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Effect of a zinc amino acid complex on egg production, egg shell quality and external appearance in two laying hen genotypes housed in two different production systems

Malin Boyner

Examensarbete / SLU, Institutionen för husdjurens utfodring och vård, **543**

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Effekt av ett zinkaminosyrakomplex på äggproduktion, skalkvalitet och exteriör hos två värphönsgenotyper inhysta i två olika produktionssystem

Malin Boyner

Handledare:	Helena Wall, SLU, Department of Animal Nutrition and Management
Supervisor:	
Examinator:	Ragnar Tauson, SLU, Department of Animal Nutrition and Management
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Abstract

With an extended production cycle up to 100 weeks of age, the yearly number of layer chickens hatched and reared can be reduced, and in turn so can the euthanasia of day-old male chickens. Lately, the interest for keeping laying hens for a prolonged production cycle has increased in Sweden as well as in other European countries. However, concerns exist regarding how prolonged laying cycles affect egg quality, animal welfare as well as producer economy. In this trial, the two commercial hybrids LSL Classic and Bovans Robust were housed in furnished cages and a single tier floor production system. In addition to the effects of age, an organic mineral complex with a hypothesized positive effect on egg quality, integument and skeletal condition was evaluated. The study was conducted during 20-61 weeks of age. There were no consistent effects of the mineral amino acid complex supplementation on either egg quality, plumage condition or keel bone deformities at a hen age of 55 weeks. However, there were a lot of differences between hybrids regarding egg composition and layer performance in the two production systems. As expected and concluded in earlier research; egg shell quality, plumage condition and keel bone deformities were negatively affected by increased hen age.

Introduction

The egg production in layers as well as some welfare conditions deteriorate with the aging of the layers. Research states that aged laying hens are more prone to bone weaknesses as well as laying eggs with shells more susceptive to cracks. After peak production at approximately 28-30 weeks of age, egg production declines and the laying hen is subsequently producing fewer but larger eggs. These eggs have proportionally thinner egg shells (Bar & Hurwitz, 1987; Joyner *et al*., 1987; Bar *et al*., 1988) which accounts for significant losses to the industry yearly (Roberts, 2004; Zamani *et al*., 2005; Solomon, 2010; Nasr *et al*., 2012b). In fact, 8 % of Europe's egg production is lost every year due to broken egg shells (Nys, 1995). The interior egg quality traits are also affected by hen age. Egg weight, egg size and yolk weight increase, while albumen weight along with shell weight decrease with hen age when considered as a proportion of the total egg (Silverside & Scott, 2001). The quality of the egg shell's covering cuticle also deteriorates with hen age, making the egg more susceptible to bacterial contamination (Rodrígues-Navarro *et al*., 2013).

Lately, the interest for keeping laying hens for a prolonged production cycle is increasing in Sweden as well as in other European countries. One reason for this is the economic losses that termination of a laying cycle entails. The ending of a laying cycle results in several weeks without economic return for the farmer, as the new layer pullets need time for acclimatization and maturation to achieve profitable production results. Moreover, because it is not yet practicably possible to sort chickens by sex on embryo stage, the egg production industry involves euthanizing of day old male chickens (Weissmann *et al*., 2013). If keeping laying hens for an extended production period (e.g. 100 weeks of age instead of the custom of approximately 75 weeks of age) is possible with maintained egg quality and laying percentage the yearly number of layers needed would be decreased. It would also be a way to confine euthanasia of male chickens, hence improving animal welfare and tending to consumers' ethical standards. The resource conservation and decrease in items of expenditure that would follow, like the number of pullets needed yearly, heating of stables, electricity and feed would also be of great importance to the sustainability of the industry as well as the farmer's economy.

Along the laying cycle, the egg laying hen successively replaces structural bone with weaker medullary bone to be able to maintain calcium supply for egg shell production (Whitehead & Fleming, 2000; Riczu *et al*., 2004). With a prolonged laying cycle, the laying hen will replace even more of her structural bones (Sandilands *et al*., 2009), making her more receptive to fractures (Whitehead, 2004). Apart from producing fewer eggs, fractured laying hens are also a significant animal welfare issue (Nasr *et al*., 2013). Therefore, concerns exist regarding how prolonged laying cycles also might affect animal welfare in practice.

One potential solution investigated is the supplementation of zinc to the hens' diet. Zinc is a trace element involved in over 300 different enzyme systems in the body (Tabatabaie *et al*., 2007). In the laying hen, it is a component of the uterine enzyme carbonic anhydrase, which supports egg shell formation (Roberts, 2004; Nys *et al*., 2011b). Zinc is also considered important with regard to bone development, (Hess *et al*., 2001) the integrity of the integument (Suttle, 2010) as well as the function of the immune system (Forbes, 1984). In other words, zinc is essential for the normal function of the body. It has also been shown to alleviate the negative effect of hen age on egg shell quality (Swiatkiewicz & Koreleski, 2008).

In the pursuit to optimize nutrient utilization among aged layers, the interest to use different organic trace minerals in poultry feed has increased. A lot of research has been conducted to evaluate if the supplementation of different zinc sources (organic or inorganic) could facilitate a prolonged laying cycle by alleviating the negative effects of hen age on welfare parameters as well as production performance traits (Swiatkiewicz & Koreleski, 2008).

There are patented molecules on the market consisting of a metal ion bound to an amino acid ion, also referred to as zinc amino acid complexes. These molecules, referred to as organic, are predicted to have good bioactive functions, meaning it is easily absorbed by the animal ingesting it.

Aim of the study

The aim of this master's thesis is to investigate the effect of a zinc amino acid complex on egg production, egg shell quality and external appearance - such as plumage condition, bumble foot and keel bone deformities, in two laying hen genotypes kept in two different housing systems during the layer age span of 20-61 weeks.

Literature review

In this section the central mechanisms regarding egg formation and egg quality will be presented with support in relevant scientific literature. The effects of the laying hens' age and aging will be addressed with respect to egg quality and animal welfare. The literature review is ended with a segment discussing zinc's effects on the laying hen and egg quality, as well as its bioavailability.

The avian egg – the ideal food product

The egg consists of a mixture of nutrients, all needed for the embryo development. As all nutrients are dedicated to make the growing chick survive and develop properly, it is also a great source of nourishment for humans (Etches, 1996). The hen egg consists to 30-33 % of yolk, 60 % albumen and 9-12 % egg shell (*Figure 2*) (Coutts & Wilson, 1995; Etches, 1996; Roberts, 2004). The formation of the egg in the laying hen's body is a complicated process that takes a day and a night to transact, in which a lot of organs participate to convert nutrients into egg mass (follow the egg's formation in *Figure 1*) (Coutts & Wilson, 1995; Roberts, 2004).

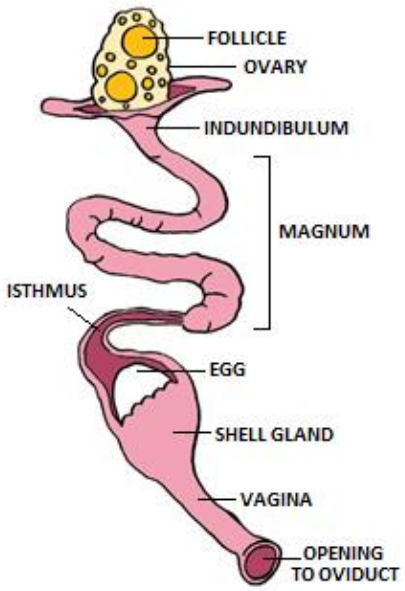

Figure 1 - Schematic illustration of the reproductive tract of the hen. Illustration by Malin Boyner, inspiration from Sjaastad et al. (2003a)

The egg formation process starts by the yolk being ovulated from the ovary into the oviduct, and further into the infundibulum. Here the yolk is enveloped with the vitelline membrane and the chalazae, which holds the yolk in place - develops in a process that takes about 15 minutes (*Figure 2*) (Roberts, 2004). It is also here that fertilization occurs in fertilized hens (Coutts & Wilson, 1995). Thereafter, the forming egg is residing in the magnum for three hours, where the albumen proteins, which serve as mechanical and bacterial protection of the yolk, are produced. The shell membranes (inner and outer) thereafter develop in the isthmus for about an hour (Roberts, 2004). At the same time, water and mineral salts are added (Coutts & Wilson, 1995).

The developing egg then enters the tubular shell gland where the albumen thickens due to addition of water and electrolytes. The egg remains here for five hours (Roberts, 2004). Next, the egg spends 15 hours in the shell gland pouch where calcium carbonate is added and the egg shell develops. The egg is thereafter laid (Coutts & Wilson, 1995; Roberts, 2004).

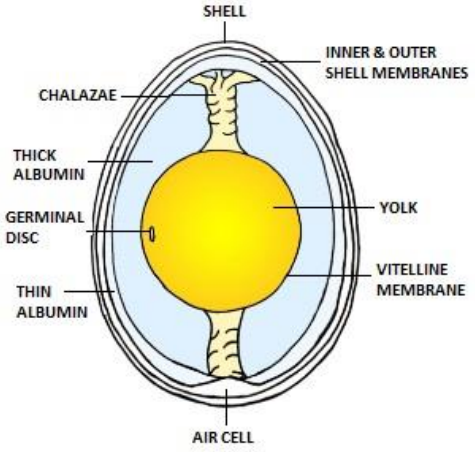

Figure 2 - The anatomy of the egg

The yolk

The laying hen has over 4000 reproductive cells in her ovaries as day-old. These cells later on develop into yolks which are released when mature (Coutts & Wilson, 1995). The yolk is surrounded by four different layers, which all together are called the vitelline membrane (Etches, 1996). The embryonic development occurs on the surface of the yolk, where the germinal disc resides (*Figure 2*) (Sjaastad *et al*., 2003a). Most of the egg's nutrients reside in the yolk. The yolk consists of 50 % water and 30 % lipids. The remaining portion is mainly protein (Burley & Vadehra, 1989). A good yolk should have a strong surrounding vitelline membrane and desirable colour. The preferences for yolk colour differ between consumers in different countries (Roberts, 2004). The yolk is of great importance to the developing embryo (Matos, 2008). Of the egg's total energy content, 80 % is found in the yolk in the form of easily digestible fat (Coutts & Wilson, 1995). Close to hatching, calcium is transferred from the egg shell to the yolk to provide the chick with a reliable calcium depot the first days of life (Matos, 2008).

The albumen

The albumen is the main source of water for the developing embryo (Etches, 1996). In fact, 88 % of the albumen consists of water and the albumen as a whole occupies about 60 % of the egg's volume (Burley & Vadehra, 1989). The albumen is a mixture of as many as 40 different proteins that together make up 12 % of the albumen content (Etches, 1996; Roberts, 2004). The proteins are primarily ovotransferrin, ovomucoid, lysozyme, ovalbumen, globulins and ovomucin. Ovomucin is the main protein responsible for the height of the albumen (Silversides & Scott, 2001). The albumen is not as thick all the way though, and is therefore divided into thin or thick albumen. The thick innermost albumen touches the vitelline membrane where the chalazae is attached (*Figure 2*) (Burley & Vadehra, 1989). One trait important to the functional properties of the albumen is the albumen viscosity. High viscosity is crucial to the result when whipping or emulsifying the albumen. It also affects albumen gelling properties (Kemps *et al*., 2010). The height of the albumen is a common assessment method for egg quality and freshness as well as the viscosity of the thick albumen (Roberts, 2004) and is often measured in Haugh Units (HU). The Haugh unit formulae include a logarithmic scale and adjust for the size of the egg to be able to compare albumen height in eggs of different size (Silversides & Scott, 2001). Lately this method has however been questioned because it is affected by age and strain of the hen (Roberts, 2004). The quality and

viscosity of the albumen is not only important for the freshness of the egg, it also has clear impact on functional properties.

The shell

The egg shell consists of both organic and inorganic materials (Roberts, 2004; Solomon, 2010). Shell membranes, mammillary cores, shell matrix and the cuticle make up for the organic portion, while the inorganic part of the shell consists of calcium carbonate (Roberts, 2004). The shell matrix mainly consists of protein. Its function is not fully understood, however, the shell matrix is thought to be working as a shock absorber in the shell, and represents 2-3 % of the calcified layer (Burley & Vadehra, 1989). The uterine enzyme carbonic anhydrase catalyzes the formation of carbonic acid from water and CO_2. Carbonic acid is the precursor of calcium carbonate which makes up the inorganic part of the egg shell (Sjaastad *et al*., 2003a). The mammillary cores in the shell serve as a connection point between the outer shell membrane and the nucleation sites of growing calcium carbonate crystals (Etches, 1996) (*Figure 3*) and consist mainly of protein (Burley & Vadehra, 1989). These calcium carbonate crystals are tightly packaged, however, the egg shell also contains 10 000 pores that enable air exchange, providing the growing chicken embryo with oxygen (Etches, 1996).

The function of the avian egg shell is to protect the egg contents and the embryo in several different ways. Primarily, it is there to serve as a barrier against bacterial penetration. It is therefore important that the egg shell is free from cracks and defects to guarantee safety for human consumption (Mabe *et al*., 2003; Zamani *et al*., 2005; Nasr *et al*., 2012b). However, the egg shell does not eliminate the risk of salmonella contamination because salmonella can contaminate the yolk of already infected layers during egg formation (Gumudavelli *et al*., 2007). Moreover, the egg shell serves as a mechanical defense barrier which protects the embryo, and prevents water loss (Etches, 1996; Rodríguez-Navarro *et al*., 2013). Furthermore, the egg shell is the main source of calcium for the developing embryo (Zamani *et al*., 2005; Matos, 2008; Rodríguez-Navarro *et al*., 2013).

Factors believed to have an impact on egg shell quality are, among others, hen age, genetics, disease, stress, stage in the production cycle, egg weight, nutrition, water quality and its salt content, microclimate, production system as well as intensity and persistence of the production (Roberts, 2004; Ledvinka *et al*., 2011). Shell thickness and shell breaking strength are two traits that are highly correlated (Roberts, 2004; Solomon, 2010; Ledvinka *et al*., 2011). Moreover, breakage of the shell occurs easier along the longitude of the egg than the latitude and the broad pole cracks easier than the narrow one. Heat stress can alter the uptake of calcium for egg shell formation due to loss of bicarbonate, which in turn impairs egg shell strength (Roberts, 2004). It is not only the shell that provides strength, the membranes also acts as stabilizing structures in the egg shell (Solomon, 2010).

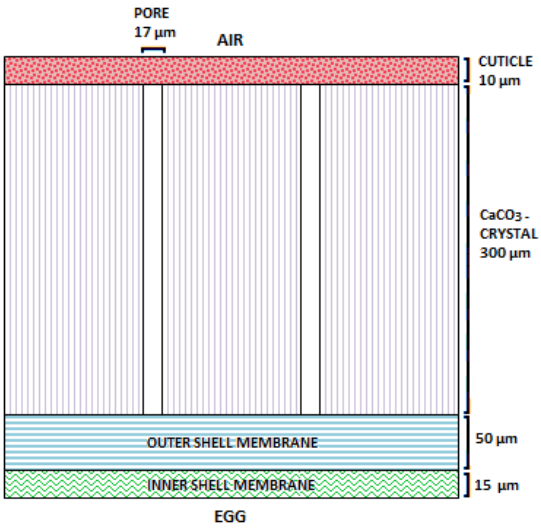

Figure 3 - Schematic illustration of the anatomical structure of the egg shell

As previously mentioned, the egg shell is a thin mineral structure, outermost coated by a cuticle layer (Figure 3). The cuticle, which is mainly composed by proteins, is produced in the uterus, and functions to regulate the egg shell's permeability by plugging the pores in the shell (Etches, 1996; Rodríguez-Navarro *et al*., 2013). The cuticle protects the egg from bacterial penetration mechanically, but also chemically, as it contains antibacterial agents (Rodríguez-Navarro *et al*., 2013). However, the protective effects last only a short time, as researchers have observed an increased permeability of the egg shell after 24 hours when the cuticle had dried, increasing the risk of bacterial penetration (Rodríguez-Navarro *et al*., 2013).

The calcium metabolism

The mineral calcium is required for many biochemical processes in the body. In the avian body it is essential for the structure of bones and the egg shell. As the hen is in need of high blood concentrations of calcium during the egg shell calcification process, there are strict regulatory mechanisms in the body of the hen that help to maintain calcium supply. The calcium needed for the production of one egg represents 10 % of the hen's body reserve of calcium (Matos, 2008). Calcium appears to 99 % in the bones of the bird, while the remaining 1 % exists as intracellular and extracellular calcium. Only 0.1 % of the total calcium is present as extracellular calcium which is the physiological active form that the hen can use in different processes such as egg shell calcification.

How much calcium that is resorbed from either source (bone or diet) is dependent on the available amount of calcium in the feed. A greater proportion of calcium is resorbed from the bones to calcify the egg shell at night when the hen is not ingesting any feed, or if the feed is low in calcium. In contrast to mammals, birds respond very quickly to decreased calcium levels (Matos, 2008). To adapt to suddenly increased calcium requirements, hormones efficiently increase absorption of calcium from the intestine and medullary bone (Whitehead & Fleming, 2000; Whitehead, 2004; Matos, 2008). Parathyroid hormone (PTH) is one of these calcium regulatory hormones, which sets in when plasma calcium levels are too low. PTH then increases calcium reabsorption, decreases calcium loss through the urine, increases the formation of calcitriol (the active form of vitamin D in the body) by the kidney and speeds up bone reabsorption. In this way, the avian body is capable of increasing calcium levels in a few minutes. In mammals, this process could take hours or days to transact (Matos, 2008).

The steroid hormone vitamin D is important in the calcium metabolism. Vitamin D_3 is always included in poultry feed to birds kept indoors, because they are not able to synthetize vitamin D from UV-light. It is converted to its most physiological active form 1,25-dihydroxycholecalciferol (calcitriol) in the kidney. The primary function of calcitriol is to

enhance mobilization of calcium from medullary bone and absorption of calcium from the feed in the intestines by stimulating production of the calcium binding protein calbindin. A similar form of calbindin is also present in the uterus, and is thought to locate calcium and assist in transferring it into the uterus. Vitamin D is also needed for bone mineralization at the onset of sexual maturity, which in turn is mediated by estrogen (Matos, 2008).

The aging hen – the challenge

Effects of age on exterior egg quality

At onset of lay, the egg weight is rather low (Etches, 1996) but thereafter it increases gradually throughout the laying cycle. The rate of egg production increases progressively until peak production. Peak production occurs at approximately 28-30 weeks of age (ISA, Hendrix Genetics, 2014). From then on, the hens are subsequently producing fewer but larger eggs with thinner egg shells (Bar & Hurwitz, 1987; Joyner *et al*., 1987, Bar *et al*., 1988). The proportion of egg shell also decreases with age (Silversides & Scott, 2001). A lot of eggs are downgraded due to different quality issues such as thin and damaged egg shells, accounting for severe economic losses to the producer and industry yearly (Roberts, 2004; Zamani *et al*., 2005; Solomon, 2010). Due to shell weakness, 8 % of Europe's egg production is lost every year (Nys, 1995). Broken egg shells accounts for as much as 80-90 % of the downgrades (price reduced eggs), making egg shell quality a major topic of interest to the producer and the industry (Mabe *et al*., 2003). If the egg is of incorrect size, the egg shell is abnormally calcified or has dirt marks it will also be downgraded (Nys *et al*., 2011a).

Many researchers have concluded that rate of egg production and egg quality decline as the hens grow older (Joyner *et al*., 1987; Alodan & Mashaly, 1999; Bar *et al*., 1999). As hens grow older they do not have the same capacity to produce eggs with as high egg shell density as younger hens. Hence, the aging hen is not as capable to respond to changes in her need for calcium, which results in a higher proportion of cracked eggs in older animals (Bar & Hurwitz, 1987; Bar *et al*., 1999). Because the hen's eggs are getting larger with her age, the calcium carbonate secretion is not enough to support the egg shell development of a larger egg (Etches, 1996). It has been suggested that this could be due to an age induced decline in the vitamin D dependent process of utilizing calcium from the intestine, as old hens often have a greater calcium deposit in their bones accumulated over time (Bar *et al*., 1999).

On the contrary, old and young hens have been observed to have similar hormonal status, indicating that the mechanisms responsible for regulating calcium levels in the blood are unimpaired in aged layers. Instead, the shell gland and its efficiency in utilizing calcium for egg shell production has been pointed out as the potentially weak link in aged laying hens (Joyner *et al*., 1987). The egg shell gland and intestine have great potential of providing and transporting calcium for egg shell production in the laying hen (Bar & Hurwitz, 1987). Why the egg shell gland fails to provide calcium in the aged layer is still a matter of speculation regarding several possible factors such as reduced blood flow to the shell gland with increased age, impaired function of the gland or simply the increased surface area of an aged layer's larger egg.

In a study by Franco-Jimenez & Beck (2005) old laying hens (>100 weeks old) were observed with regard to calcium uptake. The researchers found that there was a hybrid effect in the ability to utilize calcium, even though circulating estrogen (which mediates the onset of sexual maturity) and egg shell thickness did not differ significantly between strains. These results are interesting, because the advantage in calcium metabolism detected in one genotype was not reflected in their eggs' shell strength (Franco-Jimenez & Beck, 2005).

The enveloping cuticle layer is also important in guaranteeing food safety and internal egg quality. The quality and degree of coverage by the cuticle layer are known to decrease with hen age, making eggs from aged layers more prone to bacterial penetration (Rodríguez-Navarro *et al*., 2013). Because the cuticle coverage is also important for egg safety and egg

quality, cuticle coverage has been investigated in old laying hens (Rodríguez-Navarro *et al*, 2013). Thus, cuticle coverage was thinner, more variable and more irregular in older laying hens (70 weeks of age) than in younger. The researchers also observed that when the cuticle dried, pores in the egg shell were left open, allowing for albumen water to diffuse out of the egg. The cuticle composition was altered as the hens aged; implying declined mechanical strength and resistance of the cuticle to microbial penetration with hen age. The freshness of the egg also affected cuticle composition (Rodríguez-Navarro *et al*., 2013)

Effects of hen age and time in storage on interior egg quality

When considered as a percentage of the total egg weight, yolk weight increases with hen age, while albumen content decreases (Silverside & Scott, 2001; Van Den Brand *et al*., 2004). The hen's age also influences other interior quality traits of the egg. The albumen height decreases with hen age (Silversides & Scott, 2001; Roberts, 2004). The albumen in eggs laid by aged laying hens contains more water, and as a consequence the albumen is thinner (Rodríguez-Navarro *et al*., 2013). Along with increased hen age, egg size and albumen weight also increase. Regarding albumen pH, Silversides & Scott (2001) found no effect of hybrid or age. Therefore, they suggested that albumen pH could be used as a general freshness quality measurement instead of the commonly used parameter albumen height. According to Silversides & Scott (2001), the measuring of albumen height is biased by age and strain of the hen.

The quality of the membranes enveloping the yolk decreases during storage, increasing the risk for the yolk to burst (Roberts, 2004). The albumen height decreases with time in storage as well (Silversides & Scott, 2001). Moreover, nutrition plays only a little part with regard to the height of the albumen, while storage time and storage condition as well as age and strain of the hen are of high relevance (Silverside & Scott, 2001; Samli *et al*., 2005). Egg and albumen weight decreases with storage, whereas yolk weight increases (Coutts & Wilson, 1995; Silversides & Scott, 2001).

Due to the interaction of different albumen proteins, where ovomucin is considered to be the main actor, the viscosity and height of the albumen deteriorates with time in storage (Silversides & Budgell, 2004). The pores in the egg shell then enables water from the albumen to diffuse out of the egg (Rodrígues-Navarro *et al*., 2013).

The egg shell's ability to exchange gases also increases albumen pH and albumen dry matter with time of storage (Samli *et al*., 2005). According to the results presented in the study by Silversides & Scott (2001), effect of storage on shell weight is unclear and the effect of age on albumen pH was small, although it increased with longer storage time (Silversides & Scott, 2001; Roberts, 2004). The air cell in the egg has also been shown to increase with increased storage time (Samli *et al*., 2005).

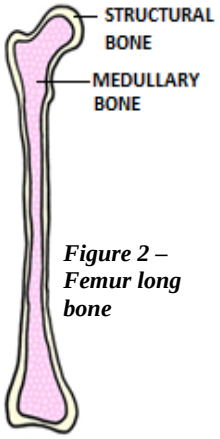

Figure 2 – Femur long bone

Bone formation and strength

At sexual maturity, the mechanisms for bone formation in the hens' body changes to provide the hen with a reliable calcium source for egg shell production. The formation of structural bone is replaced by medullary bone formation due to a rise in estrogen (*Figure 4*). Medullary bone is a bone type unique to crocodilians and birds, serving as a calcium depot (Whitehead & Fleming, 2000; Matos, 2008). The bone is needed as a reliable calcium source

because the egg shell gland is much dependent on calcium supply during egg shell formation at night, at a time when the hen is not ingesting any dietary calcium (Whitehead, 2004). The medullary bone is weaker in its structure and the onset of its formation may therefore provide a gateway to osteoporosis and other bone weaknesses, in particular, if combined with poor nutrition and inactivity (Whitehead & Fleming, 2000; Whitehead, 2004).

Bone integrity and egg production

Even though resorption of structural bone occurs during the laying cycle, the total bone content can be the same, or even increase due to the accumulation of medullary bone. The problem is that it is weaker in its structure (Whitehead, 2004). The structural bone regression during lay is however reversible, hence the structural bone builds up again when the hen goes out of lay and the estrogen levels descend (Matos, 2008). According to Whitehead (2004) the number of eggs laid during a production cycle is not the sole cause of osteoporosis. The number of eggs laid in a sequence before a pause day differs between hens and is of high relevance too. This is explained by the hen's decrease in circulating estrogen when she is out of lay. These pauses make it possible for the structural bones to reform themselves (Whitehead, 2004). However, in today's modern production, the laying hen has been selected and bred for high egg production during a prolonged laying cycle, excluding the long pauses that arises from the molting process in nature, making her even more susceptible to osteoporosis (Riczu *et al*., 2004; Sandilands *et al*., 2009). For this reason, osteoporosis is primarily a problem in aged laying hens (Whitehead & Fleming, 2000).

Keel bone deformities

Poor bone quality facilitates fractures such as keel bone fractures which are considered painful for the bird, impairing animal welfare (Whitehead & Fleming, 2000; Nasr *et al*., 2012a). It has been suggested that the high demands of calcium for egg production is one reason for the loss of keel bone strength and an increased incidence of fractures of the keel at peak production (Nasr *et al*., 2013). See *Figure 5* and *Figure 6* for schematic pictures of the keel bone and the skeleton of a bird.

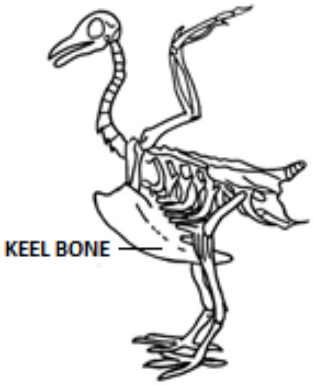

Figure 4 -
Skeleton of a bird.

Figure 3 - Keel bone.
Illustration: Malin Boyner.
***Inspiration: Fleming et al*. (2004).**

In a study performed by Nasr *et al*. (2012b; 2013) it was found that hens with healed keel bone fractures produced fewer eggs with a lower egg quality than birds with no fractures (Nasr *et al*., 2012b; 2013). Also, Nasr *et al*. (2013) found that hens with keel bone fractures had a higher feed and water intake compared to hens with intact keels. The authors speculates

that this could be due to a higher demand for calcium to heal the fracture, stress, or that the birds had trouble reaching feed and water, giving them an incitement to eat more when they were near these resources. These findings are incentives to reduce bone weakness disorders considering both animal welfare and economic return. Besides the losses in the number of eggs produced, these birds actually ate more. Hence the negative economic impact of keel fractures is twofold (Nasr *et al*., 2013).

In contrary to keel bone fractures a keel bone deviation is not as severe as a fracture, and therefore normally less harmful to the bird. Nevertheless, perch design is a topic of concern as long-term use of perches can result in deformed keel bones (Abrahamsson & Tauson, 1996). This was also concluded by Tauson (1998) who found keel bone deformities to depend on perch shape. The incidence of keel bone deformities has also been shown to increase with age due to the progressive loss of structural bone. Moreover, layer genotype is of great importance for the incidence of keel bone deformities (Fleming *et al*., 2004; Nasr *et al*., 2013). On the contrary, keel bone deformities were found to be influenced by hybrid only to a limited extent in a study performed by Tauson & Abrahamsson (1996). Hens provided with perches generally exhibit more fractures and deformities of the keel bone. This is probably due to the pressure that the keel bone is subjected to during roosting (Sandilands *et al*., 2009) but may also be caused by collision at flying in loose housed multi-tiered systems. Incorporation of soft material at the edge of furnishings was suggested by Sandilands *et al*. (2009) as a solution that could decrease the incidence of keel bone deformities when hens roost (Sandilands *et al*., 2009). Using a round shaped perch profile with a flat upper part reduce the incidence of keel bone deformities compared to fully round perches or rectangular perches with sharper edges (Tauson & Abrahamsson, 1994; 1996). The most commonly used perch commercially today is the round shaped perch. However, according to the European food safety authority (EFSA, 2015) the literature covering layer preferences for perch shapes is inconclusive. The authors of the report do however suggest perches with rounded edges in agreement with Tauson & Abrahamsson (1994;1996).

Bumble foot

If the laying hen incurs wounds on the foot pad or toes that are deep, it can result in abscesses and develop into a condition called bumble foot. The metatarsal foot pad swells and is often visible when looking at the foot from above (*Figure 7*) (Wilcox *et al*., 2009). Bumble foot is considered very painful for the bird, affecting animal welfare, as birds suffering from the condition have been observed to be unable to roost or stand on the affected foot (Tauson & Abrahamsson, 1996). Moreover, as it impairs the mobility of the hen, it can make it difficult for the hen to reach food and water (Wilcox *et al*., 2009). The problem with bumble foot is often occurring at 35-45 weeks of age (Blokhuis *et al*., 2007), thereafter the feet are healing and the incidence and severity declines.

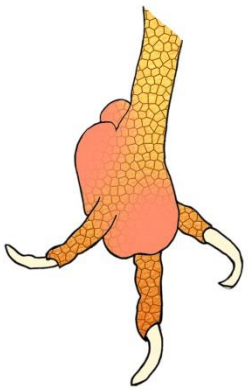

Figure 5 - Bumble foot. Illustration: Malin Boyner

Bumble foot is believed to originate from poor hygiene and perch design (Tauson & Abrahamsson, 1996; Tauson, 1998). Often, bacteria such as *Staphylococcus aureus* enters through damaged skin on the feet, causing inflammation (Wilcox *et al*., 2009; Lay *et al*., 2011). Perch design has been discussed to be the primary reason for the occurrence of bumble foot, skin erosions being of less importance (Wang *et al*., 1998). Abrahamsson & Tauson (1998) proposed the negative effect of moisture on the development of bumble foot to be of higher importance than other hygienic conditions.

Scores for bumble foot have been shown to differ between hybrids, indicating a variety of the susceptibility to the condition. Tauson & Abrahamsson (1994; 1996) found the LSL layer to be inferior with regard to the incidence of bumble foot compared to two other white hybrids used (Shaver 288 and Dekalb XL) when investigating perch material. They also found a significant interaction between hybrid and perch design as well as production system. Tauson *et al*. (1999) and Tauson (2005) reported bumble foot disorders to be more commonly occurring in floor housing systems were perches are used. This was also confirmed by Wilcox *et al*. (2009) and Lay *et al*. (2011) who reported higher incidences of bumble foot in floor production systems compared to cage systems. A generally poorer foot hygiene and higher moisture in litter are believed to be the main cause (Tauson, 2005).

Because bumble foot at a high degree affects animal welfare (Tauson & Abrahamsson, 1996), it has been suggested by the participants of the Laywel project to evaluate laying hens and score them for bumble foot regularly in order to compare outcomes from different trials across countries (Blokhuis *et al*., 2007).

Plumage condition

In nature, birds are replacing their worn feathering once or twice a year in a process called moulting. The wear of plumage is often the result of social interactions and activities such as fighting, foraging and mating. Birds that are replacing their integument are in need of sulfur containing amino acids to be able to produce keratin in this very energy demanding process. Producing eggs and feathers at the same time is therefore not possible due to the high energy requirements (Wingfield & Silverin, 2010). A natural molt is induced by hormonal changes following from shortened day length. In commercial laying hen flocks a constant day length is maintained the whole year by the use of light programs. Consequently, a damaged feather cover is difficult to recover during the egg laying cycle.

Feather pecking is still a large problem that is believed to cause pain to the animal being pecked (Gentle & Hunter, 1991). Furthermore, feather pecking causes great losses to the producer because poorly feathered birds consume more feed to be able to stay warm. If almost completely defeathered, a naked hen can consume up to 40 % more feed than a hen with a complete plumage (Tauson, 1980; Tauson, 1998; Swedish Board of Agriculture, 2005).

It is thus important to keep hens in a good plumage condition. Poor feathering and poor feather condition may also encourage additional feather pecking, as hens seem keener to peck on already damaged feathers, resulting in hens with poor plumage condition (Freire *et al*., 1998; McAdie & Keeling, 2000). Feather pecking is not the same as aggressive pecking, the latter being directed downwards and toward the head. Feather pecking is sometimes divided into gentle (light pecks) or severe (forceful pecks) depending on force and the recipient hen's reaction to the peck (Bilčík & Keeling, 1999). Bilčík & Keeling (1999) concluded that severe pecks caused the most feather damage in comparison with gentle and aggressive pecks. There was also a correlation between feather pecking and group size, with more feather damage in large group sizes (Bilčík & Keeling, 1999).

The problems derived from feather pecking are many and the behaviour is multifactorial. The behaviour has also been suggested to work as a gateway to more severe problems such as cannibalism. When the hen becomes defeathered as a consequence of feather pecking, the bare skin is more susceptive to injuries. Consequently, when blood is visible it might trigger cannibalistic behaviour (McAdie & Keeling, 2000), which is a welfare problem (McAdie & Keeling, 2002) and may cause high mortality rates. In the earlier mentioned study, McAdie &

Keeling (2000) found that hens with manipulated feathers received three times more pecks than the control hens with intact feathers. Furthermore, the manipulation of feathers resulted in a cannibalistic outbreak, where the hens attacked were injured in the areas of damaged feathers in 92 % of the registered cases (McAdie & Keeling, 2000).

Early on, Blokhuis (1986) highlighted the probability of feather pecking being a foraging behaviour redirected from ground pecking. Another hypothesis is that feather pecking originates from redirected dust bathing behaviour (Vestergaard & Lisborg, 1993). The rearing of young pullets and chickens are crucial. For instance, suitable substrate must be provided to prevent this problematic behaviour from occurring (Bilčík & Keeling, 1999; McAdie & Keeling, 2000).

Among other findings regarding nutrition; mineral deficiency, protein deficiency and improper amino acid levels can all lead to feather pecking. Moreover, the coarseness of the feed impacts the time the layers spend on feed intake and foraging, ultimately affecting feather pecking behaviour. Diets also containing fibre and roughages have been observed to decrease feather pecking (Van Krimpen *et al*., 2005).

The frequency of feather pecking behaviour also differs between laying hen genotypes, thus genetic factors must be included in the work to reduce the incidence of the behaviour. Moreover, feather pecking has been observed to be copied and imitated among hens. Therefore, removal of an antagonistic hen might not be the solution because its behaviour may already have been copied by others (McAdie & Keeling, 2000; McAdie & Keeling, 2002). It is also hard to locate and remove an antagonistic hen in a large flock of loose housed layers. Considering this, feather pecking can spread more rapidly, affecting more animals in a loose production system (McAdie & Keeling, 2002).

Evaluation of plumage condition is an indirect method to estimate the level of feather pecking in a laying hen flock. Earlier, there were different methods in use; evaluation of the body and plumage as a whole, or scoring of individual parts of the body and plumage. A standardized method is now often in use where the hen is scored at neck, breast, back, wings, cloaca and tail (Tauson *et al*., 2005). Often, the hen is simultaneously scored for pecking wounds on the rear part of the body and on the comb, keel bone deformities and the occurrence of bumble foot (Kjaer *et al*., 2011).

Zinc – the key to a solution?

Function

Zinc is a trace mineral required for a lot of functions in the animal body (Onderci *et al*., 2003) because it is involved in over 300 different enzyme systems (Tabatabaie *et al*., 2007). In the laying hen, it is a necessary component of the important uterine enzyme carbonic anhydrase, among other things. Carbonic anhydrase supports shell formation trough supply of carbonate ions (Roberts, 2004; Nys *et al*., 2011b). When this enzyme is inhibited, bicarbonate ion secretion is decreased, resulting in lower egg shell weights. Also, it has been discussed that trace minerals might be able to alter the morphology of the calcite crystals in the egg shell, and mechanical properties accordingly (Mabe *et al*., 2003; Zamani *et al*., 2005). Because of this, zinc is always included in commercial poultry feed today (Tabatabaie *et al*., 2007; Lai *et al*., 2010; Nys *et al*., 2011b).

Zinc is important to all species and deficiency is characterized by growth problems, integument lesions, lack of appetite (Suttle, 2010), severe dermatitis, slow wound healing, delayed sexual maturity, impaired sexual function, embryo abnormalities and problems with the immune system (Forbes, 1984). Moreover, zinc deficiency disturbs the metabolism of lipid, protein and carbohydrates (Forbes, 1984). Already in 1984, it was stated that problems occurring from zinc deficiency may be due to that zinc deficiency inhibits nucleic acid metabolism, resulting in inadequate cellular replication. Zinc was also proposed to be important with regard to membrane structure and function, growth, skin resistance, feathering and bone development (Forbes, 1984, Hess *et al*., 2001).

Egg shell quality

It has been proposed that zinc supplementation in the form of zinc amino acid complexes may help limiting the negative effect of hen age on egg shell quality (Swiatkiewicz & Koreleski, 2008). Resistance to shell fracture and shell breaking strength was improved in aged laying hens (60-73 and 69-82 weeks old) when supplemented with zinc, manganese and copper irrespective of the source in a study by Mabe *et al*. (2003). However, there was no increase in the total egg shell material deposited during the egg shell formation process regardless of zinc source indicating that the combination of trace elements may improve some of the properties important to mechanical integrity of the shell and not the amount of deposited materials needed for shell formation (Mabe *et al*., 2003).

On the contrary, Kim & Patterson (2005) found no significant differences in shell thickness between hens fed zinc sulfate (3000 ppm) and the control groups at the lower zinc inclusion (1000 ppm). Also, at the higher zinc inclusion treatment, egg shell thickness and egg production was significantly reduced, indicating that 3000 ppm of zinc is too high for laying hens (Kim & Patterson, 2005). Apart from Mabe *et al*. (2003), Zamani *et al*. (2005) did find a significant effect on the amount of egg shell material deposited during shell formation when supplementing laying hens with combined zinc oxide and manganese oxide. The authors suggest that supplementation of zinc and manganese may help improve mechanical egg shell properties, thereby decreasing the amount of cracked eggs (Zamani *et al*., 2005). The same authors also observed a significantly higher egg production in laying hens receiving the higher level of zinc in their diet (Zamani *et al*., 2005).

Growth and skeletal integrity

As mentioned earlier, zinc is essential for enzyme function, the immune system's integrity, as well as bone development and maintenance (Lai *et al*., 2010). Furthermore, zinc is an important factor regarding embryo development and growth. Embryos and chicks from mother hens fed zinc deficient diets show many disturbances. In a study conducted by Kienholz *et al*. (1961) it was seen that chicks from zinc deficient mothers that were able to hatch were unable to stand, eat and drink, and had trouble breathing. Zinc deficient mothers also resulted in embryos with underdeveloped skeletons (Kienholz *et al*., 1961).

Skeletal and bone development is thus dependent on a sufficient zinc administration. Hence, leg abnormalities in broiler chickens have been shown to be cured when the chickens were supplemented with copper, manganese and zinc of both inorganic and organic source at higher levels than the one recommended by the National Research Council (1994), indicating that today's fast growing broiler strains are in need of a higher level of minerals to function well (Mondal *et al*., 2009). Interestingly, trace mineral requirements for poultry are still based on the National Research Council's recommendations set in 1994, comprising results from research derived in the 1950s (Attia *et al*., 2013). In the earlier mentioned study by Mondal *et al*. (2009), plethoric supplementation of zinc, copper and manganese also increased live weight and feed conversion ratio in broilers (Mondal *et al*., 2009).

Plumage and skin condition

As zinc deficiency is characterized by integument lesions, and impaired wound healing (Suttle, 2010) there are different studies investigating the effects of zinc on plumage condition and feathering. Additionally, feathers are mainly composed of keratin (Sjaastad, 2003b) which in its synthesis is dependent on zinc (Suttle, 2010). Zinc deficient chicks were observed to have retarded feather development at hatching and frizzled feathers later in life in a study performed by Kienholz *et al*. (1961). Moreover, Hess *et al*. (2001) observed improvements in back and wing bruising in broilers fed zinc amino acid complexes (Hess *et al*., 2001).

Calcium – the zinc antagonist

Calcium and zinc have an antagonistic relationship (Kienholz, *et al*., 1961; Forbes, 1984; Lai *et al*., 2010), making it even more important to ensure zinc utilization in producing animals that also require a high amount of calcium, such as laying hens (Matos, 2008). The bioavailability of zinc is also affected by the feed's content of plant proteins, which contain phytic acid. This acid is known to inhibit zinc uptake (Forbes, 1984). The feed content of fiber, phytate, calcium and different chelating agents is thus crucial to the level of zinc that can be utilized (Lai *et al*., 2010).

In a study by Kienholz *et al*. (1961) laying hens were unable to maintain a normal weight gain when given a diet low in either calcium or zinc. However, when fed higher levels of zinc and calcium in combination, they gained significantly more. Moreover, high-calcium diets provided to mother hens increased zinc deficiency symptoms in their chicks (Kienholz, *et al*., 1961).

Environment

Supplementation of zinc is also thought to have positive features in more areas than production and health. Zinc can also be a useful tool to decrease the negative effects of ammonia and nitrogen losses to the environment when housing laying hens. High indoor levels of ammonia is a health issue to both animals and workers in the stable, causing drops in

production and impairing both working conditions and animal welfare (Kim & Patterson, 2005). The authors found significantly increased room ammonia levels in hens fed control feed compared to the hens given zinc sulfate supplementation, indicating a positive reduction in ammonia volatilization in zinc supplemented hens. Furthermore, zinc supplemented hens had a higher nitrogen retention, due to reduced decomposition of uric acid in the manure, than did the control groups. However, too high zinc levels in the manure could lead to zinc accumulation in soil and plants, which is not good for the environment (Kim & Patterson, 2005).

Bioavailability – Relevance of zinc source

Zinc can be supplemented in different forms, which are either organic or inorganic. Inorganic zinc is provided as sulfates, oxides, carbonates or chlorides while organic zinc is available as metal ion complexes joined to organic molecules (Attia *et al*., 2013). The bioavailability and source of zinc supplements have been discussed by many authors, with the absorption differences between organic and inorganic zinc supplements being one of the largest topics of interest. Some have even stated that the theory of organic sources' higher bioavailability is a scientifically unsupported one, originating from the industries' fear of zinc deficiency (Suttle, 2010).

In general, organic chelates of trace elements are considered to have higher tissue availability than the inorganic sources (Mondal *et al*., 2009) and this theory is currently investigated in different trials. Why complexed trace minerals are believed to have a higher availability in the animal body is because they have been assessed to have higher water and lipid solubility, as well as a lower incidence of interactions with other nutrients during absorption (Hess *et al*., 2001). Although, contrary to the general hypothesis, Mondal *et al*. (2009) found no significant effect regarding increased absorption when partially replacing inorganic trace minerals with their organic counterparts. Nor did Mabe *et al*. (2003) find any critical differences between inorganic and organic sources of zinc at the predetermined three measuring occasions; however regardless of origin, zinc, manganese and copper supplementation improved shell breaking strength and fracture resistance in aged laying hens (Mabe *et al*., 2003).

The effect of substitution of inorganic zinc obtained from zinc oxide with their sulfate or organic sources (metal amino acid complexes) with respect to performance and egg shell quality in laying hens has been studied by Gheisari *et al*. (2011). The study demonstrated that the hens had trouble utilizing zinc from oxide sources and that full substitution with sulfate zinc improved feed intake and egg production while decreasing the percentage of cracked eggs. In addition, it was suggested that zinc provided in its organic form is preferable in cases when dietary levels of trace elements are marginalized (Gheisari *et al*., 2011).

Furthermore, the effects of organic zinc (provided as metal amino acid complexes) on young laying hens had a large improving effect on feed conversion ratio, broken egg percentage, shell thickness and HU. Therefore, the authors emphasizes the need for further research which should also include old and force molted birds (Gheisari *et al*., 2011). Tabatabaie *et al*. (2007) found no effect on egg production, egg weight or feed conversion ratio between treatments with organic and inorganic zinc sources in laying hens. Feed intake was lower in the group fed organic zinc and no differences were seen between treatments regarding shell thickness. The authors discuss that the minor differences between treatments could be due to sufficient zinc level in the basal diet or the short trial period at 50-56 weeks of hen age (Tabatabaie *et al*., 2007).

Swiatkiewicz & Koreleski (2008) found no differences in neither bone quality nor egg shell thickness or egg shell density when inorganic zinc was substituted with zinc amino acid complexes. However, they found a significant effect of improved shell breaking strength when supplementing organic zinc to layers at an age of 62 and 70 weeks. These findings indicate an alleviating effect of zinc on the negative effect of hen age on egg shell quality (Swiatkiewicz & Koreleski, 2008).

There are different ways to determine the bioavailability and tissue uptake of trace minerals. Among these methods are blood and plasma sampling, although they tend to be time consuming and expensive. An alternative to more invasive techniques is to look at the feathering score of hens, as visual observation in combination with feather mineral analysis can reflect the zinc status of poultry. This method might also be useful in combination with others or when the feed is known to contain dietary antagonists. In a study on broilers performed by Lai *et al*. (2010) the authors found the feathers on the back and wings to be a useful tool to detect zinc deficiency.

The scientific community presents inconsistent research results regarding the impact of source when feeding supplemental zinc to laying hens (Swiatkiewicz & Koreleski, 2008; Gheisari *et al*., 2011). It is discussed that these contradictory findings are due to differences in the research models used regarding hen age, the composition of the experimental and basal diet, relative substitution and also the origin of the organic trace mineral used (Gheisari *et al*., 2011). Because it is not unanimously adopted that zinc supplementation improves egg quality and counters the adverse effects of hen age, the additional costs of buying feed supplements is proposed to be carefully considered (Roberts, 2004).

Method and materials

Layers and housing systems

A total of 3276 laying hens were used in this study. At 15 weeks of age, the layer pullets were delivered to the poultry facilities (furnished cages and single tiered floor systems) at the Swedish Livestock Research Centre of the Swedish University of Agricultural Sciences (SLU), located outside Uppsala. A total number of 1440 layer pullets whereof 720 Lohmann Selected Leghorn Classic (LSL) and 720 Bovans Robust hybrids were placed in 180 furnished cages (by Victorsson Poultry) giving eight layer pullets per cage. In the floor system, the remaining 1836 birds, half of each hybrid, were divided into 18 floor pens, resulting in 102 birds per pen. The birds were not beak-trimmed because the procedure is prohibited in Sweden. The pullets were reared in housing systems similar to those they were to be placed in at the research center. Both hybrids were reared at the same farm and fed the same feed prior to the study.

The lighting program was set in consultation with the principle investigator and maintained by the personnel in the stables to avoid outbreaks of feather pecking. Dead birds or birds with low body weight or other abnormalities were replaced with healthy animals until 20 weeks of age when the trial began and the groups in the floor system were equalized in number of birds per pen.

At arrival, two randomly chosen pullets out of eight placed in every cage received leg rings on both legs. These layers were later on chosen as focal individuals when scoring birds' exterior appearance. In case two birds marked with leg rings could not be found due to either loss of rings or mortality, a new focal bird was randomly chosen.

The floor system

The floor system was a Vencomatic one-tier system[1] with its classic elevated slatted area and littered floor area (*Figure 8*). Colony nests were attached to the slatted area with the back directed towards the aisle to facilitate the collecting of eggs. Each group had four conventional circular feed hoppers and one bell drinker. Wood shavings were used as litter material in the littered floor area of each pen.

[1] http://www.vencomaticgroup.com/en

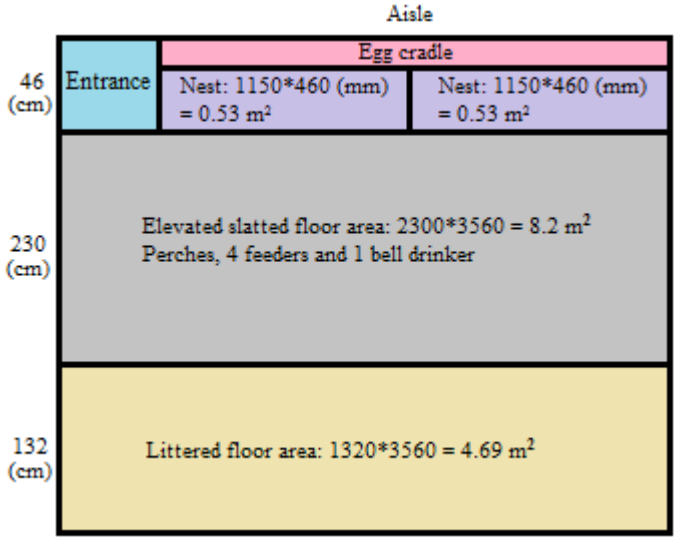

Figure 8 - Schematic picture illustrating a floor pen. One pen housed 102 layers and had a littered floor area, an elevated slatted floor area, two nests, four feeders and a bell drinker. Eggs were collected from the egg cradle in the aisle outside the pen. Each pen was also equipped with plastic perches at a low height on the slatted area and additional elevated wooden perches at the short end of the pen.

The furnished cage

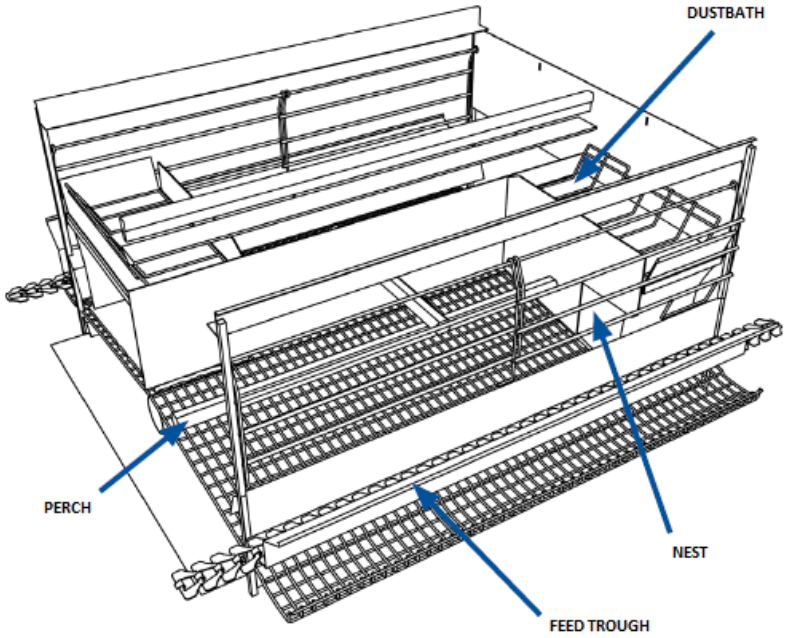

Figure 9 - Schematic picture of the Victorsson Poultry furnished cage. Every cage housed eight layers and was equipped with a perch, dust bath, nest, two available nipple drinkers and a feed through with a blocked falt chain and a manure deflector lined with a claw abrasive. Manure belts were run twice a week. Illustration: Victorsson Poultry

The furnished cage used in this trial was of the brand Victorsson Poultry[2]. The cage had a width of 1207 mm and a depth of 498 mm. (*Figure 9*). Each cage housed 8 hens resulting in an area of 600 cm^2 (or 750 cm^2 including the nest area) per hen. Each hen had access to 15 cm perch, as well as 15 cm of feed trough. The cage's bottom inclination was 12 %. Nests were lined with a plastic net[3] with hexagonal check pattern. Litter boxes were available from the beginning of the trial and replenished with saw dust at least twice a week. Access to the litter

[2] http://www.victorssonpoultry.se/
[3] Netlon mesh matting, Impex Barnevald B.V., Barnevald, the Netherlands.

boxes was adjusted to follow the lighting program. Maintained from 23 weeks of age to the end of the study, the lights were turned on at 02.00 a.m. and switched off at 16.00 p.m. Litter boxes opened at 10 a.m. and closed again at 15 p.m.

Feed and water

The control feed and the experimental feed were produced at the same feed mill by the Swedish feed company Svenska Foder. In the experimental feed the zinc amino acid complex was added, while the control layer groups received zinc in the form of zinc oxide. For composition and nutrient content of the feed see *Table 9* in the appendix.

The structure of the feed was a 4 mm crumbled pellet, fed manually once a day in the furnished cage stable and automatically via a compressed air system in the floor system. The nutrient content was altered along the experiment according to a phase feeding program. Drinkers were cleaned and inspected daily.

Experimental design

On arrival at the research facilities, the layer pullets were distributed into the two housing systems using a predetermined experimental protocol. In the facility with furnished cages, there were six cage batteries with furnished cages in three tiers. Each tier comprised a total of 10 cages, and five cages in row at the same side of the cage battery constituted a replicate. Four combinations of genotype and feed allowed nine replicates of each treatment (*Table 1*; *Figure 10*). In the floor system facility there were 9 groups on each side of the aisle, (see *Figure 11*), and the number of replicates per combination of genotype and feed were four or five (*Table 2*).

Table 1 - Treatments and replicates in the furnished cage system

LSL Classic – Experimental feed (9)	Bovans Robust – Experimental feed (9)
LSL Classic – Control feed (9)	Bovans Robust – Control feed (9)

Table 2 - Treatments and replicates in the floor production system

LSL Classic – Experimental feed (5)	Bovans Robust – Experimental feed (4)
LSL Classic – Control feed (4)	Bovans Robust – Control feed (5)

Cage battery 6		Cage battery 5		Cage battery 4		Cage battery 3		Cage battery 2		Cage battery 1	
6:1: L LSL Control	6:1: R LSL Exp.	5:1: L Bovans Exp.	5:1: R Bovans Control	4:1: L LSL Exp.	4:1: R LSL Control	3:1: L Bovans Exp.	3:1: R Bovans Control	2:1: L LSL Control	2:1: R LSL Exp.	1:1: L Bovans Control	1:1: R Bovans Exp.
6:2: L Bovans Control	6:2: R Bovans Exp.	5:2: L LSL Exp.	5:2 R LSL Control	4:2: L Bovans Exp.	4:2: R Bovans Control	3:2: L LSL Control	3:2: R LSL Exp.	2:2: L Bovans Control	2:2: R Bovans Exp.	1:2: L LSL Exp.	1:2: R LSL Control
6:3: L LSL Exp.	6:3: R LSL Control	5:3: L Bovans Exp.	5:3: R Bovans Control	4:3: L LSL Control	4:3: R LSL Exp.	3:3: L Bovans Exp.	3:3: R Bovans Control	2:3: L LSL Control	2:3: R LSL Exp.	1:3: L Bovans Exp.	1:3: R Bovans Control

Figure 10 - Schematic picture illustrating the furnished cage housing system's treatments and replicates. Cage battery 5 was situated immediately next to the entrance to the furnished cage facility. Each cage battery contained six experimental units comprising five cages in a row. The schematic picture shows the cage batteries from the short end sides.

Group 10	Group 11	Group 12	Group 13	Group 14	Group 15	Group 16	Group 17	Group 18

LSL Control	LSL Exp.	Bovans Control	Bovans Exp.	Bovans Control	LSL Exp.	Bovans Exp.	LSL Control	Bovans Control
Group 9	**Group 8**	**Group 7**	**Group 6**	**Group 5**	**Group 4**	**Group 3**	**Group 2**	**Group 1**
Bovans Control	LSL Control	LSL Exp.	Bovans Exp.	LSL Exp.	LSL Control	Bovans Control	Bovans Exp.	LSL Exp.

Figure 11 - Schematic picture illustrating the floor housing system's treatments and replicates. Group 1 and 18 were situated next to the entrance to the floor housing facility. One group contained the same hybrid and feed treatment.

Recordings

Cracked and dirty eggs – ocular grading

At 42 and 60 weeks of age in the furnished cage system, and at 43 and 61 weeks of age in the floor system, the numbers of cracked and dirty eggs were registered using a small version of a commercial egg candling machine for ocular grading. Four days of accumulated production from the furnished cage system, and three days of accumulated production in the floor system were candled. Prior to statistical analyses the percentages of cracked and dirty eggs were calculated for each replicate.

Sampling, collection and storage of eggs for laboratory analysis

Exterior and interior egg quality were analyzed at 44 and 59 weeks of age in the furnished cages, and at 45 and 60 weeks of age in the floor system. All eggs collected were to be of normal size, with no visible dirt or cracks. In the furnished cage production system, five eggs per replicate, 180 eggs in total, were analyzed. From each of the five cages in a replicate, the fourth egg counted from the left in the egg cradle was collected. If the fourth egg was damaged in any way, the next egg in order was collected. From each replicate two spare eggs were collected in case one or two eggs turned out not to be fit for analyses. All eggs were labeled with replicate and sequence number, using a water proof pen.

In the floor production system, ten eggs as well as four extra eggs were collected from every pen. As in the furnished cage system, the total number of eggs analyzed was 180. Counted from the left, every tenth egg was collected in the egg cradle. Eggs laid in the slatted or littered floor section were not included. The eggs were labeled with group and sequence number.

Laboratory egg analysis

Both interior and exterior egg quality parameters were recorded in the laboratory egg quality analysis. Interior egg quality traits investigated were dry matter content of the albumen, height of the albumen and Haugh units, yolk weight, albumen weight as well as pH of the albumen. The following formulae was used to calculate Haugh Units: $HU = 100 \times \log (H - 1.7W^{0.37} + 7.57)$ where H stands for albumen height and W for egg weight (Silversides & Villeneuve, 1994). Exterior parameters were shell breaking strength, shell thickness, dry shell weight and egg weight. To avoid biases due to effect of storage, eggs from all replicates were analyzed every day. About 45 eggs were analyzed per day, giving a distribution of 1-2 eggs per replicate from the furnished cages per day or 2-3 eggs per replicate and day in the floor production system.

At the laboratory, eggs were stored at a temperature of 4-6 °C. Prior to analysis eggs were kept in room temperature for a minimum of 30 min.

Below, the steps in the egg analysis process are described in detail.

I. The weight of the egg was recorded.

II. The shell breaking strength was tested using the Egg Force Reader™[4] (*Figure 12*).

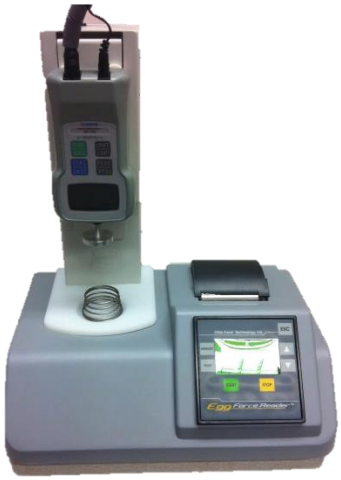

Figure 12 - Photography showing the Egg Force Reader that was used to evaluate egg shell breaking strength

III. The egg was broken onto a glass plate. The height of the albumen was measured approximately 0.5 cm from the edge of the yolk using a micrometer.

IV. Yolk and albumen were separated and weighed. The albumen was poured into an aluminum form that had been weighed to be able to subtract the weight from the dried albumen later on. The pH of the albumen was measured and registered.

V. When the daily numbers of eggs had been analyzed according to the procedures described above, the albumens were dried in the oven at 60°C overnight. The following morning, they were moved to another oven to dry for additional 24 hours at 103 °C. After at least 34 hours of drying, the albumens were weighed after adapting to room temperature within a desiccator. The weight of the remaining dry matter was registered, and recalculated into percentage DM in the results presented. The weight of the aluminum form was subtracted before calculating.

VI. The egg shell membranes were gently peeled off and shell thickness was thereafter measured at three points around the egg's equator using a micrometer. The mean value of these three measurements was used in the statistical analyses. The egg shells were labeled with a water proof pen and put in aluminum forms on a tray for overnight drying in the oven at 103 °C. The morning after, egg shells were adapted to room temperature within a desiccator before weighing. The dry shell weight was then registered in the results as a percentage of the total egg weight.

[4] Orka Food Technology, Ltd. http://www.eggtester.com/

Scoring of bird's exterior appearance

The layers were scored for exterior appearance at 35 and 55 weeks of age, according to the standard method described by Tauson *et al.* (2005) The scoring system assigned values of 1 to 4 points for each trait, where a higher scored implied a better condition. The traits scored for were feather cover, live weight, cleanliness of plumage and feet, claw length, claw damage, pecking wounds on the comb and rear part of the body, the incidence of bumble foot as well as keel bone deformities. The scoring was conducted by the same person on both occasions.

In the furnished cages eight birds from each replicate were scored for external appearance, implying a total of 288 birds. From four out of five cages in each replicate, the two hens marked with leg rings at arrival to the facility were scored. If a hen had lost its leg ring, an un-marked hen was used instead.

In the floor system, 20 hens from each pen were randomly collected from different sections of the pen for scoring. In total, 360 layers were scored in the floor housing facility.

Statistical analysis

All data were evaluated by analysis of variance, using the Procedure Mixed, SAS[5], with fixed effects of genotype (n=2), dietary treatment (n=2) and age (n=2) as well as the two-way interactions between the fixed effects included in the model. In the laboratory egg quality analyses the day of analysis was corrected for in the model to avoid biases due to storage effects. In the furnished cage system replicate within battery (n=6), tier (n=3) and side in the cage battery (left or right) were also included in the model.

Effects with $P \leq 0.05$ were considered as of statistical significance, while results with $0.05 < P < 0.10$ were considered a trend.

All parameters were tested for normal distribution before statistical analyses. The percentages of cracked and dirty egg were arcsin-transformed prior to analyses to improve the normal distribution.

[5] SAS (Statistical Analysis System) Instruments 9.4

Results

Below, the results from the egg analysis, the egg candling procedure and exterior evaluation of birds are presented. General production data are available in the appendix (*Table 10*; *Table 11*).

Egg quality

The results from the laboratory egg quality analyses conducted at 44 and 59 weeks of age in the furnished cages and at 45 and 60 weeks of age in the floor system are presented in *Table 3* and *Table 4* respectively. Additional results from each system at the separate sampling periods are available in the appendix (*Table 11*; *Table 12*; *Table 13*; *Table 14*).

Treatment

There were no general effects of dietary treatment on any of the egg quality traits observed in either production system. However, there were a few significant interactions between hybrid and treatment as well as treatment and age in the furnished cage system. These interactions indicate that the experimental feed did not seem to alleviate the effect of age on breaking strength because both feed treatments resulted in a similar decrease of breaking strength with age. There was even a tendency to higher breaking strengths with the control feed at 59 weeks of age. There were also a few treatment-hybrid interactions where the hybrids reacted differently to the feed treatments with regard to egg composition. The Bovans layers tended to have a higher proportion of albumen in their eggs when fed the experimental feed while the LSL layers experienced an increased proportion of albumen in their eggs with the control feed. When looking at the effect of feed treatment on the proportion of yolk, the relationship was the opposite; the LSL layers had a greater proportion of yolk when fed the experimental feed, while the Bovans layers had a larger proportion of yolk when fed the control feed.

Hybrid

There were some effects of hybrid on egg quality parameters in both production systems. In the furnished cage system, the LSL layers had significantly heavier eggs with higher breaking strength, thicker egg shells and higher egg shell weight (both proportionally and in absolute numbers) compared to the Bovans layers. Moreover, the LSL layers also had heavier yolks in their eggs compared to the Bovans layers. The Bovans layers, on the other hand, had a larger proportion of albumen in their eggs compared to the LSL layers in the furnished cage system.

As in the furnished cage system, the LSL layers were superior to the Bovans layers with regard to the external egg quality parameters of breaking strength, shell thickness as well as egg shell weight when expressed as a proportion of the whole egg in the floor production system.

Age

Egg weight, albumen weight, shell weight and yolk weight did all increase with age in absolute weight in the furnished cage system. There was also a tendency to a hybrid and age interaction observed with regard to yolk content due to that the Bovans layers had a larger proportion of yolk in their eggs at the first sampling, while the LSL layers had a larger proportion of yolk in their eggs the second time around. On the contrary, breaking strength, shell thickness, albumen pH and albumen dry matter content decreased with age. Moreover,

the LSL layers had a tendency to a larger proportion of dry matter content in their albumens compared to the Bovans layers at the younger age. Moreover, there were two significant interactions between hybrid and age observed in the furnished cage production system. The albumen proportion of the Bovans layers' eggs increased significantly with age. Also, at 59 weeks of age the albumen pH values of the LSL layers' eggs were significantly higher compared to the Bovans layers' eggs.

As in the furnished cage system; egg weight, albumen weight and yolk weight did all increase significantly with age in the floor production system. Additionally, in the floor production system, the proportion of albumen as well as the proportion of shell decreased with age, while the proportion of yolk increased with age.

Day

Some expected effects of storage on egg components were detected. The egg weight, albumen height as well as HU decreased with days in storage, while albumen pH increased with storage time.

In the floor production system, the effects of storage were also significant and in general consistent with the results derived from the furnished cage system. However, the results were not quite as consistent, as the values deviated from the trend on the fourth day of analysis (data not shown).

Table 1 - Egg quality in the furnished cage production system at 44 and 59 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as least squares means.

	Treatment		Hybrid		Age		P-value				Interactions		
	Exp.feed[4]	Contr.feed[5]	LSL[1]	Bovans[2]	44 weeks	59 weeks	Treatment	Hybrid	Age	Day[3]	Hybrid* treatment	Hybrid* age	Treatment* age
Trait													
Egg weight (g)	64.9	64.9	65.7	64.1	63.9	65.9	0.9	**	***	0.1	0.3	0.8	0.3
Breaking strength (kg)	4.3	4.4	4.5	4.2	4.4	4.2	0.6	***	**	0.6	0.6	1.0	*
Albumen height (mm)	7.2	7.3	7.3	7.3	7.3	7.2	0.5	0.8	0.2	***	0.2	0.2	0.5
Haugh Unit (HU)	83.2	83.5	83.2	83.5	83.9	82.8	0.6	0.5	0.1	***	0.3	0.2	0.3
Albumen pH	8.7	8.7	8.7	8.7	8.7	8.6	0.2	0.1	**	***	0.7	**	0.8
Albumen weight (g)	36.8	36.7	37.0	36.5	36.1	37.5	0.8	0.2	***	0.2	*	0.3	0.4
Albumen weight (%)	56.6	56.6	56.3	56.9	56.4	56.8	0.7	*	0.1	0.5	**	*	1.0
Shell thickness (10^{-2} mm)	34.6	34.4	34.9	34.1	34.8	34.2	0.6	*	*	0.2	0.7	0.9	0.7
Shell weight (g)	6.0	6.0	6.1	5.9	5.9	6.1	0.6	***	***	0.7	0.7	0.9	0.6
Shell weight (%)	9.3	9.2	9.3	9.2	9.2	9.3	0.6	*	0.1	0.8	0.7	0.9	0.1
Yolk weight (g)	17.9	18.0	18.3	17.7	17.8	18.2	0.6	***	*	0.7	0.1	0.2	0.2
Yolk weight (%)	27.6	27.8	27.9	27.6	27.9	27.6	0.3	0.1	0.1	0.3	***	0.1	0.6
Albumen DM (%)	11.2	11.2	11.3	11.1	11.4	11.1	0.8	0.1	***	0.4	0.9	0.1	0.8

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Day= The day of analysis was corrected for to avoid biases due to storage effects.

[4] Exp.feed = Experimental feed containing the zinc amino acid complex

[5] Contr.feed = Control feed containing zinc oxide

Table 2 - Egg quality in the floor production system at 45 and 60 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as least squares means.

	Treatment		Hybrid		Age		P-value				Interactions		
Trait	Exp.feed[4]	Contr.feed[5]	LSL[1]	Bovans[2]	45 weeks	60 weeks	Treatment	Hybrid	Age	Day[3]	Hybrid* treatment	Hybrid* age	Treatment* age
Egg weight (g)	66.6	66.5	66.4	66.6	65.1	67.9	0.9	0.7	***	0.7	0.3	0.4	0.6
Breaking strength (kg)	4.6	4.5	4.7	4.3	4.8	4.3	0.1	***	***	0.3	0.7	0.9	0.3
Albumen height (mm)	7.4	7.4	7.4	7.3	7.4	7.4	0.9	0.3	0.5	***	0.3	0.2	0.1
Haugh Unit (HU)	83.8	83.7	84.1	83.3	84.3	83.1	0.9	0.2	0.1	***	0.3	0.1	0.1
Albumen pH	8.7	8.7	8.7	8.7	8.7	8.6	0.9	0.3	0.1	***	0.4	0.7	0.2
Albumen weight (g)	38.3	38.3	38.2	38.4	37.7	38.9	1.0	0.7	***	0.6	0.5	0.5	0.2
Albumen weight (%)	57.4	57.6	57.4	57.6	57.8	57.3	0.6	0.6	*	0.2	0.5	0.8	0.1
Shell thickness (10^{-2} mm)	35.2	34.9	35.5	34.6	36.0	34.1	0.4	*	***	0.2	0.3	0.7	1.0
Shell weight (g)	6.3	6.2	6.3	6.2	6.2	6.2	0.4	0.1	0.8	0.6	0.2	0.6	0.8
Shell weight (%)	9.4	9.3	9.5	9.3	9.6	9.2	0.3	**	***	0.9	0.5	0.8	0.5
Yolk weight (g)	18.5	18.4	18.4	18.5	17.8	19.1	0.5	0.3	***	0.6	0.4	0.2	0.5
Yolk weight (%)	27.8	27.7	27.7	27.8	27.4	28.1	0.6	0.7	***	0.1	0.6	0.5	0.3
Albumen DM (%)	11.3	11.3	11.4	11.2	11.5	11.1	0.2	0.1	***	0.1	0.3	0.7	0.3

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Day= The day of analysis was corrected for to avoid biases due to storage effects.

[4] Exp.feed = Experimental feed containing the zinc amino acid complex

[5] Contr.feed = Control feed containing zinc oxide

Cracked and dirty eggs

The results from the egg candling procedure are presented in *Table 5* and *Table 6*. Additional results from each system at every sampling period are available in the appendix (*Table 14*; *Table 15*; *Table 16*; *Table 17*).

The prevalence of cracked and dirty eggs was affected by age. In both production systems, the prevalence of cracked eggs was significantly higher at the latter inspections. The same was true for the prevalence of dirty eggs. There was also an effect of hybrid with regard to the proportion of dirty eggs in the floor production system where the Bovans layers had significantly dirtier eggs compared to the LSL layers.

Table 6 - Cracked and dirty eggs in the furnished cage production system at 42 and 60 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as a proportion of the egg yield.

	Treatment		Hybrid		Age		P-value			Interactions		
	Exp.feed[3]	Contr.feed[4]	LSL[1]	Bovans[2]	42 weeks	60 weeks	Treatment	Hybrid	Age	Hybrid* treatment	Hybrid* age	Treatment* age
Trait												
Cracked eggs	2.7	2.4	2.7	2.4	2.3	2.8	0.6	0.7	*	0.9	0.9	1.0
Dirty eggs	15.2	14.5	14.1	15.6	9.4	20.3	0.5	0.2	***	0.3	0.8	0.4

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Exp.feed = Experimental feed containing the zinc amino acid complex

[4] Contr.feed = Control feed containing zinc oxide

Table 7 - Cracked and dirty eggs in the floor production system at 43 and 61 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as a proportion of the egg yield.

	Treatment		Hybrid		Age		P-value			Interactions		
	Exp.feed[3]	Contr.feed[4]	LSL[1]	Bovans[2]	43 weeks	61 weeks	Treatment	Hybrid	Age	Hybrid* treatment	Hybrid* age	Treatment* age
Trait												
Cracked eggs	1.6	2.2	2.1	1.7	1.5	2.3	0.1	0.4	**	0.6	0.6	0.9
Dirty eggs	34.9	36.5	26.7	44.7	32.0	39.4	0.8	**	**	0.8	0.2	0.8

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Exp.feed = Experimental feed containing the zinc amino acid complex

[4] Contr.feed = Control feed containing zinc oxide

Scoring of bird's exterior appearance

The results from the scoring of bird's exterior appearance at 35 and 55 weeks of age are shown in *Table 7* and *Table 8*. The parameters bumble foot (in the furnished cages) and claw damage (in the floor system) were not normally distributed and were therefore not included in the statistical analysis.

Treatment

In general there were no continuant effects of the feed treatment across production system. Claw length was not affected by diet but when evaluating the incidence of claw damage in the furnished cage system, layers that had received the experimental feed had a significantly higher prevalence of intact claws. They also tended to have greater keel bone integrity compared to when the control feed was given. There was also an interaction between diet and genotype in the furnished cages due to that the Bovans layers had a significantly lower prevalence of pecking wounds on the rear part of the body when fed the experimental feed.

In the floor system, there was an effect of treatment on plumage condition. Layers that had received the experimental feed tended to have higher plumage condition scores in total compared to the layers receiving the control feed. Moreover, when looking at separate parameters in the exterior evaluation, layers that had received the experimental feed had significantly higher feather cover scores, indicating a better condition, when evaluating the breast. They also tended to have higher plumage condition scores when evaluating the cloaca.

Hybrid

Hybrid differences were highly dependent on which production system the birds were kept in. In the furnished cage system, the Bovans layers had significantly higher plumage condition scores than the LSL layers for all plumage parameters evaluated except for the breast. Moreover, when looking into the cleanliness of the plumage as well as the length of the claws, the LSL layers were superior to the Bovans layers in the furnished cage system. Moreover, the Bovans layers tended to have a lower prevalence of pecking wounds on the rear part of the body in the furnished cage system.

However, in the floor production system the LSL layers had significantly higher plumage condition scores compared to the Bovans layers. On the contrary to the results in the furnished cage system, the Bovans layers were the ones with the shorter claw lengths in the floor production system. While the evaluated birds all tended to get high bumble foot values in the furnished cage system, resulting in results that were not normally distributed, the Bovans layers had significantly lower prevalence of the disorder in the floor production system compared to the LSL layers. Just as in the furnished cage system, the LSL layers had significantly cleaner integument compared to the Bovans layers. Moreover, in the floor production system the LSL layers had significantly lower prevalence of pecking wounds on the rear part of the body, and a tendency to fewer pecking wounds on the comb compared to the Bovans layers.

Age

Aging highly affected plumage condition and other exterior parameters evaluated. In both production systems, the plumage condition of the birds significantly deteriorated with age for all evaluated parts of the body. The effects of aging seemed larger than the effects of hybrid,

as the best hybrid at 55 weeks of age was less feathered than the most poorly feathered hybrid at 35 weeks in both systems.

In both production systems the cleanliness of the feet improved significantly with age. The cleanliness of the integument decreased with age in the furnished cage system, while it increased with age in the floor production system.

The length of the claws, the incidence of pecking wounds on the rear part of the body, as well as the incidence of keel bone deformities all increased with age in both production systems. In the floor production system, the incidence of pecking wounds on the comb decreased with age, while it increased with age in the furnished cage system. Also, the incidence of bumble foot decreased with age in the floor production system. Confined to the furnished cage system was the increase in body weight as the layers aged.

Table 8 - Exterior scoring in the furnished cage production system at 35 and 55 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as least squares means.

	Treatment		Hybrid		Age		P-value			Interactions		
	Exp.feed[5]	Contr.feed[6]	LSL[1]	Bovans[2]	35 weeks	55 weeks	Treatment	Hybrid	Age	Hybrid* treatment	Hybrid* age	Treatment* age
Trait												
Body weight	1820.8	1855.3	1829.6	1846.4	1806.9	1869.2	0.1	0.4	***	0.3	0.7	0.5
Sum of plumage scores[3]	17.5	18.0	16.6	18.9	19.9	15.6	0.4	***	***	*	*	0.7
Neck[4]	3.0	3.1	2.7	3.4	3.3	2.8	0.3	***	***	0.1	0.1	0.6
Breast[4]	2.7	2.7	2.6	2.8	3.0	2.4	1.0	0.1	***	0.1	0.1	0.2
Cloaca[4]	2.8	2.9	2.6	3.1	3.4	2.4	0.4	***	***	0.1	0.3	1.0
Back[4]	3.1	3.2	3.0	3.4	3.5	2.9	0.4	*	***	*	0.2	0.5
Wings[4]	3.4	3.5	3.3	3.5	3.8	3.0	0.3	**	***	*	0.2	0.9
Tail[4]	2.5	2.6	2.4	2.7	2.9	2.2	0.7	*	***	*	*	0.7
Plumage cleanliness[4]	3.2	3.2	3.3	3.1	3.2	3.2	0.3	***	*	0.3	0.1	0.6
Claw length[4]	3.4	3.4	3.5	3.3	3.6	3.2	0.6	***	***	0.2	0.1	0.4
Claw damage[4]	3.9	3.8	3.8	3.8	3.8	3.8	*	0.8	0.8	0.5	0.2	0.7
Bumble foot[4]*	/	/	/	/	/	/	/	/	/	/	/	/
Feet cleanliness[4]	3.9	3.9	3.9	3.9	3.8	4.0	0.5	0.7	***	1.0	0.6	0.8
Pecking wound rear[4]	3.7	3.7	3.6	3.7	3.8	3.6	0.7	0.1	***	*	0.8	0.5
Pecking wound comb[4]	3.2	3.3	3.2	3.3	3.4	3.1	0.7	0.7	***	0.8	0.8	0.5
Keel bone deformities[4]	3.7	3.6	3.7	3.6	3.8	3.5	0.1	0.2	***	0.6	0.5	0.7

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Scores from 6-24 where a higher score indicates a better plumage condition

[4] Scores from 1-4 where a higher score indicates a better condition

[5] Exp.feed = Experimental feed containing the zinc amino acid complex

[6] Contr.feed = Control feed containing zinc oxide

* Not normally distrubuted and excluded from the statistical analysis

Table 9 - Exterior scoring in the floor production system at 35 and 55 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as least squares means.

	Treatment		Hybrid		Age		P-value			Interactions		
	Exp.feed[5]	Contr.feed[6]	LSL[1]	Bovans[2]	35 weeks	55 weeks	Treatment	Hybrid	Age	Hybrid* treatment	Hybrid* age	Treatment* age
Trait												
Body weight	1835.2	1806.3	1823.5	1818.0	1816.7	1824.8	0.1	0.7	0.5	0.5	0.3	0.2
Sum of plumage scores[3]	14.5	12.7	15.1	12.1	17.5	9.7	0.1	*	***	0.1	*	0.9
Neck[4]	2.4	2.1	2.5	1.9	2.7	1.7	0.1	**	***	0.1	***	0.2
Breast[4]	2.3	2.0	2.3	2.0	2.8	1.5	*	*	***	0.1	0.6	0.8
Cloaca[4]	2.3	1.8	2.4	1.7	2.8	1.3	0.1	*	***	0.1	*	0.3
Back[4]	2.3	2.0	2.4	1.8	2.8	1.4	0.3	*	***	0.3	0.1	0.5
Wings[4]	3.1	2.9	3.2	2.8	3.7	2.4	0.1	**	***	*	0.2	0.1
Tail[4]	2.2	1.9	2.3	1.8	2.8	1.3	0.2	*	***	0.6	0.1	0.7
Plumage cleanliness[4]	3.3	3.3	3.4	3.2	3.2	3.4	0.8	**	**	0.9	0.2	0.8
Claw length[4]	3.7	3.6	3.5	3.8	3.8	3.6	0.8	**	***	0.6	*	*
Bumble foot[4]	3.7	3.8	3.6	3.8	3.7	3.8	0.3	*	*	0.9	0.1	0.9
Claw damage[4]*	4.0	4.0	4.0	4.0	4.0	4.0	/	/	/	/	/	/
Feet cleanliness[4]	3.0	3.0	2.9	3.0	2.9	3.1	0.8	0.1	**	0.8	0.5	0.4
Pecking wound rear[4]	3.3	3.2	3.4	3.1	3.4	3.1	0.1	**	**	0.7	0.1	0.8
Pecking wound comb[4]	2.7	2.7	2.8	2.7	2.6	2.9	1.0	0.1	***	0.7	*	0.9
Keel bone deformities[4]	3.9	3.9	3.9	3.9	4.0	3.8	0.8	0.7	***	0.8	0.7	1.0

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Scores from 6-24 where a higher score indicates a better plumage condition

[4] Scores from 1-4 where a higher score indicates a better condition

[5] Exp.feed = Experimental feed containing the zinc amino acid complex

[6] Contr.feed = Control feed containing zinc oxide

* Not normally distrubuted and excluded from the statistical analysis. Only least squares means shown.

Discussion

In general, there were no clear significant effects of the experimental feed treatment on egg quality traits observed in either of the production systems. One possible reason for this could be that the zinc included in the control feed was sufficient supplementation for the layers at the observed stages of the production cycle. Gheisari *et al*. (2011) suggested that organic zinc is preferable in cases when dietary levels of trace minerals are marginalized. Hence, if the amount of zinc was already adequately high in the control feed, the uptake might have been enough even though the bioavailability is considered to be higher in zinc from organic sources (Mondal *et al*., 2009). Another factor to consider is that the layers were still within the normal age span of commercial egg production. Alleviating effects of the experimental feed on age related impairments of egg quality might be revealed later on during the prolonged part of the production cycle (80-100 weeks of age). However, the prolonged production period was outside the scope of this master's thesis.

Consequently, the organic mineral complex in the experimental feed did not prevent the age dependent decrease in egg shell breaking strength in either of the two production systems. Surprisingly, in the furnished cages, there was a tendency of superior breaking strengths with the control feed compared to the experimental feed at 59 weeks of age. These results deviate from the research conducted by Swiatkiewicz & Koreleski (2008), who found that supplementation of organic zinc to aged layers at 62 and 70 weeks of age increased egg shell breaking strength. On the other hand, Mabe *et al*. (2003) stated that zinc supplementation increases shell breaking strength regardless of origin.

The fact that dietary treatment affected egg composition with regard to the proportions of albumen and yolk in the layers housed in the furnished cage system is hard to explain. Worth noting is that while the LSL layers experienced an enhanced proportion of yolk with the experimental feed, these layers also had a tendency to a higher proportion of yolk in their eggs in general in this production system, regardless of feed treatment.

In general, the LSL hybrid was superior to the Bovans hybrid in both production systems regarding the exterior egg quality parameters. It is therefore possible to state that according to the results of this study, the LSL genotype has an advantage to the Bovans genotype regarding egg shell robustness.

In consistency with previous research (Coutts & Wilson, 1995; Silversides & Scott, 2001) the absolute weight of the whole egg, the albumen and the yolk all increased with age in both production systems. Breaking strength, shell thickness and the dry matter content of the albumen decreased with age in both production systems. A lot of research has demonstrated an age related decrease in breaking strength and shell thickness (Bar & Hurwitz, 1987; Joyner *et al*., 1987; Bar *et al*., 1988). However, Silversides & Scott (2001) stated that albumen pH was not affected by either hybrid or age of the hen. These conclusions deviate from the results of this master's thesis because albumen pH did decrease with hen age in the furnished cage system.

The prevalence of cracked and dirty eggs was, as expected, highly affected by hen age. In general, the proportion of dirty eggs present in both production systems were much higher compared to the levels of dirty eggs tolerated in commercial circumstances. Heated floors were installed prior to the trial in the floor production system. An unfortunate and unexpected consequence of this was that the heated floors, possibly in combination with insufficient

management actions, attracted many layers to lay their eggs in the litter section of the pens, making the eggs more susceptive to dirt. This correlation was previously concluded by Abrahamsson & Tauson (1998) who highlighted the connection between the proportion of misplaced and dirty eggs. Moreover, variations between production cycles are more commonly occurring and harder to predict in floor production systems compared to cage systems (Abrahamsson & Tauson, 1998), in this case furnished cages.

The shell weight expressed in grams increased significantly with age in the furnished cages while it remained almost the same in the floor production system. Interestingly, while the egg shell proportion increased with age in the furnished cage system, in the floor production system it decreased with age. The results from the furnished cage system was not in accordance with those of Franco-Jimenez & Beck (2005) among others, who argued that older hens do not have the same capacity to produce eggs with as high egg shell density as younger hens. However, the increase in total egg weight over time was higher in the floor production system, which could explain the decreased proportion of egg shell. Another answer could be that the egg shell mineral structure of the eggs laid in the furnished cage system was altered over time, making the egg shells heavier. According to Mabe *et al*. (2003), supplementation of trace elements might alter the structure of the egg shell, hence modifying the mechanical strength of the egg shell. Why this would have appeared solely in the furnished cage system remains unclear.

The fact that the proportion of albumen tended to increase with age in the furnished cage system deviate from the research conducted by Silversides & Scott (2001) and Coutts & Wilson (1995) who stated that albumen weight decreases with hen age when considered as a proportion of the whole egg. The results from the floor system were however in line with those of the latter study. Yolk weight increased more rapidly with hen age compared to other egg components, resulting in eggs with a higher yolk weight percentage in the floor production system. These results from the floor production system are in agreement with the research conducted by Silversides & Scott (2001), while the furnished cage results contradict the same. Although, the results concerning yolk proportion in the furnished cage system were not statistically significant. The increased mean weights of all egg components are in line with previous findings (Amem & Al-Daraji, 2011). However, the reasons for the difference in egg composition between the production systems remain unclear and require further research.

In both production systems, there were expected effects of storage in terms of an effect of day of analysis on the height of the albumen, HU as well as albumen pH. The albumen height as well as HU decreased with storage time while the albumen pH increased with storage time in agreement with Coutts & Wilson (1995) and Roberts (2004).

When considering the exterior evaluation of the layers there were some effects of treatment in both production systems. The experimental feed reduced the occurrence of claw damage as well as keel bone deformities in the furnished cage system, implying that the organic zinc complex had a positive impact on these parameters. This is in accordance with Hess *et al*. (2001) and Lai *et al*. (2010) who argued the importance of zinc with regard to bone development and maintenance. In the floor production system, the layers that were fed the experimental feed tended to have a more intact integument in general, demonstrating the positive effect of the organic zinc compound on plumage condition. These results correspond to the research carried out by Kienholz *et al*. (1961), Hess *et al*. (2001) as well as Suttle (2010) that emphasized the positive effects of zinc on plumage condition and highlighted the negative effects of deficiency when excluded.

There were some interesting interactions in the furnished cage system with regard to the total sum of the plumage parameters. The LSL layers' plumage condition was negatively affected by the experimental feed whereas the Bovans layers were unaffected. The experimental feed had a positive preventive effect on the prevalence of pecking wounds to the rear part of the body for the Bovans layers. Hess *et al*. (2001) found that broilers fed complexed zinc showed improvements with regard to back and wing bruising. If zinc supplementation improves the skin's resistance and increases the healing of the skin, this could explain why the Bovans layers given experimental feed had a lower prevalence of visible pecking wounds on the rear part of the body.

There was an interesting difference between hybrids depending on which production system they were kept in. The Bovans layers were superior with regard to all plumage condition parameters compared to the LSL layers in the furnished cage system. In the floor system on the other hand, the LSL layers were superior with regard to all plumage condition parameters evaluated. Even though the LSL layers were superior with regard to plumage condition in the floor production system, when looking at the mean values, they were still less feathered compared to when housed in the furnished cage system. These results prove that different hybrids can be more or less adaptable to different environments, and that if one hybrid is performing well in one system, it does not mean that it will deliver as good results in another production system. The fact that one hybrid cannot be as well adapted to every housing system was earlier discussed by Lay *et al*. (2011).

This was also the case with claw length, where the LSL layers had the shortest claws in the furnished cage system, while the Bovans layers had the shortest claws in the floor production system. Moreover, the Bovans layers had a lower prevalence of pecking wounds on the rear of the body in the furnished cage system, while the LSL layers had a lower occurrence in the floor production system. The fact that the LSL layers also had a lower prevalence of pecking wounds on the comb suggests that maybe, given that they were also superiorly feathered, the LSL layers were generally better adapted to the floor production system than the Bovans layers.

The Bovans layers were inferior with regard to plumage and egg cleanliness compared to the LSL layers in the floor production system. This was probably due to that they laid their eggs in the littered floor section of the pens to a higher extent.

Generally, the floor housed layers experienced a more extensive deterioration of their plumage condition with age, compared to the caged layers. This was expected, and in accordance with the research comprised by Bilčík & Keeling (1999) and McAdie & Keeling (2000) among others, that stated the higher risk of feather pecking behaviour appearing and spreading in large group sizes of layers.

As expected, the plumage condition was better for all scored parts of the body in both production systems at 35 weeks of age compared to 55 weeks of age, which is in accordance with earlier research (Freire *et al*., 1998; McAdie & Keeling, 2000). At 55 weeks of age the layers were significantly heavier in the furnished cage system, which could be explained by the fact that the caged layers were not as mobile as the floor housed layers, who may have had a harder time gaining weight due to a higher level of activity. Opposed to conventional cages, increased activity and locomotion in floor production systems increases the layers feed conversion ratios (Tauson, 2005). Moreover, deteriorated plumage in laying hens results in an

increased amount of energy required to maintain body temperature (Peguri & Coon, 1993), which could explain why the floor housed layers did not put on as much weight as did the furnished cage layers.

In both production systems, the layers had a lower occurrence of keel bone deformities at 35 weeks of age. This was in accordance with Abrahamsson & Tauson (1996) who found that the long term use of perches is often the cause of keel bone deformities. As described by Whitehead (2004), medullary bone development takes place due to resorption of structural bone to maintain calcium supply. The fact that medullary bone is weaker in its structure may be a gateway to keel bone deformities later on in the laying cycle (Fleming *et al*., 2004).

In the furnished cage system, the prevalence of bumble foot was not included in the statistical analysis because it was not normally distributed, due to high scores in all scored birds. The prevalence of bumble foot was however of statistical significance in the floor production system and was significantly more frequent at 35 weeks of age, suggesting that the problems decreased with age. This finding is in accordance with the research conducted by Blokhuis *et al*. (2007) who described bumble foot as a problem arising at 35-45 weeks of age, subsequently declining thereafter. Also, the cleanliness of the foot pad was better at 55 weeks of age in both production systems which could imply a correlation between the occurrence of bumble foot and hygiene, as suggested in previous research (Tauson & Abrahamsson, 1996; Tauson, 1998). The layers had shorter claws in both production systems at 35 weeks of age, which indicates that the wear of claws was not enough to keep the layers' claws of short length as they grew older.

Regarding the cleanliness of the plumage, the layers had the cleanest integument at 35 weeks in the furnished cage system, whereas plumage hygiene was superior at 55 weeks of age in the floor production system. Maybe the floor housed layers had higher cleanliness of their integument at an older age because at that time, they had lost so much of their feathers there was not much of the plumage left to soil. Another reason could be that the floor housed layers learned to roost more over time, and that by using the perches they might not have been as exposed to other hens' manure. However, incorrect placement of perches can lead to a greater exposure to dirt. Additionally, hygiene and quality of the litter may have changed over the production cycle, making the layers more or less susceptive to dirt.

In both production systems, the layers had a higher prevalence of pecking wounds on the rear part of the body at 55 weeks of age. This could be due to that open wounds attract attention as described by McAdie & Keeling (2000), and therefore, pecking could spread within a flock of layers.

Conclusion

In the present study there were no consistent positive effects of supplementing complexed organic zinc to laying hens on egg quality, plumage condition score or keel bone deformities during the observed period of production. It cannot be excluded that differences would have been detected if the master's thesis had spanned also the prolonged part of the laying cycle. A lot of differences between hybrids were shown, and it was evident that the hybrids used differed in their ability to adapt in the two production systems used in this study. Therefore, future research should focus on the adaptability of hybrids, to find and develop hybrids that are suitable to the production system they are to be housed in. It could also be of interest to investigate optimization of feed for specific hybrid and productions system combinations. As expected and concluded in earlier research; egg shell quality, plumage condition and keel bone deformities were negatively affected by increasing hen age. On average production figures including mortality and feed conversion ratio as well as plumage condition scores were inferior in the floor system compared to the furnished cages.

References

Abrahamsson, P. and Tauson, R. (1996). Foot and Keel Bone Disorders in Laying Hens. *Aacta Agriculturae Scandinavica Section A-Animal science*, vol. 46, pp. 239-246

Alodan, M.A. & Mashaly, M.M. (1999). Effect of Induced Molting in Laying Hens on Production and Immune Parameters. *Poultry Science*, vol. 78, pp. 171-177.

Amem, M.H.M and Al-Daraji, J.H. (2011). Zinc Improves Egg Quality in Cobb500 Broiler Breeder Females. *International Journal of Poultry Science*, vol. 10 (6), pp. 471-476.

Attia, Y.A., Al-Hamid, A., Zeweil, H.S., Qota, E.M., Bovera, F., Monastra, G. and Sahledom, M.D. (2013). Effect of Dietary Amounts of Inorganic and Organic Zinc on Productive and Physiological Traits of White Peking Ducks. *Animal*, vol. 7 (6), pp. 895-900.

Bar, A & Hurwitz, S. (1987) Vitamin D metabolism and calbindin (calcium binding protein) in aged laying hens. *The Journal of Nutrition*, vol. 117, pp: 1775-1779.

Bar, A., Striem, S., Rosenberg, J. and Hurwitz, S. (1988). Egg Shell Quality and Cholecalciferol Metabolism in Aged Laying Hens. *The Journal of Nutrition*, vol. 118, pp. 1018-1023.

Bar, A., Vax, E. and 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*, vol. 123, pp. 147-154.

Bilčík, B. & Keeling, L.J. (1999). Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. *British Poultry Science*, vol. 40, pp. 444-451.

Blokhuis, H.J. (1986). Feather-pecking in Poultry: Its Relation With Ground-Pecking. *Applied Animal Behaviour Science*, vol. 16, pp. 63-67.

Blokhuis, H.J., Fiks Van Niekerk, T., Bessei, W., Elson, A., Guémené, D., Kjaer, J.B., Maria Levrino, G.A., Nicol, C.J., Tauson, R., Weeks, C.A. and Van De Weerd, H.A. (2007). The LayWel project: welfare implications of changes in production systems for laying hens. *World's Poultry Science Journal*, vol. 63, pp. 101-114.

Burley, R.W. and D.V. Vadehra. (1989). *Introduction: The Macroscopic Structure, Physical Properties, and Chemical Composition of Avian Eggs*. In: The Avian Egg: Chemistry and Biology. John Wiley & Sons, Inc, USA, 1-16.Coutts, J.A. and Wilson, G.C. (1995). Egg Quality – A Practical Approach. 2. ed. Queensland: Department of Primary Industries.

European Food Safety Authority (EFSA). (2015). Scientific opinion on welfare aspects of the use of perches for laying hens. *EFSA Journal*, vol. 13 (6): 4131, pp. 1-69.

Etches, R.J. (1996). *Reproduction in Poultry*. Cambridge: CAB International.

Fleming, R.H., McCormack, H.A., McTeir, L. and Whitehead, C.C. (2004). Incidence, pathology and prevention of keel bone deformities in the laying hen. *British Poultry Science*, vol. 45, pp. 320-330.

Franco-Jimenez, D.J. & Beck, M.M. (2005). Intestinal calcium uptake, shell quality and reproductive hormones levels of three laying hen varieties after prolonged egg production. *International Journal of Poultry Science*, vol. 4 (8), pp. 518-522.

Freire, R., Walker, A. and Nicol, C.J. (1998). The relationship between trough height, feather cover and behaviour of laying hens in modified cages. *Applied Animal Behaviour Science*, vol. 63, pp. 55-64.

Forbes, R.M. (1984). Use of Laboratory Animals to Define Physiological Functions and Bioavailability of Zinc. *Federation Proceedings*, vol. 43 (13), pp. 2835-2839.

Gentle, M.J. and Hunter, L.N. (1991). Physiological and behavioural responses associated with feather removal in *Gallus gallus* var. *domesticus*. Research in Veterinary Science, vol. 50 (1), pp. 95–101.

Gheisari, A.A., Sanei, A., Samie, A., Gheisari, M.M. and Toghyani, M. (2011). Effect of Diets Supplemented with Different Levels of Manganese, Zinc and Copper from their Organic or Inorganic Sources on Egg Production and Quality Characteristics in Laying Hens. *Biological Trace Element Research*, vol. 142, pp. 557-571.

Gumudavelli, V., Subbiah, J., Thippareddi, H., Velugoti, P. R. and Froning, G. (2007). Dynamic Predictive Model for Growth of *Salmonella* Enteritidis in Egg Yolk. *Journal of Food Science*, vol. 72 (7), pp. 254-262.

Hess, J.B., Bilgili, S.F., Parson, A.M. and Downs, K.M. (2001). Influence of Complexed Zinc Products on Live Performance and Carcass Grade of Broilers. *Journal of Applied Animal Research*, vol. 19 (1), pp. 49-60.

ISA, Hendrix Genetics. (2014). Bovans White - Production Report. [Electronic] The Netherlands: ISA – Hendrix Genetics. [Brochure] Available: http://www.isapoultry.com/~/media/Files/ISA/ISA%20new/product%20information/Bovans/Commercials/White/bovans_white_production_report_cage_production_system_vs1408a.pdf

Joyner, C.J., Peddie, M.J. and Taylor, T.G. (1987). The effect of age on egg production in the domestic hen. *General and Comparative Endocrinology*, vol. 65, pp. 331-336.

Kemps, B.J., Bamelis, F.R., Mertens, K., Decuypere, E.M., De Baerdemaeker, J.G. and De Ketelaere, B. (2010). The assessment of viscosity measurements on the albumen of consumption eggs as an indicator for freshness. *Poultry Science*, vol. 89, pp. 2699-2703.

Kienholz, E.W., Turk, D.E., Sunde M.L, Hoekstra, W.G. (1961). Effects of zinc deficiency in diets of hens. *The Journal of Nutrition*, vol. 75, pp. 211-221.

Kim, W.K. and Patterson, P.H. (2005). Effects of Dietary Zinc Supplementation on Hen Performance, Ammonia Volatilization, and Nitrogen Retention in Manure. *Journal of Environmental Science and Health Part B*, vol. 40, pp. 675-686.

Kjaer, J.B., Glawatz, H., Scholz, B., Rettenbacher, S. and Tauson, R. (2011). Reducing stress during welfare inspection: validation of a non-intrusive version of the LayWel plumage scoring system for laying hens. *British Poultry Science*, vol. 52 (2), pp. 149-154.

Ledvinka, Z., Zita, L., Hubený, M., Tůmová, E., Tyller, M., Dobrovolný, P. and Hruška, M. (2011). Effect of genotype, age of hens and K/k allele on eggshell quality. *Czeck Journal of Animal Science*, vol. 56 (5), pp. 242-249.

Lai, P.W., Liang, J.B., Hsia, L.C., Loh, T.C and Ho, Y.W. (2010). Effects of Varying Dietary Zinc Levels and Environmental Temperatures on the Growth Performance, Feathering Score and Feather Mineral Concentrations of Broiler Chicks. *Asian-Australasian Journal of Animal Sciences*, vol. 23 (7), pp. 937-945.

Lay Jr, D.C., Fulton, R.M., Hester, P. Y., Karcher, D.M., Kjaer, J.B., Mench, J.A., Mullens, B.A., Newberry, R.C., Nicol, C.J., O'Sullivan, N.P. and Porter, R.E. (2011). Hen welfare in different housing systems. *Poultry Science*, vol. 90, pp. 278-294.

Mabe, I., Rapp, C., Bain, M.M and Nys, Y. (2003). Supplementation of a Corn-Soybean Meal Diet with Manganese, Copper and Zinc from Organic or Inorganic Sources Improves Eggshell Quality in Aged Laying Hens. *Poultry Science*, vol. 82, pp. 1903-1913.

Matos, de. R. (2008). Calcium Metabolism in Birds. *Veterinary Clinics Exotic Animal Practice*, vol. 11, pp. 59-82.

McAdie, T.M & Keeling, L.J. (2000). Effect of manipulating feathers of laying hens on the incidence of feather pecking and cannibalism. *Applied Animal Behaviour Science*, vol. 68, pp. 215-229.

McAdie, T.M. & Keeling, L.J. (2002). The social transmission of feather pecking in laying hens: effects of environment and age. *Applied Animal Behaviour Science*, vol. 75, pp. 147-159.

Mondal, S., Haldar, S., Saha, P and Ghosh, T.K. (2009). Metabolism and Tissue Distribution of Trace Elements in Broiler Chickens' Fed Diets Containing Deficient and Plethoric Levels of Copper, Manganese, and Zinc. *Biological Trace Element Research*, vol. 137, pp. 190-205.

Nasr, M.A.F., Nicol, C.J. & Murrell, J.C. (2012a). Do laying hens with keel bone fractures experience pain? *Plos One*, vol. 7 (8): e42420. doi:10.1371.

Nasr, M.A.F., Murrell, J., Wilkins, L.J., Nicol, C.J. (2012b) The effect of keel fractures on egg-production parameters, mobility and behavior in individual laying hens. *Animal Welfare*, vol. 21, pp. 127-135.

Nasr, M.A.F., Murrell, J. and Nicol, C.J. (2013). The effect of keel fractures on egg production, feed and water consumption in individual laying hens. *British Poultry Science*, vol. 54 (2), pp. 165-170.

National Research Council. (1994). Nutrient Requirements of Poultry, 9th edition. National Research Council, National Academy Press, Washington, DC, USA.

Nys, Y. (1995). Influence of nutritional factors on eggshell quality at high environmental temperature. In: Dr. R, Cepero Briz, *Egg and egg products quality. Proceedings of the VI European Symposium on the Quality of Eggs and Egg Products*. 25-29 September 1995, Zaragoza, Spain. pp. 209-220.

Nys, Y., Bain, M & Immerseel, F. van. (2011a). Profiling the egg consumer: attitudes, perceptions and behaviours. *Improving the safety and quality of eggs and egg products. Volume 1: Egg chemistry, production and consumption*, pp. 39-61.

Nys, Y., Bain, M & Immerseel, F. van. (2011b). Hen nutrition for sustained egg quality. *Improving the safety and quality of eggs and egg products. Volume 1: Egg chemistry, production and consumption*, pp. 261-299.

Onderci, M., Sahin, N., Sahin, K. and Kilic, N. (2003). Antioxidant Properties of Chromium and Zinc. In Vivo Effects on Digestibility, Lipid Peroxidation, Antioxidant Vitamins, and Some Minerals Under a Low Ambient Temperature. *Biological Trace Element Research*, vol. 92, pp. 139-149.

Peguri, A. and Coon, C. (1993) Effects of feather coverage and temperature on layer performance. *Poultry Science*, vol. 72, pp. 1318-1329.

Riczu, C.M., Saunders-Blades, J.L., Yngvesson, Å.K., Robinson, F.E. and Korver, D.R. (2004). End-of-Cycle Bone Quality in White- and Brown-Egg Laying Hens. *Poultry Science*, vol. 83, pp. 375-383.

Roberts, R.J. (2004). Factors affecting egg internal quality and egg shell quality in laying hens. *Journal of Poultry Science*, vol. 41, pp. 161-177.

Rodríguez-Navarro, A.B., Domínguez-Gasca, N., Muñoz, A. and Ortega-Huertas, M. (2013). Change in the chicken eggshell cuticle with hen age and egg freshness. *Poultry Science*, vol. 92, pp. 3026-3035.

Samli, H.E., Agma, A. and Senkoylu, N. (2005). Effects of Storage Time and Temperature on Egg Quality in Old Laying Hens. *Journal of Applied Poultry Research*, vol. 14, pp. 548-553.

Sandilands, V., Moinard, C. and Sparks, N.H.C. (2009). Providing laying hens with perches: fulfilling behavioural needs but causing injury? *British Poultry Science*, vol. 50 (4), pp. 395-406.

Silversides, F.G. & Budgell, K. (2004). The Relationships Among Measures of Egg Albumen Height, pH and Whipping Volume. *Poultry Science*, vol. 83, pp. 1619-1623.

Silversides, F.G. & Villeneuve, P. (1994). Is the Haugh Unit Correction for Egg Weigh Valid for Eggs Stored at Room Temperature? *Poultry Science*, vol. 73 (1), pp. 50-55.

Silversides, F.G & Scott, T.A. (2001). Effect of storage and layer age on quality of eggs from two lines of hens. *Poultry Science*, vol. 80, pp. 1240-1245.

Sjaastad, Ø.V., Sand, O. & Hove, K. (2003a). Avian reproduction. In: Physiology of domestic animals. 2nd edition. Scandinavian Veterinary Press, Oslo, pp. 730-734.

Sjaastad, Ø.V., Sand, O. & Hove, K. (2003b). The Skin. In: Physiology of domestic animals. 2nd edition. Scandinavian Veterinary Press, Oslo, pp. 646-650.

Solomon, S.E. (2010). The eggshell: strength, structure and function. *British Poultry Science*, vol. 51 (1), pp. 52-59.

Suttle, N.F. (2010). Zinc. In: Suttle, N.F., *Mineral Nutrition of Livestock*. 4th edition. Oxfordshire, UK: CAB International, pp. 426-458.

Swedish Board of Agriculture (Jordbruksverket). (2005). Äggproduktion i ekologiskt lantbruk. [Electronic] Jönköping: Jordbruksverket (Jordbruksinformation 2005:21) [Brochure] Available: http://www2.jordbruksverket.se/webdav/files/SJV/trycksaker/Pdf_jo/jo05_21.pdf (2014-08-11).

Swiatkiewicz, S & Koreleski, J. (2008). The effect of zinc and manganese source in the diet for laying hens on egg shell and bones quality. *Veterinarni Medicina*, vol. 53 (10), pp. 555-563.

Tabatabaie, M.M., Aliarabi, H., Saki, A.A., Ahmadi, A. and Siyar, S.A.H. (2007). Effect of Different Sources and Levels of Zinc on Egg Quality and Laying Hen Performance. *Pakistan Journal of Biological Sciences*, vol. 10 (19), pp. 3476-3478.

Tauson, R. (1980). Cages: How could they be improved? *Current topics in veterinary medicine and animal science*, vol. 8, pp. 269-304.

Tauson, R. (1998). Health and Production in Improved Cage Designs. *Poultry Science*, vol. 77, pp. 1820-1827.

Tauson, R., Wahlström, A. and Abrahamsson, P. (1999). Effect of two floor housing systems and cages on health, production, and fear response in layers. *Journal of Applied Poultry Research*, vol. 8, pp. 152-159.

Tauson, R. (2005). Management and housing systems for layers – effects on welfare and production. *World's Poultry Science Association*, vol. 61, pp. 477-490.

Tauson, R., Kjaer, J., Maria, G., Cepero, R., Holm, K.E. (2005). Applied scoring of integument and health in laying hens. *Animal Science Papers and Reports*, vol. 23 (1), pp. 153-159.

Van Den Brand, H. Dr., Parmentier, H.K & Kemp, B. (2004) Effects of housing system (outdoor vs cages) and age of laying hens on egg characteristics. *British Poultry Science*, vol. 45 (6), pp. 745-752.

Van Krimpen, M.M., Kwakkel, R.P., Reuvekamp, B.F.G., Van Der Peet-Schwering, C.M.C., Den Hartog, L.A. and Verstegen, M.W.A. (2005). Impact of feeding management on feather pecking in laying hens. *World's Poultry Science Journal*, vol. 61. pp. 663-686.

Vestergaard, K.S. and Lisborg, L. (1993). A Model of Feather Pecking Development Which Relates to Dustbathing in the Fowl. *Behaviour*, vol. 126 (3), pp. 291-308.

Wang, G., Ekstrand, C. and Svedberg, J. (1998). Wet litter and perches as risk factors for the development of foot pad dermatitis in floor-housed hens. *British Poultry Science*, vol. 39, pp. 191-197.

Weissmann, A., Reitemeier, S., Hahn, A., Gottschalk, J. and Einspanier, A. (2013). Sexing domestic chicken before hatch: A new method for in ovo gender identification. *Theriogenology*, vol. 80, pp. 199-205.

Whitehead, C.C. & Fleming, R.H. (2000). Osteoporosis in cage layers. *Poultry Science*, vol 79, pp. 1033–1041.

Whitehead, C.C. (2004). Overview of Bone Biology in the Egg-Laying Hen. *Poultry Science*, vol. 83, pp. 193-199.

Wilcox, C.S., Patterson, J. and Cheng, H.W. (2009). Use of thermography to screen for subclinical bumblefoot in poultry. *Poultry Science*, vol. 88, pp. 1176-1180.

Wingfield, J.C. and Silverin, B. (2010). Molt in Birds and Mammals: Hormones and Behavior in: Breed, M.D and Moore, J., *Encyclopedia of Animal Behavior*, vol. 1. London: Elsevier, pp. 462-467.

Zamani, A, Rahmani, H.R. and Pourreza, J. (2005). Effect of Different Levels of Manganese and Zinc on Performance Traits and Breaking Eggs in Laying Hens. *Pakistan Journal of Biological Sciences*, vol. 8 (7), pp. 1035-1040.

Appendix

Table 10 - Scheme describing the composition of the control and experimental feed

	Phase 1	*Phase I*	*Phase II*	*Phase II*
	Control	*Experimental*	*Control*	*Experimental*
Ingredients, %				
Wheat	39.3	38.9	41.1	40.3
Soybean meal	19.6	19.7	15.7	15.9
Rapeseed meal	1.39	1.39	3.44	3.44
Rape seed	3.00	3.00	3.00	3.00
Oats	15.00	15.0	15.00	15.0
Barley	5.00	5.00	5.00	5.00
Soy bean oil	1.30	1.45	-	-
Linoleic acid C18:2	2.47	2.53	1.88	1.91
CF	4.47	4.48	4.47	4.48
AK-standard[6]	2.94	2.84	3.7	3.85
Maize gluten meal	-	-	0.75	0.75
Limestone	9.77	9.44	10.2	9.90
Mono calcium phosphate[7]	1.05	1.05	0.55	0.87
Methionine	0.43	0.42	0.40	0.40
Met+Cystine	0.74	0.74	0.71	0.71
Threonine	0.59	0.60	0.58	0.58
DL-Methionine	0.19	0.18	0.16	0.15
Lysine	0.86	0.85	0.79	0.78
Premix[8]				
Sodium chloride	0.28	0.28	0.24	0.24
Chemical composition (calculated, g/kg diet)				
ME MJ/ kg diet	11.6	11.6	11.7	11.7
DM (analyzed)	87.8 ± 0.43	87.8 ± 1.16	88.9 ± 0.10	88.8 ± 0.45
Ash (analyzed)	128 ± 7.34	127 ± 11.7	128 ± 6.59	128 ± 5.45
CP (analyzed)	168 ± 4.51	170 ± 7.15	168 ± 3.72	167 ± 3.46
EG (fat) (analyzed)	69.3 ± 3.07	72.5 ± 1.52	72.63 ± 1.37	69.8 ± 1.69
Ca (analyzed)	36.5 ± 7.70	36.1 ± 4.69	39.5 ± 4.90	41.5 ± 5.20
P (analyzed)	4.93 ± 1.88	5.00 ± 1.83	3.30 ± 1.99	3.7 ± 2.52
K (analyzed)	8.30 ± 1.25	8.20 ± 1.00	7.34 ± 0.72	7.77 ± 0.36
Mg (analyzed)	2.92 ± 2.02	2.95 ± 1.97	4.07 ± 2.07	4.68 ± 2.24
S (analyzed)	2.00 ± 0.24	2.14 ± 0.31	1.82 ± 0.22	1.74 ± 0.11
Cl (analyzed)	2.55 ± 0.49	2.52 ± 0.38	2.85 ± 0.14	2.83 ± 0.05
Zn, total, mg/kg feed	78	78	60	60
Zn, organic, mg/kg feed	-	40	-	60

[6] Liquid vegetable fat comprising a mixture of fatty acids
[7] Included to satisfy the need for phosphorus
[8] Contains a mixture of vitamins (A, D, E), trace elements (iron, cupper, manganese) and phytase

Table 10 - Production figures for the furnished cage production system during the layer age of 20-67 weeks. The results are presented as mean values.

Hybrid	Feed	N	Egg laying %	Egg weight g	Egg mass per hd g	Feed per henday g	Kg feed per kg eggs	Mortality %
Bovans	Expt	9	92,3	63,1	58,2	113,9	1,96	4,2
Bovans	Kontr	9	92,6	62,8	58,2	114,5	1,97	3,6
LSL	Expt	9	92,0	63,4	58,3	116,4	2,00	1,4
LSL	Kontr	9	92,3	63,5	58,6	115,4	1,97	2,8
Statistics	Hy	p<	0,28	0,05	0,79	0,03	0,03	0,07
	Feed	p<	0,34	0,58	0,63	0,75	0,47	0,69
	HyxFeed	p<	0,99	0,17	0,44	0,27	0,08	0,36

Table 11 - Production figures for the floor production system during the layer age of 20-67 weeks. The results are presented as mean values.

Hybrid	Feed	N	Egg laying %	Egg weight g	Egg mass per hd g	Feed per henday g	Kg feed per kg eggs	Mortality %	Eggs in litter, %	Eggs on slats, %
Bovans	Expt	4	81	63	51	151	2,94	7,8	21	0,2
Bovans	Kontr	5	84	63	53	151	2,86	6,1	20	0,3
LSL	Expt	5	90	64	58	131	2,27	2,5	9	0,3
LSL	Kontr	4	89	64	57	140	2,46	6	11	0,3
Statistics	Hy	p<	0,001	0,001	0,001	0,001	0,001	0,29	0,05	0,61
	Feed	p<	0,44	0,81	0,54	0,23	0,55	0,66	0,93	0,77
	HyxFeed	p<	0,27	0,86	0,30	0,25	0,14	0,24	0,72	0,39

Table 11 - Egg quality in the furnished cage production system at 44 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as least squares means.

	Treatment		Hybrid		P-value			
	Exp.feed[4]	Contr.feed[5]	LSL[1]	Bovans[2]	Treatment	Hybrid	Hybrid x treatment	Day[3]
Trait								
Egg weight (g)	63.8	64.2	64.8	63.2	0.6	*	0.2	0.1
Breaking strength (kg)	4.5	4.4	4.6	4.3	0.2	**	0.9	*
Albumen height (mm)	7.3	7.4	7.4	7.3	1.0	0.3	0.3	***
Haugh Unit (HU)	84.3	84.0	84.4	83.9	0.8	0.6	0.4	***
Albumen weight (g)	36.1	36.2	36.5	35.7	0.8	0.1	0.1	0.3
Albumen weight (%)	56.4	56.4	56.3	56.5	0.8	0.7	0.1	0.8
Albumen pH	8.7	8.7	8.7	8.7	0.4	0.3	0.4	***
Shell thickness (10^{-2} mm)	34.8	34.6	35.1	34.3	0.5	*	0.3	**
Shell weight (g)	5.9	5.8	6.0	5.7	0.4	***	0.1	0.9
Shell weight (%)	9.2	9.1	9.2	9.1	0.2	0.1	0.7	0.2
Yolk weight (g)	17.7	17.9	18.0	17.6	0.2	*	0.5	0.7
Yolk weight (%)	27.7	28.0	27.9	27.9	0.3	1.0	*	0.3
Albumen DM (%)	11.4	11.4	11.5	11.3	0.8	*	0.9	0.4

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Day= The day of analysis was corrected for to avoid biases due to storage effects.

[4] Exp.feed = Experimental feed containing the zinc amino acid complex

[5] Contr.feed = Control feed containing zinc oxide

Table 12 - Egg quality in the floor production system at 45 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as least squares means.

	Treatment		Hybrid		P-value			
	Exp.feed[4]	Contr.feed[5]	LSL[1]	Bovans[2]	Treatment	Hybrid	Hybrid x treatment	Day[3]
Trait								
Egg weight (g)	65.3	65.0	64.9	65.4	0.8	0.6	0.4	0.2
Breaking strength (kg)	4.8	4.7	5.0	4.5	0.5	***	0.3	0.8
Albumen height (mm)	7.5	7.3	7.5	7.3	0.2	0.1	0.4	*
Haugh Unit (HU)	84.9	83.7	85.2	83.5	0.2	0.1	0.5	*
Albumen weight (g)	37.8	37.5	37.4	37.9	0.6	0.6	0.8	0.1
Albumen weight (%)	57.9	57.6	57.6	57.8	0.4	0.6	0.2	0.1
Albumen pH	8.7	8.7	8.7	8.7	0.5	0.3	0.2	***
Shell thickness (10^{-2} mm)	36.2	35.9	36.4	35.6	0.5	0.1	0.6	**
Shell weight (g)	6.3	6.2	6.3	6.2	0.6	0.2	0.2	*
Shell weight (%)	9.6	9.6	9.7	9.4	0.7	*	0.6	0.3
Yolk weight (g)	17.8	17.8	17.7	17.9	1.0	0.2	0.2	0.7
Yolk weight (%)	27.3	27.4	27.3	27.5	0.8	0.5	0.6	*
Albumen DM (%)	11.4	11.5	11.5	11.4	0.7	0.1	0.2	0.3

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Day= The day of analysis was corrected for to avoid biases due to storage effects.

[4] Exp.feed = Experimental feed containing the zinc amino acid complex

[5] Contr.feed = Control feed containing zinc oxide

Table 13 - Egg quality in the furnished cage production system at 59 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as least squares means.

	Treatment		Hybrid		P-value			
	Exp.feed[4]	Contr.feed[5]	LSL[1]	Bovans[2]	Treatment	Hybrid	Hybrid x treatment	Day[3]
Trait								
Egg weight (g)	66.4	65.9	66.9	65.4	0.4	*	0.9	0.5
Breaking strength (kg)	4.2	4.3	4.4	4.1	0.1	**	0.4	0.8
Albumen height (mm)	7.3	7.4	7.3	7.4	0.3	0.4	0.5	***
Haugh Unit (HU)	83.4	84.3	83.3	84.4	0.2	0.1	0.5	***
Albumen weight (g)	37.8	37.5	37.7	37.6	0.4	0.7	0.2	0.6
Albumen weight (%)	56.9	56.8	56.4	57.4	0.8	**	*	0.6
Albumen pH	8.6	8.6	8.6	8.5	0.3	*	0.9	***
Shell thickness (10^{-2} mm)	34.4	34.3	34.8	33.9	0.8	*	0.1	0.9
Shell weight (g)	6.1	6.1	6.3	6.0	0.8	**	0.4	0.4
Shell weight (%)	9.3	9.3	9.4	9.2	0.5	*	0.3	0.9
Yolk weight (g)	18.3	18.2	18.6	17.8	0.8	**	0.1	0.9
Yolk weight (%)	27.5	27.6	27.9	27.2	0.7	*	*	0.6
Albumin DM (%)	11.1	11.1	11.1	11.1	1.0	0.9	1.0	0.1

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Day = The day of analysis was corrected for to avoid biases due to storage effects.

[4] Exp. feed = Experimental feed containing the zinc amino acid complex

[5] Contr.feed = Control feed containing zinc oxide

Table 14 - Egg quality in the floor production system at 60 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as Least squares means.

	Treatment		Hybrid		P-value			
	Exp.feed[4]	Contr.feed[5]	LSL[1]	Bovans[2]	Treatment	Hybrid	Hybrid x treatment	Day[3]
Trait								
Egg weight (g)	67.9	68.0	68.0	67.9	0.8	0.9	0.3	0.2
Breaking strength (kg)	4.4	4.2	4.5	4.1	0.1	**	0.7	0.1
Albumen height (mm)	7.3	7.5	7.4	7.4	0.2	0.9	0.4	***
Haugh Unit (HU)	83.0	84.0	83.4	83.5	0.2	0.9	0.5	***
Albumen pH	8.6	8.6	8.6	8.6	0.3	0.5	0.8	***
Albumen weight (g)	38.8	39.2	39.0	39.0	0.3	1.0	0.3	0.8
Albumen weight (%)	57.0	57.6	57.3	57.3	0.1	0.8	0.3	0.7
Shell thickness (10^{-2} mm)	34.4	34.1	34.8	33.8	0.4	*	0.2	0.6
Shell weight (g)	6.3	6.2	6.3	6.2	0.4	0.1	0.4	0.7
Shell weight (%)	9.3	9.1	9.3	9.1	0.2	*	0.6	0.5
Yolk weight (g)	19.1	19.0	19.1	19.1	0.4	0.9	0.5	0.8
Yolk weight (%)	28.2	27.9	28.1	28.1	0.3	0.9	0.1	0.6
Albumen DM (%)	11.0	11.2	11.2	11.1	0.1	0.3	0.7	0.1

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Day= The day of analysis was corrected for to avoid biases due to storage effects.

[4] Exp.feed = Experimental feed containing the zinc amino acid complex

[5] Contr.feed = Control feed containing zinc oxide

Table 15 - The proportion of cracked and dirty eggs in the furnished cage production system at 42 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as a proportion of the egg yield.

	Treatment		Hybrid		P-value		
	Exp.feed[3]	Contr.feed[4]	LSL[1]	Bovans[2]	Treatment	Hybrid	Hybrid x treatment
Trait							
Cracked eggs	2.4	2.2	2.5	2.1	0.7	0.8	0.6
Dirty eggs	10.2	8.6	8.9	9.9	0.2	0.4	1.0

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Exp.feed = Experimental feed containing the zinc amino acid complex

[4] Contr.feed = Control feed containing zinc oxide

Table 16 - The proportion of cracked and dirty eggs in the floor production system at 43 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as a proportion of the egg yield.

	Treatment		Hybrid		P-value		
	Exp.feed[3]	Contr.feed[4]	LSL[1]	Bovans[2]	Treatment	Hybrid	Hybrid x treatment
Trait							
Cracked eggs	1.3	1.6	1.7	1.2	0.3	0.4	0.9
Dirty eggs	30.8	33.0	24.4	39.4	0.6	**	1.0

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Exp.feed = Experimental feed containing the zinc amino acid complex

[4] Contr.feed = Control feed containing zinc oxide

Table 17 - The proportion of cracked and dirty eggs in the furnished cage production system at 60 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as a proportion of the egg yield.

	Treatment		Hybrid		P-value		
	Exp.feed[3]	Contr.feed[4]	LSL[1]	Bovans[2]	Treatment	Hybrid	Hybrid x treatment
Trait							
Cracked eggs	3.0	2.6	3.0	2.6	0.5	0.6	0.7
Dirty eggs	20.3	20.3	19.3	21.4	1.0	0.3	0.1

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Exp.feed = Experimental feed containing the zinc amino acid complex

[4] Contr.feed = Control feed containing zinc oxide

Table 18 - The proportion of cracked and dirty eggs in the floor production system at 61 weeks of age. * P< 0.05; ** P< 0.01; *** P< 0.001. Results within 0.05 < P < 0.10 are considered a tendency. Values are expressed as a proportion of the egg yield.

	Treatment		Hybrid		P-value		
	Exp.feed[3]	Contr.feed[4]	LSL[1]	Bovans[2]	Treatment	Hybrid	Hybrid x treatment
Trait							
Cracked eggs	1.9	2.8	2.4	2.2	0.1	0.7	0.3
Dirty eggs	39.0	40.1	29.0	50.1	0.9	*	0.7

[1] LSL = Lohmann Selected Leghorn

[2] Bovans = Bovans Robust

[3] Exp.feed = Experimental feed containing the zinc amino acid complex

[4] Contr.feed = Control feed containing zinc oxide

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