soybean meal	MCP, DCP	3.53	0.23, 0.24, 0.32, 0.33, 0.41, 0.43	0	49–61	Skrivan et al., 2010
13	ISA-Brown	120	6	Corn-soybean meal, Wheat-soybean meal	MCP, DCP	3.53	0.18, 0.23, 0.27, 0.29, 0.43	0	47–59	Skri van et al., 2010
14	Lohmann	540	5	Corn-soybean meal	DCP	3.3	0.14, 0.26	0, 300	56–76	Lei et al., 2011

[1]MDCP = mono-dicalcium phosphate; DCP = dicalcium phosphate; MCP = monocalcium phosphate.

[2]In all phytase-containing treatments, the source of phytase was Natuphos 600 (BASF Corporation, Mt. Olive, NJ).

4.2.3 Phytase supplementation

The role and efficiency of phytase on P, Ca Zn, amino acids and energy has been largely discussed in pigs and poultry diets (Kornegay, 2001). This author explained that Ca and P have a high capacity to bind to phytic acid and to form unavailable phytate complexes. The utilization of PP by monogastrics requires that phytate is hydrolyzed to inositol and phosphate (Ballam et al., 1984). This is accomplished with phytase (myoinositol hexaphosphate phosphohydrolase). Phytase is a special type of phosphatase that catalyzes the stepwise removal of inorganic orthophosphate from phytate (Nelson, 1976).

Low-P diets (0.1-0.15% aP)

However, compared to broilers, phytase supplementation in diets for laying hens has been the subject of fewer publications. The use of microbial phytase in laying hens diets has been reviewed by Selle & Ravindran (2007). In layers, the authors confronted 14 studies including (Gordon & Roland, 1997; Carlos & Edwards, 1998; Boling, Douglas, Johnson, et al., 2000; Keshavarz, 2000; Jalal & Scheideler, 2001; Ceylan et al., 2003; Francesch et al., 2005) whose characteristics and main conclusions are presented in Table 6. As a consequence, only studies published after 2007 are considered in this part with some referencing to the review of Selle & Ravindran (2007).

Nine studies not discussed in the review of Selle & Ravindran (2007) will be discussed below and their Material & Methods are presented in Table 7.

According to Wu et al. (2006), the inclusion of phytase in a P-deficient diet (0.11% nPP) restored levels of egg production and egg mass to levels similar to hens fed the control diet (0.38% NPP). It is generally agreed that P-deficient diets result in lower production performance and that phytase supplementation can reverse the performance. The low-P diets presented in this part vary from 0.1 to 0.15% nPP.

In this situation, studies agree that feed intake is improved by the use of phytase (Rama Rao et al., 1999; Punna & Roland, 1999; Jalal & Scheideler, 2001; Musapuor et al., 2006; Wu et al., 2006; Lei et al., 2011). Supplemental phytase also increases weight gain (Van der Klis et al., 1997; Sari et al., 2012).

Most studies also show that egg production is increased by phytase inclusion in low-P diets (Van der Klis et al., 1997; Rama Rao et al., 1999; Punna & Roland, 1999; Keshavarz, 2000; Lim et al., 2003; Wu et al., 2006; Sari et al., 2012). However, Jalal & Scheideler (2001) did not find any significant influence of supplemental phytase on egg production of hens fed 0.25, 0.15 or 0.10% aP-diets, confirmed by Musapuor et al. (2006) using diets of 0.175 or 0.25g/kg aP.

The same trend was observed in egg weight, which is generally increased by the use of phytase in low-P diets (Van der Klis et al., 1997; Rama Rao et al., 1999; Punna & Roland, 1999; Wu et al., 2006). Nevertheless, Jalal & Scheideler (2001) found an improvement of phytase on egg mass but no significant difference on egg weight confirmed by Musapuor et al. (2006).

Table 6. Summary of phytase supplementation of diets for laying hens (Selle & Ravindran, 2009)

Summary of phytase supplementation of diets for laying hens

Reference	Phytase (FTU kg^{-1})	Non-phytate-P (g kg^{-1})	P source	Ca (g kg^{-1})	Comments
Gordon and Roland (1997)	300	1.0–5.0	DCP	40.0	Phytase supplementation of 1.0 g kg^{-1} npP corrected adverse effects. Phytase did not improve performance in diets with higher npP levels
Gordon and Roland (1998)	300	1.0 and 3.0	DCP	25.0–31.0	Phytase × npP interactions observed for eggshell quality, feed consumption and egg production. Phytase compensated/reduced adverse effects of low dietary npP and Ca
Carlos and Edwards (1998)	600	3.3 tP	Nil	30.0	Phytase and 5 µg kg^{-1} 1.25-$(OH)_2D_3$ and phytase assessed in two experiments. Phytase had positive effects on bodyweight, plasma P, tibia ash and phytate-P retention
Scott et al. (1999)	250, 500	2.0 and 4.0, 1.1 and 2.2	M/DCP	37 and 40	From 55 to 67 weeks, 500 FTU kg^{-1} phytase in 2.2 g kg^{-1} npP diets depressed body wt., egg wt. and FCR. Ca:available P impacted on shell quality. Ca impacted on phytase
Scott et al. (2000)	250, 500, 250, 500	2.0 and 4.0, 1.1 and 2.2	M/DCP	37 and 40	Wheat-based diets, average 629 g kg^{-1}. Phytase had little effect, which was attributed to plant phytase activity in wheat (~821) or mean of 516 FTU kg^{-1} in complete diets
Scott et al. (2001)	300	2.0–4.2	M/DCP	~37.5	Standard and modified (matrix values) maize or wheat-based diets. From 50 to 62 weeks. Phytase accommodated reductions to energy, CP, P and Ca with maize (not wheat)
Boling et al. (2000a)	300	1.0–4.5	NA[a]	38.0	Diets containing 1.5 or 1.0 g kg^{-1} available P plus 300 FTU kg^{-1} supported optimal egg production and the latter reduced P excretion by ~50% *vs.* 4.5 g kg^{-1} avail P diets
Keshavarz (2000)	300	1.0–4.0	M/DCP	38.0	From 30 to 66 weeks layers on the lowest P regimen + phytase performed as well as controls. Phytase increased P retention by 15% and reduced P excretion by 34–47%
Keshavarz (2003)	300	1.0–4.0	M/DCP	38.0	Phytase supplementation completely addressed the adverse effects of 2.0 g kg^{-1} npP; at 1.0 and 1.5 g kg^{-1} effects were partially addressed. P excretion was 21–43% less
Sohail and Roland (2000)	300	3.0	DCP	31.0–37.0	Study designed to determine phytase effect on Ca. Phytase improved Ca availability, and eggshell quality at 34 g kg^{-1} Ca: as indicated by improved egg specific gravity
Jalal and Scheideler (2001)	A 250, B 300	1.0–3.5	DCP	38.5	Phytase improved feed intake, conversion, egg mass in normal diets and shell quality and egg components at 1.0 g kg^{-1} npP. A *vs.* B: differences in Ca and P digestibility
Ceylan et al. (2003)	300	2.0–4.0	DCP	38.0	HAP and standard maize. Phytase supplementation of 2.0 g kg^{-1} npP diet did not improve egg production parameters. HAP maize permits reductions in DCP levels
Francesch et al. (2005)	150–450	1.1–3.2	DCP	36.0	Experimental phytase, barley and maize-based diets. Phytase compensated for lower npP levels and reduced P excretion by 49%. Phytase linearly increased P absorption
Panda et al. (2005)	500	1.2–3.0	DCP	34.8	There was no advantage in increasing npP above 1.8 g kg^{-1} or adding phytase. Phytase permits 1.2 g kg^{-1} npP diets, eliminates added iP and reduces P excretion

[a] Not available.

Table 7. Material & Methods of comparable studies regarding use of phytase supplementation

Author (s)	Age of hens (wk)	aP levels (% aP or nPP)	Phytase level (FTU/kg)	Feed intake (g/day)	mg aP/day	Ca level (%)
Deniz et al., 2013	64	0.33	0	116	383	3.9
		0.115	300	117	135	
Tahmasbi et al., 2012	72	0.36	0/300	138	497	5.9/6.3
		0.39	0/300	141	550	
Sari et al., 2012	23	0.11	0	105.8	116	3.8
			500	112.6	155	
Lei et al., 2011	56	0.26	0	117.7	306	3.4/3.3
		0.14	0	112.4	157	
		0.14	5000	117	164	
Liu et al., 2007	23	0.28	0	97.4	272.7	3.3
		0.15	0	94.1	141	
		0.15	300	98	147	
Musapuor et al., 2006	30	0.175	0/500/ 1000	96.3	168.5	2.3 / 3.3
		0.25		97.7	244.25	
Wu et al., 2006	21	0.26	0	92.8	241	3.8/4.9
		0.26	300	91.5	238	
		0.11	0	84.2	92.6	
		0.11	300	91.5	100.6	
Lim et al., 2003	21	0.25	0/300	111	277	3.0/4.0
		0.15		110	165	
Punna & Roland, 1999	19	0.1	0	69.1	69.1	4.0
		0.1	300	83.4	83.4	
		0.2	0	83.7	167.4	
		0.2	300	83.6	167.2	
		0.3	0	85.7	257.1	
		0.3	300	79.8	239.4	
		0.4	0	82.5	330	
		0.4	300	83.2	332	

Supplemental phytase also improves absorption and retention of P in low-P diets (Van der Klis et al., 1997; Lim et al., 2003; Musapuor et al., 2006; Wu et al., 2006). Phytase inclusion improves plasma P (Sari et al., 2012). As well as for P, Ca absorption and retention is increased by addition of phytase in the diet (Gordon & Roland, 1997; Van der Klis et al., 1997; Lim et al., 2003; Wu et al., 2006).

The most interesting finding regarding phytase benefits concerns the reduction of fecal P in manure. Excretion of P has been proven to be reduced with inclusion of phytase in the diets compared to higher aP-diets (Deniz et al., 2013; Lim et al., 2003). For instance, Wu et al. (2006) showed a decrease of 56% of P excretion when hens fed low-P diets is supplemented with phytase and Boling et al. (2000) discovered that excretion is reduced by 50% without having depressive effects on performances when changing from a 0.45% P diet to a 0.1%+phytase diet.

Egg quality is very controversial and mostly depends on the amount of Ca included in the basal diet. According to several studies, eggshell quality is not influenced by supplementary phytase (Boling, Douglas, Johnson, et al., 2000; Lim et al., 2003; Francesch et al., 2005; Musapuor et al., 2006; Lei et al., 2011; Deniz et al., 2013), whereas Gordon and Roland (1998) reported that phytase supplementation in diets with low nPP (0.1%) improved egg specific gravity which is confirmed by Liu et al. (2007) with hens fed 0.15% P diet. Lim et al. (2003) also reported superior eggshell quality through a reduced number of broken eggs due to phytase inclusion. In addition, according to Liu et al (2007) phytase improved eggshell thickness but not eggshell hardness which is supported by Um & Paik (1999). High eggshell thickness is not always resulting in eggshell hardness.

It is commonly admitted that there is an improvement in bone quality when phytase is added to diets containing between 0.10 and 0.15% nPP (Carlos & Edwards, 1998; Punna & Roland, 1999; Boling, Douglas, Johnson, et al., 2000). Musapuor et al. (2006) also showed an increase in bone P content because of phytase supplementation. Addition of phytase improves tibial mineralization in hens in late lay given available Ca and P diets (Deniz et al., 2013) and phytase restores the impaired bone quality of hens fed P-deficient diet (Lei et al., 2011).

Phytate increases endogenous releases of amino acids and phytase improves energy digestibility in laying hens (Cowieson et al., 2004; Francesch et al., 2005; Liu et al., 2007). Van der Klis and Versteegh (1991) indicated that phytase supplementation improved the ileal absorption of nitrogen. Agbede et al. (2009) denied the latter because they did not find any significant effect of phytase on AA digestibility. Snow et al. (2003) showed that phytase depressed the digestibility of 17 amino acids by 2.3%. However, when the diet included meat-and-bone meal or wheat middlings, phytase increased average amino acid digestibility by , 3.1% and 3.7%, respectively.

High P-diets (>0.20% aP)

As stated by Selle & Ravindran (2007), the effect of phytase supplementation is directly influenced by the dietary levels of non-PP. For instance, Hughes et al. (2008) found that the addition of phytase to a diet with 0.15% nPP significantly increased total egg production but had no effect when a diet with 0.25% nPP was fed. This is also supported by Punna & Roland (1999) who observed that phytase restored all deficiency symptoms in hens consuming 0.1% aP but showed no influence on hens fed aP levels >0.2%. Snow et al. (2003) reported that phytase supplementation of diets with 0.25% nPP did not affect hen performance. Lastly, in the study from Tahmasbi et al. (2012) who used phytase supplementation on diets containing more than 0.36% aP, no effect was observed on egg production, shell thickness or broken eggs, eggshell specific gravity, tibia bone weight or tibia P content.

When using phytase, supplementary inorganic P can be reduced without adversely affecting the hen performances. In addition, providing phytase to a P-deficient diet can reduce P excretion in manure and therefore the environmental impact of poultry production. It is thus recommended by most studies to feed hens with low P-diets ranging from 0.1 to 0.15% available P supplemented by 300 or 500FTU/kg (Gordon & Roland, 1997; Carlos & Edwards, 1998; Punna & Roland, 1999; Boling, Douglas, Johnson, et al., 2000; Lim et al., 2003; Wu et al., 2006; Liu et al., 2007; Lei et al., 2011; Deniz, Gezen, et al., 2013). Ahmadi & Rodehutscord (2012) delivered a more precise recommendation of laying hens P requirements when supplemental phytase is added to the diet. They recommend a diet containing 0.22%, 0.18%, 0.15% or 0.14% nPP when the phytase level is, respectively, 0, 150, 300 or 400 FTU/kg feed.

The low P-diets (0.10-0.15% aP) have depressive effects on hen performance such as egg production or egg quality. However, the effects are reversed by use of supplemental phytase (300-500FTU/kg). Phytase has been proven to have no effect when hens are fed high P-diets (>0.20%). Finally, literature recommends levels of 0.16 to 0.20% aP to sustain egg production or egg quality and levels of 0.10 to 0.15% aP when phytase is added to the diets. The last remarkable finding is that supplemental phytase can reduce the P excretion of hens fed low P-diets (0.15% aP) compared to high P-diets (>0.45% aP) without phytase.

5 Conclusion

Age and housing are two crucial parameters of the system which have significant effects on laying hens P utilization and requirement. For hens aged from 20 to 70 weeks, the P requirements are well documented and poultry scientists generally agree on 0.16 to 0.20% aP in the diet.

When hens get older, studies show depressed performances, especially egg production and eggshell quality. Unfortunately, in the literature, only physiological changes regarding Ca are explained but not regarding P. As hens get older, they lose their capacity to respond to Ca deficiency. As a consequence, when Ca is lacking at the end of the day, the old hens have reduced stimulation of Ca absorption and mobilization from the bone. The Ca utilization seems to be the limiting factor compared to P of good technical results in old hens. However, even though the P utilization as affected by age has not been studied so far, researchers have highlighted that current dietary P levels are insufficient when hens get older and advice to feed hens with diets containing between 0.2 and 0.25% aP.

The effects of new housing systems are controversial in the literature. The enriched cages and non-cage systems present improved bone quality, egg production and eggshell strength. However, the presence of perches results in an increase of broken eggs. In general, the alternative housing systems induce a better P and Ca utilization with reduced P excretion, which might lower the P-requirements.

P utilization can be optimized by feeding poultry closer to their requirement. For example, the use of particulate forms of Ca and the reduction of dietary P in the afternoon are ways to reduce the excess of P in the diet. P utilization can also be improved by feeding hens with enzymes or feed additives. Phytase is the most efficient feed additive to improve availability of P. Finally, HAP corn and CDDGS are examples of ingredients which can be added to the diet to reduce the use of inorganic P because they contain less PP compared to other plant ingredients.

6 Summary

Phosphorus is an important nutrient for laying hens, e.g. for bone development and egg formation, but also plays an important role in environmental contamination. Research on the phosphorus requirement of laying hens is dated. Schothorst Feed Research and Wageningen Livestock Research, therefore, started a research project to determine the phosphorus requirements of modern laying hens. The research was subsidised by the Dutch Ministry of Economic Affairs and the Dutch Product Board for Poultry and Eggs. The outcome of the literature study will be described below. The results of a related animal experiment will follow later.

The modern laying hen with a high egg number and laying persistency probably has a different phosphorus requirement than birds from 20 to 30 years ago. Besides, laying hens housed in non-cage housing systems have a better bone development. It might be that P supply by feed can be lowered without negative effects on bone quality and production performance. Determination of the P-requirement, -utilisation, and -excretion is important to support the lump phosphorus excretion and to reduce the phosphorus supply by the feed.

Importance of phosphorus for the laying hen

Laying hens will use the phosphorus (supplied by the feed) e.g. for bone development and egg formation. The use of phosphorus is closely related with the calcium metabolism of the birds. Calcium binds to phosphorus and will be in this form deposited in bone. As soon as calcium is mobilised from the bone for egg shell formation, phosphorus will be come available either.[1] Only a small amount of phosphorus will be deposited during egg shell formation. The other part is excreted by the laying hens almost immediately.

Factors affecting the phosphorus requirement

A majority of the P present in cereal grains and seeds is bound to phytate. Poultry does not have enzymes in the gastro-intestinal tract to hydrolyze the phytate. Therefore, the phytate bound phosphorus has a low availability. To improve availability, the enzyme phytase is often added to the feed. Furthermore, inorganic phosphorus from minerals can be added to the feed to satisfy the requirement of the birds.

The phosphorus requirement differs for different (production) criteria. The phosphorus requirement for optimal laying rate and egg weight is lower compared to high bone quality, and this is lower than for high egg shell quality. However, age of the birds affects phosphorus requirement and production performance. Aged laying hens have a lower egg production and often a poorer egg shell quality. Poor egg shell quality is often ascribed to a reduced calcium absorption from the intestine and bones. Utilisation of calcium seems to be the limiting factor for

[1] Formation of the egg shell will be around 18 hours, of which 8 hours will be during the dark period and laying hens will not eat during this period. Therefore, calcium from the feed is not available during the total dark period. As a result, calcium mobilisation from the bone will take during the last part of the dark period.

egg shell quality and not the utilisation of phosphorus. However, effect of age on phosphorus utilisation is not studied well yet. Several researchers argue that the current recommendations for phosphorus level in feed are too low for aged laying hens.

Since 2012, laying hens have to be housed in systems were they can express more natural behaviours like walking and foraging. Studies on the effect of housing system on production performance and bone strength are difficult to compare, because use of different systems (enriched cages, floor housing, aviary, with and without outdoor) and because of differences in floor space per hen. However, the general tendency is that laying hens housed in non-cage systems lay more high quality eggs. Besides, bone strength of these birds is improved. The role of phosphorus in the improved production and egg shell quality is not clear yet. Only two studies were performed to test the phosphorus requirement in (enriched) cages with different floor space per hen. Laying hens housed at a lower density had a higher feed intake and as a consequence a higher phosphorus intake, suggesting that phosphorus level in feed can be reduced when birds are housed at a lower density. Laying hens in enriched cages with a comparable density as in battery cages did not have a better production performance, but did have a better bone strength and a lower excretion of calcium and phosphorus in manure. Utilisation of calcium and phosphorus seems to be improved in laying hens housed in enriched cages.

Effects of diseases on phosphorus requirement is unknown. A bird that is ill will reduce feed intake resulting in a lower phosphorus intake. Egg production will drop or even stop, reducing the phosphorus requirement. It is only known that birds with fatty liver syndrome, with a normal feed intake, have an increased phosphorus blood level, being higher in aged birds than young birds with fatty liver syndrome.

Conclusion

Age and housing system probably are twee important factors in the phosphorus requirement and utilisation of laying hens. On the one hand, aged laying hens have a less active bone metabolism to utilise calcium and phosphorus well. On the other hand, egg production will decrease in aged birds, thereby reducing the requirement for phosphorus. Laying hens housed in non-cage systems seem to have an improved phosphorus utilisation and perhaps a lower phosphorus requirement. The performed animal experiment has to prove the effect of age and breed on phosphorus requirement and utilisation of the modern laying hen housed in an aviary system.

The experiment

The current knowledge on phosphorus requirement and utilisation is based on dated results from circa 20 to 30 years ago and were determined in laying hens housed in battery cages until 60 weeks of age. To determine the requirement and utilisation of the modern laying hen in a non-cage system an experiment was performed with LSL Classic and Dekalb White birds. Birds are housed in the layer facility of Schothorst Feed Research containing 36 aviary pens, with 330 birds per pen. The experiment consists of six dietary treatments differing in phosphorus level. Production performance are followed from 36 to 90 weeks of age. Furthermore, phosphorus level in manure, eggs and carcasses is determined at several moments to clarify the phosphorus deposition and excretion. Results are expected in the course of 2014.

7 Samenvatting

Fosfor is een belangrijke voedingsstof voor leghennen, onder andere voor botontwikkeling en eivorming, maar speelt ook een grote rol bij milieubelasting. Onderzoek naar de fosforbehoefte van leghennen is gedateerd. Schothorst Feed Research en Wageningen Livestock Research zijn daarom om een onderzoek gestart naar de fosforbehoefte van de huidige leghennen. Het onderzoek in mogelijk gemaakt met subsidie van het ministerie van EL&I en Productschap Pluimvee en Eieren. De uitkomst van de literatuurstudie zal hieronder worden toegelicht. De resultaten van het bijbehorende dierexperiment zullen later volgen.

De moderne leghen met een hoge productie en goede legpersistentie heeft waarschijnlijk een andere fosforbehoefte dan de hennen van 20 à 30 jaar geleden. Daarnaast hebben leghennen in alternatieve huisvestingssystemen een betere botontwikkeling, waardoor ze mogelijk toe kunnen met een lagere fosforvoorziening via het voer. Vaststellen van de fosforbehoefte, -benutting en -uitscheiding is noodzakelijk ter onderbouwing van de forfaitaire fosfaatuitscheiding en om de fosforvoorziening verantwoord te kunnen verlagen.

Belang van fosfor voor de hen

Leghennen nemen fosfor op via het voer en gebruiken dit vervolgens voor onder meer botontwikkeling en eivorming. Het gebruik van fosfor is nauw verbonden met het calciummetabolisme van leghennen. Calcium bindt aan fosfor en wordt in deze vorm opgeslagen in de botten. Zodra het calcium uit de botten nodig is voor vorming van de eischaal wordt dit vrijgemaakt en hiermee komt ook fosfor weer vrij.[2] Slechts een klein deel van de fosfor wordt gebruikt en vastgelegd bij de eischaalvorming. De rest van de fosfor scheiden de leghennen vrijwel direct uit.

Factoren van invloed op de fosforbehoefte

Het grootste deel van fosfor in granen en zaden is gebonden aan fytaat. Pluimvee heeft geen enzymen in het maag-darmkanaal om dit fytaat af te breken. Hierdoor komt het aan fytaat gebonden fosfor niet beschikbaar. Om de fosfor beschikbaar te maken wordt veelal het enzym fytase toegevoegd aan het voer. Daarnaast wordt vaak niet-plantaardige fosfor uit mineralen aan het voer toegevoegd om aan de behoefte van de leghennen te voldoen.

De fosforbehoefte is verschillend voor verschillende (productie)criteria. De fosforbehoefte voor een goed legpercentage en eigewicht is lager dan voor een goede botkwaliteit, en deze is weer lager dan voor een goede schaalkwaliteit. Echter, de leeftijd van de leghennen beïnvloedt de fosforbehoefte en de productieprestaties. Oude leghennen hebben een lagere eiproductie en vaak een slechtere eischaalkwaliteit. De slechtere schaalkwaliteit wordt veelal toegeschreven aan de verminderde calciumopname vanuit de darm en de botten. De benutting van calcium lijkt dan

[2] Vorming van de eischaal duurt ongeveer 18 uur, waarvan 8 uur tijdens de donkerperiode waarin de leghennen geen voer opnemen. Hierdoor hebben de hennen niet de gehele donkerperiode beschikking over calcium uit het voer. Calciumopname vindt gedurende de donkerperiode dan ook deels plaats vanuit de botten.

ook eerder de beperkende factor voor schaalkwaliteit dan de benutting van fosfor. Echter, het effect van leeftijd op de fosforbenutting is nog niet in kaart gebracht. Verschillende onderzoekers stellen dat de huidige adviezen voor het fosforgehalte in het voer te laag zijn voor oude leghennen.

Sinds 2012 moeten leghennen in huisvestingssystemen gehouden worden, waarin ze meer bewegingsvrijheid hebben en actiever zijn. Studies naar het effect van huisvesting op productieprestaties en botsterkte zijn echter lastig te vergelijken, omdat er verschillende systemen zijn (verrijkte kooien, scharrel, volière, met of zonder uitloop) met veelal een verschillende bezettingsdichtheid. Toch is de algemene trend dat leghennen gehouden in alternatieve systemen meer eieren leggen van betere kwaliteit. Ook de botsterkte van deze hennen is beter. In hoeverre fosfor een rol speelt bij de betere productie en eischaalkwaliteit is niet duidelijk. Slechts in twee studies is gekeken naar de fosforbehoefte in (verrijkte) kooien met verschillende bezettingsdichtheid. Leghennen gehouden bij een lagere bezettingsdichtheid hadden een hogere voeropname en hierdoor ook een hogere fosforopname, waardoor hennen bij een lagere bezettingsgraad toe zouden kunnen met een lager fosforgehalte in het voer. Leghennen in verrijkte kooien met een vergelijkbare bezettingsdichtheid als in batterijkooien hadden geen betere productieprestaties, maar wel een betere botsterkte en een lagere uitscheiding van calcium en fosfor in de mest. De benutting van calcium en fosfor lijkt dan ook beter bij leghennen gehouden in verrijkte kooien.

Het effect van bepaalde ziekten op fosforbehoefte is niet duidelijk. Een zieke hen zal minder eten en hierdoor zal ook de fosforopname om laag gaan. Eiproductie zal ook omlaag gaan of zelfs stoppen, waardoor de fosforbehoefte afneemt. Alleen van leghennen met leververvetting, waarbij de voeropname niet is aangepast, is bekend dat ze een hoger fosforgehalte in het bloed hebben. Dit is tevens hoger bij oude leghennen met leververvetting dan bij jonge hennen.

Conclusie

Leeftijd en huisvesting zijn waarschijnlijk twee belangrijke factoren in de fosforbehoefte en -benutting van leghennen. Oudere hennen hebben enerzijds een minder actief botmetabolisme om calcium en fosfor goed te benutten. Anderzijds is bij een dalende productie minder fosfor voor vorming van eieren nodig. Leghennen gehouden in alternatieve systemen lijken echter een betere fosforbenutting te hebben en hebben hierdoor wellicht een lagere fosforbehoefte. Het in dit kader uitgevoerde experiment moet uitwijzen wat het effect van leeftijd en ras is op de fosforbehoefte en –benutting van de moderne leghen in een volièresysteem.

Het experiment

De basisgegevens over de fosforbehoefte en –benutting zijn gebaseerd op gegevens van circa 20 à 30 jaar geleden en zijn vastgesteld bij leghennen gehuisvest in batterijkooien tot 60 weken leeftijd. Om de behoefte en benutting van de moderne leghen in een alternatieve huisvesting vast te stellen is een experiment opgezet met LSL Classic en Dekalb White leghennen. De hennen zijn gehuisvest in de leghennenfaciliteit van Schothorst Feed Research bestaande uit 36 volièrehokken. In elk hok zijn 330 hennen opgezet. Het experiment bestaat uit zes voerbehandelingen die verschillen in fosforgehalte. De productieprestaties van de leghennen worden van 36 tot 90 weken leeftijd vastgelegd. Daarnaast wordt op verschillende momenten het fosforgehalte bepaald in mest, eieren en karkas om hiermee de vastlegging en uitscheiding van fosfor in kaart te brengen. Resultaten worden in de loop van 2014 verwacht.

8 References

Abas, I., Bilal, T., Ercag, E. & Keser, O. (2011) The Effect of Organic Acid and Zeolite Addition Alone and in Combination on the Bone Mineral Value in Broiler Fed Different Dietary Phosphorus Levels., *Asian Journal of Animal and Veterinary Advances* 6 (7): 678–687

Abe, E., Horikawa, H., Masumura, T. & Sugahara, M. (1982) Disorders of Cholecalciferol Metabolism in Old, *Journal of Nutrition* 112: 436–446

Abrahamsson, P. & Tauson, R. (1995) Aviary Systems and Conventional Cages for Laying Hens: Effects on Production, Egg Quality, Health and Bird Location in Three Hybrids Downloaded, *Acta Agriculturae Scandinavica, Section A – Animal Science* 45 (3): 191–203

Abudabos, A.M. (2012) Optimal Dietary Phosphorus for Broiler Performance, Bone Integrity and Reduction of Phosphorus Excretion, *Asian Journal of Animal and Veterinary Advances* 7 (4): 288–289

AEB (2013) Nutrient Composition, Available from: <http://www.aeb.org/food-manufacturers/egg-nutrition-and-trends/nutrient-composition>

Agbede, J.O., Kluth, H. & Rodehutscord, M. (2009) Studies on the Effects of Microbial Phytase on Amino Acid Digestibility and Energy Metabolisability in Caecectomised Laying Hens and the Interaction with the Dietary Phosphorus Level, *British poultry science* 50 (5): 583–591

Ahmadi, H. & Rodehutscord, M. (2012) A Meta-analysis of Responses to Dietary Nonphytate Phosphorus and Phytase in Laying Hens., *Poultry science* 91 (8): 2072–8

Al-batshan, H.A., Scheideler, S.E., Black, B.L., Garlich, J.D. & Anderson, K.E. (1994) Duodenal Calcium Uptake, Femur Ash, and Eggshell Quality Decline with Age and Increase Following Molt, *Poultry science* 73: 1590–1596

Amezcua, C.M., Parsons, C.M. & Noll, S.L. (2004) Content and Relative Bioavailability of Phosphorus in Distillers Dried Grains with Solubles in Chicks, *Poultry science* 80: 971–976

Atteh, J.O. & Leeson, S. (1983) Influence of Increasing Dietary Calcium and Magnesium Levels on Performance, Mineral Metabolism, and Egg Mineral Content of Laying Hens., *Poultry science* 62 (7): 1261–8

Baimbridge, K.. & Taylor, T.. (1980) The in Controlling Role of Calcitonin Hypercalcaemia in the Domestic, (1971)

Ballam, G.C., Nelson, T.S. & Kirby, L.K. (1984) Effect of Fiber and Phytate Source and of Calcium and Phosphorus Level on Phytate Hydrolysis in the Chick., *Poultry science* 63 (2): 333–8

Balnave, D., Usayran, N. & Zhang, D. (1992) Calcium and Carbonate Supply in the Shell Gland of Hens Laying Eggs with Strong and Weak Shells and During and After a Rest from Lay, *Poultry science* 71: 2035–2040

Bar, A. (2008) Calcium Homeostasis and Vitamin D Metabolism and Expression in Strongly Calcifying Laying Birds., *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology* 151 (4): 477–90

Bar, A. (2009) Calcium Transport in Strongly Calcifying Laying Birds: Mechanisms and Regulation., *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology* 152 (4): 447–69

Bar, A. & Hurwitz, S. (1984) Egg Shell Quality, Medullary Bone Ash, Intestinal Calcium and Phosphorus Absorption, and Calcium-binding Protein in Phosphorus-deficient Hens., *Poultry science* 63 (10): 1975–9

Bar, A. & Hurwitz, S. (1987) Vitamin D Metabolism and Calbindin (Calcium-Binding Protein) in Aged Laying Hens, *The Journal of nutrition*: 1775–1779

Bar, A., Razaphkovsky, V. & Vax, E. (2002) Re-evaluation of Calcium and Phosphorus Requirements in Aged Laying Hens, *British poultry science* 43 (2): 261–269

Bar, A., Rosenberg, J. & Hurwitz, S. (1984) The Lack of Relationships Between Vitamin D3 Metabolites and Calcium-binding Protein in the Eggshell Gland of Laying Birds., *Comparative biochemistry and physiology. B, Comparative biochemistry* 78 (1): 75–9

Bar, A., Striem, S., Rosenberg, J. & Hurwitz, S. (1988) Egg Shell Quality and Cholecalciferol Metabolism in Aged Laying Hens., *The Journal of nutrition* 118 (8): 1018–23

Bar, A., Vax, E. & Striem, S. (1999) Relationships Among Age , Eggshell Thickness and Vitamin D Metabolism and Its Expression in the Laying Hen, *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 123: 147–154

Barkley, G.R., Miller, H.M. & Forbes, J.M. (2004) The Ability of Laying Hens to Regulate Phosphorus Intake When Offered Two Feeds Containing Different Levels of Phosphorus., *The British journal of nutrition* 92 (2): 233–40

Belay, T., Wiernusz, C.J. & Teeter, R.G. (1992) Mineral Balance and Urinary and Fecal Mineral Excretion Profile of Broilers Housed in Thermoneutral and Heat-Distressed Environments, *Poultry science* 71: 1043–1047

Berry, W.D. & Brake, J. (1983) Comparison of Parameters Associated with Molt Induced by Fasting , Zinc , and Low Dietary Sodium in Caged Layers, *Poultry science* 64: 2027–2036

Bilal, T., Eseceli, H., Altiner, A. & Keser, O. (2010) Effect of Vitamin D3 And/or Zeolite Supplementation to Laying Hen Rations Added Microbial Phytase on Some Blood Indices. 1. Calcium and Inorganic Phosphorus Levels and Alkaline Phosphatase Activity, *Journal of Animal and Veterinary Advances* 9 (10): 1458–1462

Blaabjerg, K., Jørgensen, H., Tauson, a-H. & Poulsen, H.D. (2010) Heat-treatment, Phytase and Fermented Liquid Feeding Affect the Presence of Inositol Phosphates in Ileal Digesta and Phosphorus Digestibility in Pigs Fed a Wheat and Barley Diet., *Animal : an international journal of animal bioscience* 4 (6): 876–85

Boling, S.D., Douglas, M.W., Johnson, M.L., Wang, X., Parsons, C.M., Koelkebeck, K.W., *et al.* (2000) The Effects of Dietary Available Phosphorus Levels and Phytase on Performance of Young and Older Laying Hens., *Poultry science* 79 (2): 224–30

Boling, S.D., Douglas, M.W., Snow, J.L., Parsons, C.M. & Baker, D.H. (2000) Citric Acid Does Not Improve Phosphorus Utilization in Laying Hens Fed a Corn-soybean Meal Diet., *Poultry science* 79 (9): 1335–7

Boling, S.D., Webel, D.M., Mavromichalis, I., Parsons, C.M. & Baker, D.H. (2000) The Effects of Citric Acid on Phytate-phosphorus Utilization in Young Chicks and Pigs. *Journal of Animal Science* 78: 682–689

Boling-Frankenbach, S.D., Snow, J.L., Parsons, C.M. & Baker, D.H. (2001) The Effect of Citric Acid on the Calcium and Phosphorus Requirements of Chicks Fed Corn-soybean Meal Diets., *Poultry science* 80 (6): 783–8

Bolukbasi, S., Celebi, S. & Utlu, N. (2005) The Effects of Calcium and Vitamin D3 in Diet on Plasma Calcium and Phosphorus, Eggshell Calcium and Phosphorus Levels of Laying Hens in Late Laying Production Period, *International Journal of Poultry Science* 4 (8): 600–603

Boorman, K.N. & Gunaratne, S.P. (2001) Dietary Phosphorus Supply, Egg-shell Deposition and Plasma Inorganic Phosphorus in Laying Hens., *British poultry science* 42 (1): 81–91

Van Den Brand, H., Parmentier, H.K. & Kemp, B. (2004) Effects of Housing System (outdoor Vs Cages) and Age of Laying Hens on Egg Characteristics., *British poultry science* 45 (6): 745–52

Brenes, A., Viveros, A., Arija, I., Centeno, C., Pizarro, M. & Bravo, C. (2003) The Effect of Citric Acid and Microbial Phytase on Mineral Utilization in Broiler Chicks, *Animal Feed Science and Technology* 110 (1-4): 201–219

Brook & Marshall (2001) *Essential Endocrinology.*, Willliston: Blackwell

Buss, E.G. & Guyer, R.B. (1984) Bone Parameters of Thick and Thin Lines of Chickens, *Comparative Biochemistry and Physiology* 78 (3): 449–452

Candlish, J.K. (1971) The Formation of Mineral and Organic Matrix of Fowl Cortical and Medullary Bone During Shell Calcification, *British poultry science* 12 (1): 119–127

Carlos, a B. & Edwards, H.M. (1998) The Effects of 1,25-dihydroxycholecalciferol and Phytase on the Natural Phytate Phosphorus Utilization by Laying Hens., *Poultry science* 77 (6): 850–8

Castaldo, D.J. & Maurice, D. V (1990) Shell Gland Adenosine Triphosphatase in Hens Producing Strong and Weak Egg Shells., *British poultry science* 31 (1): 225–9

Ceylan, N., Scheideler, S.E. & Stilborn, H.L. (2003) High Available Phosphorus Corn and Phytase in Layer Diets., *Poultry science* 82 (5): 789–95

Chandramoni, Jadhao, S.B. & Sinha, R.P. (1998) Effect of Dietary Calcium and Phosphorus Concentrations on Retention of These Nutrients by Caged Layers., *British poultry science* 39 (4): 544–8

Cheng, T.K. & Coon, C.N. (1990) Effect of Calcium Source, Particle Size, Limestone Solubility In Vitro, and Calcium Intake Level on Layer Bone Status and Performance, *Poultry science* 69: 2214–2219

Chung, T.K. & Baker, D.H. (1990) Phosphorus Utilization in Chicks Fed Hydrated Sodium Calcium Aluminosilicate, *Journal of Animal Science* 68: 1992–1998

Clunies, M., Parks, D. & Leeson, S. (1992) Calcium and Phosphorus Metabolism and Eggshell Formation of Hens Fed Different Amounts of Calcium, *Poultry science* 71 (71): 482–489

Corradino, R.A., Wasserman, R.H., Pubols, M.H. & Chang, S. (1968) Vitamin DS Induction of a Calcium-Binding Protein in the Uterus of the Laying Hen, *Archives of Biochemistry and Biophysics* 125: 378–380

Cowieson, a. J., Acamovic, T. & Bedford, M.R. (2004) The Effects of Phytase and Phytic Acid on the Loss of Endogenous Amino Acids and Minerals from Broiler Chickens, *British Poultry Science* 45 (1): 101–108

Cusack, M., Fraser, a C. & Stachel, T. (2003) Magnesium and Phosphorus Distribution in the Avian Eggshell., *Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology* 134 (1): 63–9

Dacke, C.G., Arkle, S., Cook, D.J., Wormstone, I.M., Jones, S., Zaidi, M., *et al.* (1993) Medullary Bone and Avian Calcium Regulation, *Journal of Experimental Biology* 88: 63–88

Damron, B.L. & Harms, R.H. (1965) a Comparison of Phosphorus Assay Techniques With Chicks., *Poultry science* 44: 1086–9

Deluca, F. (1988) The Vitamin Basic D Story: a Collaborative Effort of Science.

DeLuca, H.F. (2008) Evolution of Our Understanding of Vitamin D., *Nutrition reviews* 66 (10 Suppl 2): S73–87

Deniz, G., Gencoglu, H., Gezen, S.S., Turkmen, I.I., Orman, a. & Kara, C. (2013) Effects of Feeding Corn Distiller's Dried Grains with Solubles with and Without Enzyme Cocktail Supplementation to Laying Hens on Performance, Egg Quality, Selected Manure Parameters, and Feed Cost, *Livestock Science* 152 (2-3): 174–181

Deniz, G., Gezen, S.S., Kara, C., Gencoglu, H., Meral, Y. & Baser, E. (2013) Evaluation of Nutrient Equivalency of Microbial Phytase in Hens in Late Lay Given Maize-soybean or Distiller's Dried Grains with Solubles (DDGS) Diets., *British poultry science* 54 (4): 494–502

Doyle, J.. (1979) Toxic and Essential Elements in Bone − a Review. *Journal of Animal Science* 49: 482–497

Driver, J.P., Pesti, G.M., Bakalli, R.I. & Edwards, H.M. (2005) Effects of Calcium and Nonphytate Phosphorus Concentrations on Phytase Efficacy in Broiler Chicks., *Poultry science* 84 (9): 1406–17

Duncan, E.T., Appleby, M.C. & Hughes, B.O. (1992) Effect of Perches in Laying Cages on Welfare and Production of Hens, *British poultry science* 33 (1): 25–35

Dupre, J.N., Keenan, M.J., Hegsted, M. & Brudevold, A.M. (1994) Effects of Dietary Boron in Rats Fed a Vitamin D-Deficient Diet, *Environmental Health Perspectives* 102: 55–58

Edwards, H.M. (1987) Effect of Dietary Calcium, Phosphorus, Chloride, and Zeolite on the Development of Tibial Dyschondroplasia, *Poultry science* 67: 1436–1448

Edwards, H.M., Elliot, M.A. & Sooncharernytng, S. (1992) Effect of Dietary Calcium on Tibial Dyschondroplasia. Interaction with Light, Cholecalciferol, 1,25-Dihydroxycholecalciferol, Protein, and Synthetic Zeolite, *Poultry science* 71: 2041–2055

Edwards, N.A. (1973) Phosphate Excretion in the Laying Fowl, *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 46 (2): 131–137

Eeckhout, W. & De Paepe, M. (1994) Total Phosphorus, Phytate-phosphorus and Phytase Activity in Plant Feedstuffs, *Animal Feed Science and Technology* 47 (1-2): 19–29

Eleroğlu, H., Yalçın, H. & Yıldırım, A. (2011) Dietary Effects of Ca-zeolite Supplementation on Some Blood and Tibial Bone Characteristics of Broilers, *South African Journal of Animal Science* 41 (4): 319–330

Elliot, M. a & Edwards, H.M. (1991a) Some Effects of Dietary Aluminum and Silicon on Broiler Chickens., *Poultry science* 70 (6): 1390–402

Elliot, M. a & Edwards, H.M. (1991b) Comparison of the Effects of Synthetic and Natural Zeolite on Laying Hen and Broiler Chicken Performance., *Poultry science* 70 (10): 2115–30

Erdman, J.W. (1979) Oilseed Phytates□: Nutritional Implications, *Journal of American Oil Chemistry Association* 56: 736–741

Eren, M. (2004) The Influence of Dietary Boron Supplementation on Egg Quality and Serum Calcium , Inorganic Phosphorus , Magnesium Levels and Alkaline Phosphatase Activity in Laying Hens, *Research in veterinary science* 76: 203–210

Etches, R.J. (1987) Calcium Logistics in the Laying Hen., *The Journal of nutrition* 117 (3): 619–28

Fleming, R.H. (2008) Nutritional Factors Affecting Poultry Bone Health., *The Proceedings of the Nutrition Society* 67 (2): 177–83

Fleming, R.H., McCormack, H. a, McTeir, L. & Whitehead, C.C. (1998) Medullary Bone and Humeral Breaking Strength in Laying Hens., *Research in veterinary science* 64 (1): 63–7

Fleming, R.H., McCormack, H. a & Whitehead, C.C. (1998a) Bone Structure and Strength at Different Ages in Laying Hens and Effects of Dietary Particulate Limestone, Vitamin K and Ascorbic Acid., *British poultry science* 39 (3): 434–40

Fleming, R.H., McCormack, H. a & Whitehead, C.C. (1998b) Bone Structure and Strength at Different Ages in Laying Hens and Effects of Dietary Particulate Limestone, Vitamin K and Ascorbic Acid., *British poultry science* 39 (3): 434–40

Francesch, M., Broz, J. & Brufau, J. (2005) Effects of an Experimental Phytase on Performance, Egg Quality, Tibia Ash Content and Phosphorus Bioavailability in Laying Hens Fed on Maize- or Barley-based Diets., *British poultry science* 46 (3): 340–8

Frost, T.J., Roland, D. a & Marple, D.N. (1991) The Effects of Various Dietary Phosphorus Levels on the Circadian Patterns of Plasma 1,25-dihydroxycholecalciferol, Total Calcium, Ionized Calcium, and Phosphorus in Laying Hens., *Poultry science* 70 (7): 1564–70

Frost, T.J. & Roland, D.A. (1990) Influence of Vitamin D3 , la-Hydroxyvitamin D3 , and 1 , 25-Dmydroxyvitarnin D3 on Eggshell Quality , Tibia Strength , and Various Production Parameters in Commercial Laying Hens., *Poultry Science* 69 (12): 2008–2016

Frost, T.J., Roland, D.A., Barnes, D.G. & Laurent, S.M. (1992) The Effect of Sodium Zeolite A and Cholecalciferol on Plasma Levels of 1 , 25-Dihydroxycholecalciferol , Calcium , and Phosphorus in Commercial Leghorns, *Poultry science* 71: 886–893

Galitzer, H., Lavi-Moshayoff, V., Nechama, M., Meir, T., Silver, J. & Naveh-Many, T. (2009) The Calcium-sensing Receptor Regulates Parathyroid Hormone Gene Expression in Transfected HEK293 Cells., *BMC biology* 7: 17

Garlich, J., Brake, J., Parkhurst, C.R., Thaxton, J.P. & Morgan, G.W. (1984) Physiological Profile of Caged Layers During One Production Year, Molt and Postmolt, *Poultry science* 63: 339–343

Garlich, J.I.M., Morris, C., Brake, J. & Carolina, N. (1982) External Bone Volume, Ash, and Fat-Free Dry Weight of Femurs of Laying Hens Fed Diets Deficient or Adequate in Phosphorus, *Poultry science* 61: 1003–1006

Gordon, R.W. & Roland, D. a (1997) Performance of Commercial Laying Hens Fed Various Phosphorus Levels, with and Without Supplemental Phytase., *Poultry science* 76 (8): 1172–7

Gregory, N.G. & Wilkins, L.J. (1996) Effect of Age on Bone Strength and the Prevalence of Broken Bones in Perchery Laying Hens., *New Zealand veterinary journal* 44 (1): 31–2

Grizzle, J., Iheanacho, M., Saxton, a & Broaden, J. (1992) Nutritional and Environmental Factors Involved in Egg Shell Quality of Laying Hens., *British poultry science* 33 (4): 781–94

Gross, M. (2010) Fears over Phosphorus Supplies, *Current Biology* 20 (9): 386–387

Guinotte, F. & Nys, Y. (1991) Effects of Particle Size and Origin of Calcium Sources on Eggshell Quality and Bone Mineralization in Egg Laying Hens, *Poultry Science* 70: 583–592

Harms, R.H., Junqueira, O.M. & Miles, R.D. (1985) Plasma Calcium, Phosphorus, 25-dihydroxyvitamin D3, and 1-25-dihydroxyvitamin D3 of Hens with Fatty Liver Syndrome., *Poultry science* 64 (4): 768–70

Härtel, H. (1990) Evaluation of the Dietary Interaction of Calcium and Phosphorus in the High Producing Laying Hen., *British poultry science* 31 (3): 473–94

Hegsted, M., Keenan, M.J., Siver, F. & Wozniak, P. (1991) Effect of Boron on Vitamin D Deficient Rats., *Biological trace element research* 28 (3): 243–55

Holcombe, D.J., Roland, D. a & Harms, R.H. (1976) The Ability of Hens to Regulate Phosphorus Intake When Offered Diets Containing Different Levels of Phosphorus., *Poultry science* 55 (1): 308–17

Hossain, S.M. & G. Bertechini, A. (1998) Effects of Varying Levels of Magnesium and Available Phosphorus on Performance of Layers, *Animal Feed Science and Technology* 71 (3-4): 363–368

Huff, W.E., Moore, P. a, Waldroup, P.W., Waldroup, a L., Balog, J.M., Huff, G.R., *et al.* (1998) Effect of Dietary Phytase and High Available Phosphorus Corn on Broiler Chicken Performance., *Poultry science* 77 (12): 1899–904

Hughes, A.L., Dahiya, J.P., Wyatt, C.L. & Classen, H.L. (2008) The Efficacy of Quantum Phytase in a Forty-week Production Trial Using White Leghorn Laying Hens Fed Corn-soybean Meal-based Diets., *Poultry science* 87 (6): 1156–61

Hughes, B.O., Dun, P. & McCorquodale, C.C. (1985) Housed in Cages or on Range in Outside Pens, *British poultry science* 26: 129–136

Hughes, B.O., Wilson, S., Appleby, M.C. & Smith, S.F. (1993) Comparison of Bone Volume and Strength as Measures of Skeletal Integrity in Caged Laying Hens with Access to Perches., *Research in veterinary science* 54 (2): 202–6

Hunt, C.D. (1989) Dietary Boron Modified the Effects of Magnesium and Molybdenum on Mineral Metabolism in the Cholecalciferol-deficient Chick., *Biological trace element research* 22 (2): 201–20

Hurwitz, S. (1996) Homeostatic Control of Plasma Calcium Concentration., *Critical reviews in biochemistry and molecular biology* 31 (1): 41–100

Hurwitz, S. & Bar, A. (1965) Absorption of Calcium and Phosphorus Along the Gastrointestinal Tract of the Laying Fowl As Influenced By Dietary Calcium and Egg Shell Formation., *The Journal of nutrition* 86 (840): 433–8

Hussein, a S., Cantor, a H. & Johnson, T.H. (1989) Effect of Dietary Aluminum on Calcium and Phosphorus Metabolism and Performance of Laying Hens., *Poultry science* 68 (5): 706–14

Hussein, A.S., Cantor, A.H. & Johnson, T.H. (1987) Use of High Levels of Dietary Aluminum and Zinc for Inducing Pauses in Egg Production of Japanese Quail, *Poultry science* 67: 1157–1165

Izat, A.L., Gardner, F.A. & Mellor, D.B. (1984) Effects of Age of Bird and Season of the Year on Egg Quality 1 . Shell Quality, *Poultry science* 64: 1900–1906

Jalal, M. a & Scheideler, S.E. (2001) Effect of Supplementation of Two Different Sources of Phytase on Egg Production Parameters in Laying Hens and Nutrient Digestiblity., *Poultry science* 80 (10): 1463–71

Jendral, M.J., Korver, D.R., Church, J.S. & Feddes, J.J.R. (2008) Bone Mineral Density and Breaking Strength of White Leghorns Housed in Conventional, Modified, and Commercially Available Colony Battery Cages., *Poultry science* 87 (5): 828–37

Jones, G., Strugnell, S.A. & Deluca, H.F. (1998) Current Understanding of the Molecular Actions of Vitamin D, 78 (4): 1193–1231

Jongbloed, a. W. & Kemme, P. a. (1990) Effect of Pelleting Mixed Feeds on Phytase Activity and the Apparent Absorbability of Phosphorus and Calcium in Pigs, *Animal Feed Science and Technology* 28 (3-4): 233–242

Kebreab, E., France, J., Kwakkel, R.P., Leeson, S., Kuhi, H.D. & Dijkstra, J. (2009) Development and Evaluation of a Dynamic Model of Calcium and Phosphorus Flows in Layers., *Poultry science* 88 (3): 680–9

Keshavarz, K. (1994) Laying Hens Respond Differently to High Dietary Levels of Phosphorus in Monobasic and Dibasic Calcium Phosphate, *Poultry science* 73: 687–703

Keshavarz, K. (1998) Investigation on the Possibility of Reducing Protein, Phosphorus, and Calcium Requirements of Laying Hens by Manipulation of Time of Access to These Nutrients., *Poultry Science* 77 (9): 1320–1332

Keshavarz, K. (2000) Nonphytate Phosphorus Requirement of Laying Hens with and Without Phytase on a Phase Feeding Program., *Poultry science* 79 (5): 748–63

Keshavarz, K. (2003) A Comparison Between Cholecalciferol and 25-OH-cholecalciferol on Performance and Eggshell Quality of Hens Fed Different Levels of Calcium and Phosphorus., *Poultry science* 82 (9): 1415–22

Keshavarz, K. & Nakajima, S. (1993) Re-Evaluation of Calcium and Phosphorus Requirements of Laying Hens for Optimum Performance and Eggshell Quality 1 K. KESHAVARZ 2 and S. NAKAJIMA 3 Department of Animal Science, Cornell University, Ithaca, New York 14853, (1984)

Khalaji, S., H. Naderi, A., N. Mousavi, S., Zaghari, M. & Malakzadegan, A. (2013) Determination of Phosphorus Requirement for Post Molted Laying Hens Using Nonlinear and Multivariate Nonlinear Mixed Effects Models, *The Journal of Poultry Science*

Kiarie, E. & Nyachoti, C.. (2010) Bioavailability of Calcium and Phosphorus in Feedstuffs for Farm Animals, *In*: Vitti, D. & Kebreab, E. eds. *Phosphorus and calcium utilization and requirements in farm animals.*, Wiltshire: CABI Publishing, pp.76–93

Kim, C.H., Paik, I.K. & Kil, D.Y. (2013) Effects of Increasing Supplementation of Magnesium in Diets on Productive Performance and Eggshell Quality of Aged Laying Hens., *Biological trace element research* 151 (1): 38–42

Kim, W.K., Donalson, L.M., Bloomfield, S. a, Hogan, H. a, Kubena, L.F., Nisbet, D.J., *et al.* (2007) Molt Performance and Bone Density of Cortical, Medullary, and Cancellous Bone in Laying Hens During Feed Restriction or Alfalfa-based Feed Molt., *Poultry science* 86 (9): 1821–30

Kim, W.K., Donalson, L.M., Herrera, P., Kubena, L.F., Nisbet, D.J. & Ricke, S.C. (2005) Comparisons of Molting Diets on Skeletal Quality and Eggshell Parameters in Hens at the End of the Second Egg-Laying Cycle, *Poultry science* 84: 522–527

Van der Klis, J.D., Versteegh, H. a, Simons, P.C. & Kies, a K. (1997) The Efficacy of Phytase in Corn-soybean Meal-based Diets for Laying Hens., *Poultry science* 76 (11): 1535–42

Knott, L. & Bailey, A.J. (1999) Collagen Biochemistry of Avian Bone□: Comparison of Bone Type and Skeletal Site Collagen Biochemistry of Avian Bone□: Comparison of Bone Type, *British poultry science* 40 (3): 371–379

Knott, L., Whitehead, C.C., Fleming, R.H. & Bailey, a J. (1995) Biochemical Changes in the Collagenous Matrix of Osteoporotic Avian Bone., *The Biochemical journal* 310, 3: 1045–51

Koksal, B., Sacakli, P. & A, E. (2012) Effects of Phytase and Vitamin D3 Addition Ot Diets Containing Distillers Dried Grains with Solubles (DDGS) on Performance and Some Egg Traits in Laying Hens, *International Journal of Poultry Science* 11 (4): 259–263

Kornegay, E.T. (2001) Digestion of Phosphorus and Other Nutrients: The Role of Phytases and Factors Influencing Their Activity, *In*: *Enzymes in Farm Animal Nutrition*, Wiltshire: CABI Publishing, pp.237–272

Leach, R.M., Heinrichs, B.S. & Burdette, J. (1990) Broiler Chicks Fed Low Calcium Diets. 1. Influence of Zeolite on Growth Rate and Parameters of Bone Metabolism., *Poultry science* 69 (9): 1539–43

Lee, K. & Moss, C.W. (1995) Effects of Cage Density on Fear-Related Behavioral Response and Activity of Layers, *Poultry science* 74: 1426–1430

Leeson, S. & Summers, J.. (1991) *Commercial Poultry Nutrition*, Guelph: University Books

Leeson, S & Summers, J.D. (1978). Voluntary Food Restriction by Laying Hens Mediated through Dietary Self-Selection, *British poultry science* 19 (4): 417–424

Lei, Q.B., Shi, L.X., Zhang, K.Y., Ding, X.M., Bai, S.P. & Liu, Y.G. (2011) Effect of Reduced Energy, Protein and Entire Substitution of Inorganic Phosphorus by Phytase on Performance and Bone Mineralisation of Laying Hens., *British poultry science* 52 (2): 202–13

Létourneau-Montminy, M.P., Narcy, a, Lescoat, P., Bernier, J.F., Magnin, M., Pomar, C., *et al.* (2010) Meta-analysis of Phosphorus Utilisation by Broilers Receiving Corn-soyabean Meal Diets: Influence of Dietary Calcium and Microbial Phytase., *Animal: an international journal of animal bioscience* 4 (11): 1844–53

Lim, H.S., Namkung, H. & Paik, I.K. (2003) Effects of Phytase Supplementation on the Performance, Egg Quality, and Phosphorous Excretion of Laying Hens Fed Different Levels of Dietary Calcium and Nonphytate Phosphorous., *Poultry science* 82 (1): 92–9

Liu, N., Liu, G.H., Li, F.D., Sands, J.S., Zhang, S., Zheng, a J., *et al.* (2007) Efficacy of Phytases on Egg Production and Nutrient Digestibility in Layers Fed Reduced Phosphorus Diets., *Poultry science* 86 (11): 2337–42

Mackie, E.J., Tatarczuch, L. & Mirams, M. (2011) The Skeleton: a Multi-functional Complex Organ: The Growth Plate Chondrocyte and Endochondral Ossification., *The Journal of endocrinology* 211 (2): 109–21

Marounek, M., Skřivan, M., Dlouhá, G. & Břeňová, N. (2008) Availability of Phytate Phosphorus and Endogenous Phytase Activity in the Digestive Tract of Laying Hens 20 and 47 Weeks Old, *Animal Feed Science and Technology* 146 (3-4): 353–359

Maynard, L., Loosli, J., Hintz, H., Warner, R. & Zapa, C.R. (1979) *Animal Nutrition*, New York: McGraw-Hill

Mazzuco, H. & Hester, P.Y. (2005) The Effect of an Induced Molt and a Second Cycle of Lay on Skeletal Integrity of White Leghorns, *Poultry science*: 771–781

McCuaig, L.W., Davies, M.I. & Motzok, I. (1972) Intestinal Alkaline Phosphatase and Phytase of Chicks: Effect of Dietary Magnesium, Calcium, Phosphorus and Thyroactive Casein., *Poultry science* 51 (2): 526–530

Mench, J. a, van Tienhoven, a, Marsh, J. a, McCormick, C.C., Cunningham, D.L. & Baker, R.C. (1986) Effects of Cage and Floor Pen Management on Behavior, Production, and Physiological Stress Responses of Laying Hens., *Poultry science* 65 (6): 1058–69

Miles, R.D., Christmas, R.B. & Harms, R.H. (1982) Dietary and Plasma Phosphorus in Hens with Fatty Liver Syndrome., *Poultry science* 61 (12): 2512–6

Miller, P.C. & Sunde, M.L. (1975) The Effect of Various Particle Sizes of Oyster Shell and Limestone on Performance of Laying Leghorn Pullets, *Poultry science* 54: 1422–1433

Mitchell, R.D. & Edwards, H.M. (1996) Additive Effects of 1,25-dihydroxycholecalciferol and Phytase on Phytate Phosphorus Utilization and Related Parameters in Broiler Chickens., *Poultry science* 75 (1): 111–9

Mohammed, a, Gibney, M.J. & Taylor, T.G. (1991) The Effects of Dietary Levels of Inorganic Phosphorus, Calcium and Cholecalciferol on the Digestibility of phytate-P by the Chick., *The British journal of nutrition* 66 (2): 251–9

Mongin, P. & Sauveur, B. (1974) Voluntary Food and Calcium Intake by the Laying Hen., *British poultry science* 15 (4): 349–59

Mongin, P. & Sauveur, B. (1979) Plasma Inorganic Phosphorus Concentration During Egg-Shell Formation: Effect of the Physical Form of the Dietary Calcium, *British poultry science* 20 (4): 401–412

Musapuor, A., Afsharmanesh, M. & Shahrbabak Moradi, H. (2006) Use of Microbial Phytase for Decrease of Pollutant Due to Environmental Poultry Excreta Phosphorus, *International Journal of Agriculture & Biology* 8 (1): 35–37

Nahm, K.H. (2008) Efficient Phosphorus Utilization in Poultry Feeding to Lessen the Environmental Impact of Excreta, *World's Poultry Science Journal* 63, 4

Neijat, M., House, J.D., Guenter, W. & Kebreab, E. (2011) Calcium and Phosphorus Dynamics in Commercial Laying Hens Housed in Conventional or Enriched Cage Systems., *Poultry science* 90 (10): 2383–96

Nelson, T.S. (1976) The Hydrolysis of Phytate Phosphorus by Chicks and Laying Hens., *Poultry science* 55 (6): 2262–4

Nelson, T.S., Shieh, T.R., Wodzinski, R.J. & Ware, J.H. (1968) The Availability of Phytate Phosphorus in Soybean Meal before and after Treatment with a Mold Phytase., *Poultry science* 47 (6): 1842–8

Neset, T.-S.S. & Cordell, D. (2012) Global Phosphorus Scarcity: Identifying Synergies for a Sustainable Future., *Journal of the science of food and agriculture* 92 (1): 2–6

Newman, S. & Leeson, S. (1997) Skeletal Integrity in Layers at the Completion of Egg Production, *World's Poultry Science Journal* 53: 265–277

Nezhad, Y.E., Shivazad, M., Taherkhani, R. & Nazeradl, K. (2007) Effect of Citric Acid Supplementation on Phytate Phosphorus Utilization and Efficiency of Microbial Phytase in Laying Hens, *Journal of Biological Sciences* 7 (4): 638–642

Ni, Y., Zhu, Q., Zhou, Z., Grossmann, R., Chen, J. & Zhao, R. (2007) Effect of Dietary Daidzein on Egg Production , Shell Quality , and Gene Expression of ER- r , GH-R , and IGF-IR in Shell Glands of Laying Hens, *Journal of Agriculture and Food Chemistry* 55: 6997–7001

Nielsen, F.H. & Shuler, T.R. (1992) Studies of the Interaction Between Boron and Calcium , and Its Modification by Magnesium and Potassium , in Rats, *Biological trace element research* 35

Nørgaard-Nielsen, G. (1990) Bone Strength of Laying Hens Kept in an Alternative System, Compared with Hens in Cages and on Deep-litter., *British poultry science* 31 (1): 81–9

NRC (1984) *Nutrient Requirement of Poultry*, Washington, D.C: National Academy Press

NRC (1994) *Nutrient Requirements of Poultry*, Washington, D.C: National Academy Press

Nys, Y. & de Laage, X. (1984) Effects of Suppression of Eggshell Calcification and of 1,25(OH)2D3 on Mg2+, Ca2+ and Mg2+HCO-3 ATPase, Alkaline Phosphatase, Carbonic Anhydrase and CaBP levels--I. The Laying Hen Uterus., *Comparative biochemistry and physiology. A, Comparative physiology* 78 (4): 833–8

Pelicia, K., Garcia, E., Faitarone, A., Silva, A., Berto, D., Molino, A., *et al.* (2009) Calcium and Available Phosphorus Levels for Laying Hens in Second Production Cycle, *Brazilian Journal of Poultry Science* 11 (1): 39–49

Persia, M.E., Utterback, P.L., Biggs, P.E., Koelkebeck, K.W. & Parsons, C.M. (2003) Interrelationship Between Environmental Temperature and Dietary Nonphytate Phosphorus in Laying Hens, *Poultry science* 82: 1763–1768

ProProfs (2012) Skeletal System, Available from: <http://www.proprofs.com/flashcards/story.php?title=skeletal-system_72>

Punna, S. & Roland, D. a (1999) Influence of Supplemental Microbial Phytase on First Cycle Laying Hens Fed Phosphorus-deficient Diets from Day One of Age., *Poultry science* 78 (10): 1407–11

Rama Rao, S.., Ravindra Reddy, V. & Ramasubba Reddy, V. (1999) Enhancement of Phytate Phosphorus Availability in the Diets of Commercial Broilers and Layers, *Animal Feed Science and Technology* 79 (3): 211–222

Rama Rao, S.V., Raju, M.V.L.N., Reddy, M.R. & Pavani, P. (2006) Interaction Between Dietary Calcium and Non-phytate Phosphorus Levels on Growth, Bone Mineralization and Mineral Excretion in Commercial Broilers, *Animal Feed Science and Technology* 131 (1-2): 135–150

Rao, K., Roland, D.A. & Orban, J.I. (1991) Influence of Dietary Cholecalciferol , Calcium , and Phosphorus on Urinary Calcium in Commercial Leghorn Hens 1 Converted to Cholecalciferol , Commonly Known as Vitamin D3 (Norman , 1987). Under Lighting In Laying Hens , Dietary Ca and P Interact to Con, *Poultry science* 70: 1921–1927

Rennie, J.S., Fleming, R.H., McCormack, H. a, McCorquodale, C.C. & Whitehead, C.C. (1997) Studies on Effects of Nutritional Factors on Bone Structure and Osteoporosis in Laying Hens., *British poultry science* 38 (4): 417–24

Roberson, K.D. & Edwards, H.M. (1994) Effects of Ascorbic Acid and 1,25-dihydroxycholecalciferol on Alkaline Phosphatase and Tibial Dyschondroplasia in Broiler Chickens., *British poultry science* 35 (5): 763–73

Roberson, K.D., Kalbfleisch, J.., Charbeneau, R.. & Pan, W. (2005) Effect of Corn Distiller's Dried Grains with Solubles at Various Levels on Performance of Laying Hens and Egg Yolk Color, *International Journal of Poultry Science* 4 (2): 44–51

Rodehutscord, M. (2012) Determination of Phosphorus Availability in Poultry, *World's Poultry Science*: 1–19

Roland, D. a, Laurent, S.M. & Orloff, H.D. (1985) Shell Quality as Influenced by Zeolite with High Ion-exchange Capability., *Poultry science* 64 (6): 1177–87

Roland, D.A. (1986) Eggshell Quality IV: Oystershell Versus Limestone and the Importance of Particle Size or Solubility of Calcium Source, *World's Poultry Science Journal* 42: 166–171

Roland, D.A., Potman, C.E. & Hilburn, R.L. (1977) The Relationship of Age on Ability of Hens to Maintain Egg Shell Calcification When Stressed with Inadequate Dietary Calcium,

Rose, S.P. (1997) *Principles of Poultry Science*, Wallingford: CAB International

Rossi, A.F., Miles, R.D. & Harms, R.H. (1990) Research Note: Influence of Aluminum on Phosphorus Availability in Laying Hen Diets 1, *Poultry science* 69: 2237–2240

Sabbagh, Y., Giral, H., Caldas, Y., Levi, M. & Schiavi, S.C. (2011) Intestinal Phosphate Transport, *Advanced Chronic Kidney Disease* 18 (2): 85–90

Sahin, N., Onderci, M., Balci, T. a, Cikim, G., Sahin, K. & Kucuk, O. (2007) The Effect of Soy Isoflavones on Egg Quality and Bone Mineralisation During the Late Laying Period of Quail., *British poultry science* 48 (3): 363–9

Sands, J.S., Ragland, D., Wilcox, J.R. & Adeola, O. (2003) Relative Bioavailability of Phosphorus in Low-phytate Soybean Meal for Broiler Chicks, *Canadian Journal of Animal Science* 83 (1): 95–100

Sari, M., Onol, A.G., Daskiran, M. & Cengiz, O. (2012) Egg Production and Calcium-phosphorus Utilization of Laying Hens Fed Diets Suppemented with Phytase Alone or in Combination with Organic Acid, *International Journal of Poultry Science* 11 (3): 181–189

Saunders-Blades, J.L., MacIsaac, J.L., Korver, D.R. & Anderson, D.M. (2009) The Effect of Calcium Source and Particle Size on the Production Performance and Bone Quality of Laying Hens., *Poultry science* 88 (2): 338–53

Schlemmer, U., Jany, K. D., Berk, a., Schulz, E. & Rechkemmer, G. (2001) Degradation of Phytate in the Gut of Pigs: Pathway of Gastrointestinal Inositol Phosphate Hydrolysis and Enzymes Involved, *Archiv für Tierernaehrung* 55 (4): 255–280

Schraer, H. & Schraer, R. (1961) Bone Mass Changes in Hens Observed in Vivo During the Egg Laying Cycle, *Experimentia* 17: 255–256

Scott, M.L., Hull, S.J. & Mullenhoff, P.A. (1971) The Calcium Requirements of Laying Hens and Effects of Dietary Oyster Shell Upon Egg Shell Quality, *Poultry science* 50: 1055–1063

Selle, P.H., Cowieson, A.J. & Ravindran, V. (2009) Consequences of Calcium Interactions with Phytate and Phytase for Poultry and Pigs, *Livestock Science* 124 (1-3): 126–141

Selle, P.H. & Ravindran, V. (2007) Microbial Phytase in Poultry Nutrition, *Animal Feed Science and Technology* 135 (1-2): 1–41

Selle, P.H. & Ravindran, V. (2008) Phytate-degrading Enzymes in Pig Nutrition, *Livestock Science* 113 (2-3): 99–122

Shafey, T.M. (1993) Calcium Tolerance of Growing Chickens: Effect of Ratio of Dietary Calcium to Available Phosphorus, *World's Poultry Science Journal* 49: 5–18

Sharpley, A. (1999) Agricultural Phosphorus, Water Quality, and Poultry Production: Are They Compatible?, *Poultry science* 78: 660–673

Shastak, Y., Witzig, M., Hartung, K. & Rodehutscord, M. (2012) Comparison of Retention and Prececal Digestibility Measurements in Evaluating Mineral Phosphorus Sources in Broilers., *Poultry science* 91 (9): 2201–9

Shi, S.R., Gu, H., Chang, L.L., Wang, Z.Y., Tong, H.B. & Zou, J.M. (2013) Safety Evaluation of Daidzein in Laying Hens: Part I. Effects on Laying Performance, Clinical Blood Parameters, and Organs Development., *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 55: 684–8

Singsen, E.P., Spandorf, A.H., Matterson, L.D., Serafin, J.A., Agricultural, S., Station, E., *et al.* (1961) Phosphorus in the Nutrition of the Adult Hen, *Poultry science* 41: 1401–1414

Skřivan, M., Englmaierová, M. & Skřivanová, V. (2010) Effect of Different Phosphorus Levels on the Performance and Egg Quality of Laying Hens Fed Wheat- and Maize-based Diets, *Czech Journal of Animal Science* 55: 420–427

Skřivan, M., Marounek, M., Bubancová, I. & Podsedníček, M. (2010) Influence of Limestone Particle Size on Performance and Egg Quality in Laying Hens Aged 24–36 Weeks and 56–68 Weeks, *Animal Feed Science and Technology* 158 (1-2): 110–114

Snow, J.L., Douglas, M.W., Batal, a B., Persia, M.E., Biggs, P.E. & Parsons, C.M. (2003) Efficacy of High Available Phosphorus Corn in Laying Hen Diets., *Poultry science* 82 (6): 1037–41

Snow, J.L., Douglas, M.W., Koelkebeck, K.W., Batal, a B., Persia, M.E., Biggs, P.E., *et al.* (2004) Minimum Phosphorus Requirement of One-cycle and Two-cycle (molted) Hens., *Poultry science* 83 (6): 917–24

Soares, J.H. (1984) Calcium Metabolism and Its Control--a Review., *Poultry science* 63 (10): 2075–83

Soares, J.S. (1995) Phosphorus Bioavailability,

Sohail, S.S., Bryant, M.M., Rao, S.K. & Roland, D. a (2001) Influence of Cage Density and Prior Dietary Phosphorus Level on Phosphorus Requirement of Commercial Leghorns., *Poultry science* 80 (6): 769–75

Sohail, S.S. & Roland, D. a (2002) Influence of Dietary Phosphorus on Performance of Hy-line W36 Hens., *Poultry science* 81 (1): 75–83

Spencer, J.D., Allee, G.L. & Sauber, T.E. (2000) Phosphorus Bioavailability and Digestibility of Normal and Genetically Modified Low-phytate Corn for Pigs., *Journal of Animal Science* 78: 675–681

Tahmasbi, a M., Mirakzehi, M.T., Hosseini, S.J., Agah, M.J. & Fard, M.K. (2012) The Effects of Phytase and Root Hydroalcoholic Extract of Withania Somnifera on Productive Performance and Bone Mineralisation of Laying Hens in the Late Phase of Production., *British poultry science* 53 (2): 204–14

Taylor, T. (1970) The Role of the Skeleton in Egg-shell Formation, *Ann. Biol. Anim. Biochim. Bio- phys* 10: 83–91

Taylor, T.G. & Moore, J.H. (1954) Skeletal Depletion in Hens Laying on a Low-calcium Diet., *The British journal of nutrition* 8 (2): 112–24

Taylor, T.G. & Moore, J.H. (1956) The Effect of Calcium Depletion on the Chemical Composition of Bone Minerals in Laying Hens., *The British journal of nutrition* 10 (3): 250–63

Taylor, T.G. & Moore, J.H. (1958) The Effect of High and Low Levels of Dietary Inorganic Phosphate on the Pre-laying Storage of Calcium and Phosphorus and on the Composition of the Medullary and Cortical Bone in Pullets., *The British journal of nutrition* 12 (1): 35–42

Tolboom, J.G. & Kwakkel, R.P. (1998) Dynamic Modelling of Ca and P Flows in Layers: Prospects to Reduce Dietary Digestible P-levels, *British Poultry Science* 39 (sup001): 43–44

Um, J.S. & Paik, I.K. (1999) Effects of Microbial Phytase Supplementation on Egg Production, Eggshell Quality, and Mineral Retention of Laying Hens Fed Different Levels of Phosphorus., *Poultry science* 78 (1): 75–9

Usayran, N., Farran, M.T., Awadallah, H.H., Al-Hawi, I.R., Asmar, R.J. & Ashkarian, V.M. (2001) Effects of Added Dietary Fat and Phosphorus on the Performance and Egg Quality of Laying Hens Subjected to a Constant High Environmental Temperature., *Poultry science* 80 (12): 1695–701

Van de Velde, J.P., Vermeiden, J.P. & Bloot, a M. (1985) Medullary Bone Matrix Formation, Mineralization, and Remodeling Related to the Daily Egg-laying Cycle of Japanese Quail: a Histological and Radiological Study., *Bone* 6 (5): 321–7

Van de Velde, J.P., Vermeiden, J.P., Touw, J.J. & Veldhuijzen, J.P. (1984) Changes in Activity of Chicken Medullary Bone Cell Populations in Relation to the Egg-laying Cycle., *Metabolic bone disease & related research* 5 (4): 191–3

Veum, T.. (2010) Phosphorus and Calcium Nutrition and Metabolism, *In*: Vitti, D. & Kebreab, E. eds. *Phosphorus and calcium utilization and requirements in farm animals.*, Wiltshire: CABI Publishing, pp.94–111

Vits, a, Weitzenbürger, D., Hamann, H. & Distl, O. (2005) Production, Egg Quality, Bone Strength, Claw Length, and Keel Bone Deformities of Laying Hens Housed in Furnished Cages with Different Group Sizes., *Poultry science* 84 (10): 1511–9

De Vries, S., Kwakkel, R.P. & Dijkstra, J. (2010) Dynamics of Calcium and Phosphorus Metabolism in Laying Hens, *In*: Vitti, D.M.S.S. & Kebreab, E. eds. *Phosphorus and calcium utilization and requirements in farm animals.*, CABI Publishing, pp.133–151

Waldroup, P.W., Kersey, J.H., Saleh, E. a, Fritts, C. a, Yan, F., Stilborn, H.L., *et al.* (2000) Nonphytate Phosphorus Requirement and Phosphorus Excretion of Broiler Chicks Fed Diets Composed of Normal or High Available Phosphate Corn with and Without Microbial Phytase., *Poultry science* 79 (10): 1451–9

Wall, H., Tauson, R. & Elwinger, K. (2002) Effect of Nest Design, Passages, and Hybrid on Use of Nest and Production Performance of Layers in Furnished Cages., *Poultry science* 81 (3): 333–9

Wamsley, K.G.S., Ii, R.E.L., Karges, K. & Moritz, J.S. (2013) The Use of Practical Diets and Regression Analyses to Determine the Utilization of Lysine and Phosphorus in Corn Distillers Dried Grains and Solubles Using Cobb 500 Male Broilers, *Journal of Applied Poultry Research* 22: 279–297

Wasserman, R.H. & Taylor, a N. (1973) Intestinal Absorption of Phosphate in the Chick: Effect of Vitamin D and Other Parameters., *The Journal of nutrition* 103 (4): 586–99

Watkins, K.L. & Southern, L.L.E.E. (1992) Effect of Dietary Sodium Zeolite A and Graded Levels of Calcium and Phosphorus on Growth , Plasma , and Tibia Characteristics of Chicks 1 Department of Animal Science , Louisiana State University Agricultural Center , Baton Rouge , Louisiana 70803 ABSTRAC, *Poultry science* 71: 1048–1058

Whitehead, C.C. (2004) Overview of Bone Biology in the Egg-laying Hen., *Poultry science* 83 (2): 193–9

Whitehead, C.C. & Fleming, R.H. (2000) Osteoporosis in Cage Layers., *Poultry science* 79 (7): 1033–41

Wilson, S. & Duff, S.R. (1991) Effects of Vitamin or Mineral Deficiency on the Morphology of Medullary Bone in Laying Hens., *Research in veterinary science* 50 (2): 216–21

Wilson, S., Duff, S.R. & 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): 52–8 Available from: <http://www.ncbi.nlm.nih.gov/pubmed/1410819> [Accessed:

Wu, G., Liu, Z., Bryant, M.M. & Roland, D. a (2006) Comparison of Natuphos and Phyzyme as Phytase Sources for Commercial Layers Fed Corn-soy Diet., *Poultry science* 85 (1): 64–9