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Fakulteten för veterinärmedicin och husdjursvetenskap

Swedish University of Agricultural Sciences
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Effect of Insoluble Fibre Enrichment on Egg Quality in Laying Hens

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Preface

This study was completed as a Master's thesis of Animal Science at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences (SLU). The subject was chosen because of my interest in animal nutritional physiology, animal welfare and human food production as well as the pleasure performing the study on the hens at SLU research centre where I have, previously and during the study, spent time carrying out the daily work in the stables. The study was a part of the SLU project “The significance of fibrous materials in poultry production”, financed by the Swedish board of agriculture and Formas among others.

I would like to thank all people at the poultry section with Ragnar Tauson in charge, for letting me do this master thesis and helping me with the upset of the experimental design. Thanks to my supervisor Helena Wall for supporting me during the work and Robin Kalmendal for sharing interesting knowledge and joining me to perform the behavioural study. I want to thank also Istvan Pamlényi for sharing experience of the practical laboratory routines concerning the tests of egg quality parameters.

Also I would like to send my appreciation to the staff at Kungsängen laboratory, especially Börje Ericsson and Eva Verner for the helpfulness and Jorge André for bringing up many interesting questions and thoughts during my experimental work at the laboratory.

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SAMMANFATTNING

Studien var en del i ett större projekt som syftade till att undersöka hur värphöns påverkas beteendemässigt och nutritionellt av ökad konsumtion av olöslig fiber. I det nu aktuella projektet undersöktes hur ökat intag av olöslig fiber påverkar äggkvalitén. Fiberkällan utgjordes av halmpellets som antingen blandades i fodret med 3 % inblandning, alternativt utgjorde strömaterial för att undersöka strömaterialets betydelse som fiberkälla. Två olika hybrider ingick i försöket, 720 Lohmann Selected Leghorn (LSL) och 720 Lohmann Brown (LB), varav halva antalet av varje hybrid hölls i frigående system och andra hälften i inredda burar. Totalt insamlades och undersöktes 180 ägg från varje inhysningssystem då hönorna var 31-32 veckor gamla. Vid 51 veckors ålder upprepades proceduren i burstallet, då ytterligare 180 ägg samlades in. Ett tiotal parametrar för inre och yttre äggkvalitet mättes på laboratorium och resultaten bearbetades med hjälp av det statistiska analysprogrammet SAS med avseende på behandling, hybrid och ålder samt interaktioner däremellan. Konsumtionen av strö mättes också och beteendestudier gjordes för att studera hur hönorna interagerade med strömaterialet. Strökonsumtionen varierade mellan 2,1 och 4,1 g/höna och dag vid 45 veckors ålder och var signifikant högre hos LB än hos LSL. Det fanns inga effekter av fiberberikning i frigående systemet, men där kunde den planerade mätningen vid äldre ålder inte genomföras vilket i sin tur resulterade i att en jämförelse mellan inhysningssystem uteblev. Inte heller i bursystemet fanns påverkan på inre äggkvalitén av ökat fiberintag. Däremot fanns en tendens till att ökat fiberintag gav sämre skalstyrka och lägre viktprocent skal i bursystemet. Skalkvalitén påverkades i högre grad hos LB som hade en högre konsumtion av strömaterial och därmed högre totalt fiberintag jämfört med LSL. Resultaten visar att 3 % halm i fodret inte försämrar äggkvalitén, men att hänsyn även behöver tas till intag av strömaterial när man bedömer totalt fiberintag och hur äggkvalitén kan påverkas. Hybrid och ålder hade signifikant påverkan på flera av kvalitetsparametrarna, i såväl inre som yttre äggkvalitet.

ABSTRACT

The study made part of a bigger project investigating behavioural and nutritional effects of insoluble fibre enrichment in laying hens. In the present part of the project, egg quality was examined whether affected by increased intake of insoluble fibre. Straw pellets constituted fibre source composing 3 % of the feed, or was used as litter material to investigate the role of litter as fibre source. A total number of 1 440 Lohmann Selected Leghorn (LSL) and Lohmann Brown (LB) were housed in furnished cages and in a traditional floor housing system, half the number of each hybrid in each housing system. A total number of 180 eggs from each housing system were collected and examined at the hen age of 31-32 weeks. At 51 weeks of age, the procedure was repeated in the cage housing system, resulting in another 180 eggs examined. Both internal and external egg quality were examined at the laboratory and data were then statistically analysed according to experimental treatment, hybrid and hen age as well as interactions between these parameters; using the computer program SAS. The consumption of litter material was measured as well and behavioural studies were pursued to examine birds´ interactions with litter material. Litter consumption varied between 2.1 and 4.1 grams per hen and day at 45 weeks of age and was significantly higher in LB compared to LSL. No effect of fibre on egg quality was found in the floor housing system, where measurements at older age could not be performed, in turn excluding comparison between housing systems in the study. Nor in the cage housing system inner egg quality was affected by fibre enrichment. However, egg shell strength and egg shell percentage tended to be reduced with increased fibre intake in the cage housing system. Furthermore, shell quality was affected to a higher extent in LB where litter consumption was higher, leading to a higher total fibre intake, compared to LSL. The results show that feed enriched with 3 % of straw does not impair egg quality but at the same time litter consumption should also be considered in terms of total fibre intake for poultry and how egg quality may be affected. Hybrid and hen age influenced several of the inner and shell quality parameters significantly.

INTRODUCTION

Keeping animals out of their natural habitat and for human commercial purposes, always leads to compromises, trying to fulfil basic needs of the animals and at the same time manage an economically sustainable production. Animals used in food production are bred and to a high extent adapted to live and produce under these conditions. However, behavioural problems can arise. In laying hens, feather pecking and cannibalism lead to welfare problems as well as economical loss for the egg producer when they occur. Studies have shown that increased fibre intake can reduce the risk of occurrence of these behavioural abnormalities (Steenfeldt *et al.*, 2007; van Krimpen *et al.*, 2009; Hammershøj and Steenfeldt, 2005; Hartini and Choct, 2010).

High quality of the eggs produced is of importance for the economic viability of the egg production. Knowledge about the effect of fibre enrichment on egg quality may constitute important information of whether fibre enrichment is to be recommended as inclusion in poultry feed. Moreover, broadening the knowledge concerning effects of fibre on egg quality may provide information that can be useful for organic egg producers in particular, due to the requirement of forage in that production system (Swedish Board of Aagriculture, 2012). Furthermore, not only feed but also litter material can constitute a nutritional fibre source. Studies have shown that hens have an appetite for litter material, in amounts depending on composition of both feed and litter material (Hetland *et al.*, 2005; Hetland and Svihus, 2007).

AIMS AND OBJECTIVES

The aim of the present study was to broaden the knowledge concerning the effect of fibre intake on egg quality in laying hens. The objectives were to investigate how shell and inner egg quality is affected by fibre enrichment in diet and litter material. The study was set up to include eggs laid at different hen ages from two different hybrids of layers; to investigate if possible effect of fibre enrichment was influenced by hybrid and age. A literature study was to be pursued, providing some basic knowledge about the egg and hen gut anatomy, followed by quality parameters used to describe egg quality and the influence of fibre enrichment on the metabolism and behaviour of the hen. In addition to the egg quality trial, a behavioural study was set up to investigate the consumption of litter material in hens.

LITERATURE SURVEY

The functional laying hens

The layers used for egg production today have, like other food producing animals, enhanced their production capacity to a very high production rate as compared to the production in earlier years. Haddon (1945) stated that the local hen breed back at that time gave 40 to 50 eggs a year, a number that could be raised to 80 eggs a year with adequate feeding, while by selective breeding the production could be raised even further to 140 eggs annually. Today the egg laying hybrids produce more than twice that quantity, approximately 320 eggs during 52 weeks in lay in LSL (Lohmann Selected Leghorn), a commonly used hybrid for egg production (Lohmann Tierzuch a).

An egg shell contains approximately two grams of calcium. To produce these large amounts of eggs, the calcium metabolism activity in the bird is very high, enabling the build-up of the egg shells. This achievement is made possible thanks to an extremely active calcium metabolism in some parts of the bone called medullary bone, where dietary calcium is stored during the day and removed for egg shell production during the night (Silversides *et al.*, 2006).

Producing eggs is also an energy demanding process. The energy requirement is considerable as energy is deposited in the egg in the form of nutrients (333 kJ/day approximately). In addition there is an energetic cost for the hen when synthesising the egg components and transporting the precursors to the egg, moving the egg through the oviduct and out at laying and for maintenance of the reproductive organs (estimated to 340-400 kJ/day). This increased energy requirement during the egg laying period is met by an increase in voluntary feed intake. The circadian rhythm influences the pattern of feed intake, resulting in an increased consumption in the end of the diurnal light period before the nocturnal fast. During night the crop and to some extent the gizzard act as feed reservoirs, gradually releasing feed during the night (Scanes *et al.*, 1987).

Egg composition

The egg is designed to create a favourable environment for chicken development, providing all the nutrients needed as well as protection from external mechanical forces and bacterial invasion. An egg is a concentrated nutrient source in relation to its energy content. The proteins as well as the fat, which is emulsified in the egg, are highly digestible. The mineral content of the internal parts of the egg is relatively high, particularly of iron and phosphorus. However, mineral composition can be altered by diets given to the laying hen. The egg also contains all vitamins except vitamin C, and particularly they are rich in the fat-soluble vitamins D and A (Rose, 1997).

Furthermore, the egg can be divided into three main parts of very different composition, representing the following quantities in percentage of total egg weight; 27 % yolk, 64 % albumen and 9 % shell (Figure 1) (Rose, 1997).

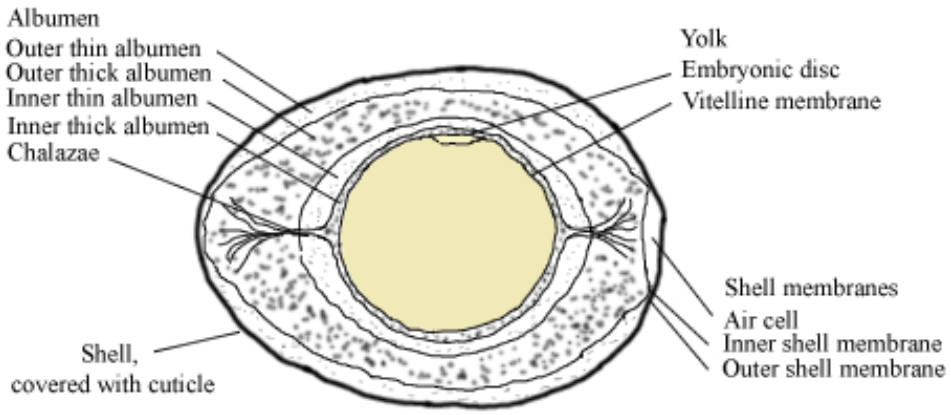

Figure 1. Structure of a laying hen egg. Modified after Stadelman (1995).

Yolk

The yolk contains the genetic code of the egg cell, present in the embryonic disc that can be seen as a paler spot on the surface of the yolk. The yolk is the part that is the richest in nutrients, containing approximately 50 % of the protein content in the egg and almost all the fat (Rose, 1997). In a fertilized egg the fatty acids of the yolk is the main nutrient source for the chick embryo, providing over 90 % of the energy needed for embryo development (Speake *et al.*, 1998).

Yolk is built up to approximately 33 % of fat and to 16 % of protein. The water content increases during storage as water is transferred from the albumen into the yolk. Depending on length of

storage, yolk water content can therefore vary between 46 and 50 %. All yolk material is enclosed in a tender, elastic membrane called the vitelline membrane, keeping this material together and separating the yolk from the albumen content surrounding it (Rose, 1997).

Albumen

The albumen surrounding the yolk has several beneficial characteristics. It protects the embryo from external forces by acting as a shock absorber as well as acting as a protective barrier against bacteria. The antibacterial effect is reached through the inclusion of an antimicrobial substance, the enzyme lysozyme, attacking the cell walls of any bacteria that may enter. The albumen is also the main water storage of the egg, containing approximately 88 % of water. Moreover, it is a supply of some nutrients; containing approximately 11 % protein, 1 % of carbohydrates but almost no fat (Rose, 1997).

Albumen is present in two types differing in viscosity; thin and thick albumen. These two types are building up four layers of albumen surrounding the yolk. The first layer closest to the yolk is represented by a layer of thick albumen which is adjacent to the vitelline membrane. The second layer is composed of thin albumen, followed by a layer of thick albumen finally surrounded by another layer of thin albumen closest to the shell membranes (Figure 1) (Stadelman, 1995). The thickness is not directly related to the water content of the albumen, whereas the concentration of the protein ovomucin is approximately four times higher in thick albumen compared to thin albumen. Ovomucin is thought to be the main reason for the higher viscosity in the thick albumen layers (Rose, 1997).

There is also a type of albumen that is formed as long twisted fibres named chalazae, keeping yolk position in the centre of the egg. They are positioned at each side of the yolk, attached with one end to the surface of the vitelline membrane and the other one interlaced with fibres in the thick albumen layer closest to the yolk (Rose, 1997).

Shell and shell membranes

Function

Also the shell structure possesses several characteristics advantageous for embryo development. The most evident is perhaps the role as a protective coating of the egg contents, protecting it from external mechanical forces. Furthermore, the shell is a source of calcium needed for the development of the embryonal skeleton (Cordts *et al.*, 2002). The inner side of the shell is covered by two shell membranes, separating shell and albumen contents. The shell membranes as well as the shell structure, provide a barrier against bacterial invasion into the egg (Rose, 1997). In addition, shell membranes at the same time reduce moisture losses by partly blocking the shell pores (Jacob *et al.*, 2011).

Both shell and shell membranes are structured in a way to enable entrance of oxygen needed for the developing embryo and loss of metabolic water and carbon dioxide that the embryo produces. Whether or not fertilised, water and carbon dioxide are lost from the egg during storage (Rose, 1997). After the egg is laid, an air cell is formed at the larger end of the egg through a separation of the two shell membranes; the outer one attaching to the shell and the innermost attaching to the albumen. The air cell formation occurs as a result of contraction of egg contents as egg temperature cools down to ambient temperature after the point of lay – at which egg temperature is the same as hen body temperature (about 40.5 ºC). During storage, the air cell is enlarged as air enters through the shell pores into the air cell, at the same time as water is lost from the albumen and evaporated through the shell pores (Jacob *et al.*, 2011).

Egg shell properties are facing a contradictory challenge. It should be strong enough to protect the embryo but at the same time porous to enable gas exchange. Also, shell strength has to be enough to support the weight of the adult hen during brooding, yet weak enough to enable the chick to hatch (Rose, 1997). These challenges have been made possible to meet through the composition of the shell structure as described below.

Structure

The components building up the shell is to 98 % calcium carbonate crystals and to 2 % protein. Also small amounts of magnesium and phosphorus are present. The shell structure is built up by columns of calcium carbonate crystals, formed around a matrix of fine protein fibres (Figure 2). These columns are closely attached to each other but between some of them there is an air space, forming a channel throughout the egg shell and an oval pore on the shell surface. By these channels it is possible for gases to enter and exit the egg, enabling gas exchange from the embryo. The size of these pores is in average about 20 μm and there are approximately 7500 pores on an egg from a domestic fowl (Rose, 1997).

The inner ends of the columns, facing the inside of the egg, are placed on top of cone shaped arrangements of calcium carbonate crystals. These cones are embedded in a matrix in which proteins are combined with polysaccharides. Small parts of this matrix are bedded in the shell membrane present closest to the inner shell surface, and the calcium carbonate cones are embedded within it (Rose, 1997).

Around the outer side of the shell, a water-insoluble cuticle covers the surface of the egg, acting as a barrier that contributes to protect the egg from entrance of bacteria. However, after the egg is laid, the cuticle starts to dry and cracks. The functional properties of this covering have been questioned and not all poultry species produce any cuticle but lay eggs without this final covering (Solomon, 2010).

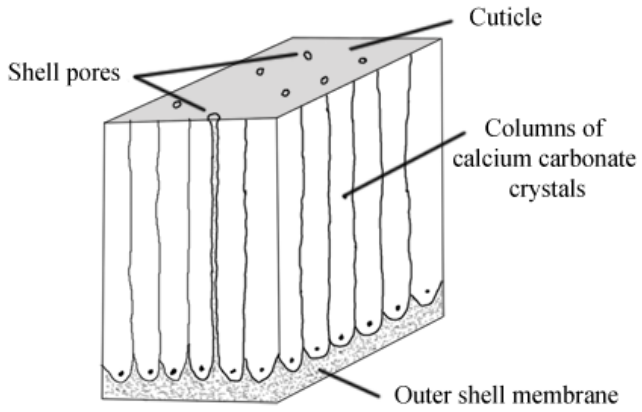

Figure 2. Cross section of the shell structure of a laying hen egg. Modified after Rose (2005).

Egg formation

The egg formation process is started through the ovulation when a yolk, produced in the ovary, is released into the oviduct (Figure 3). The yolk then passes through the egg formation tract, where the egg is formed through a series of processes along the way in the oviduct, as described below. The left side of the reproductive tract is the one in function, whereas the right ovary and oviduct don't develop in the commercial laying hen (Ahmadi and Rahimi, 2011).

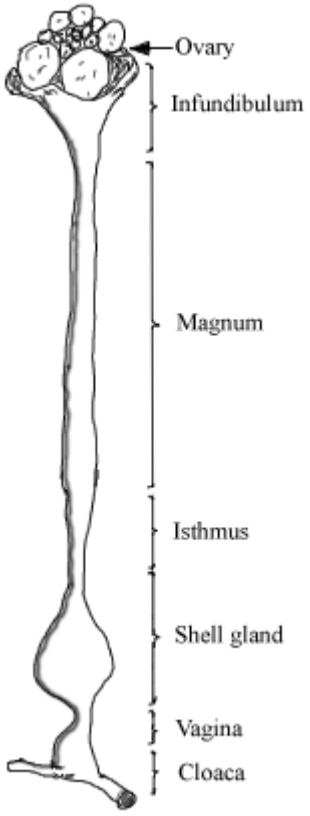

Figure 3. Hen reproductive system. Modified after Roberts (2004).

The first step of egg formation, after ovulation, is the formation of the yolk membrane and the chalazae, which takes place during 15 minutes in the infundibulum. Thereafter the developing egg moves further into the magnum where the albumen proteins (a total of approximately 40) are produced during another 15 minutes. Next step of egg formation occur further down in the oviduct in the istmus where the two shell membranes are formed from the fibres produced here, a process lasting for more than one hour (Ahmadi and Rahimi, 2011). The egg then proceeds into the shell gland where water and electrolytes hydrate the albumen proteins within the shell membranes, filling out the egg into its characteristic shape, a process called “plumping”. Shell formation is initiated by calcium deposits, coming from calcium salts released from the epithelial cells, binding onto the shell membrane. Eggs may stay in the shell gland during more than 20 hours, and during the last few hours the pigment porphyrin is secreted in brown egg layers, producing the brown colouration of their eggs. In white egg layers no pigmentation is secreted resulting in white colour of these egg shells (Rose, 1997).

The newly formed egg is laid through the cloaca by the relaxation of the powerful musculature of the vagina wall. The vaginal muscles are under the voluntary control of the hen enabling the bird to delay laying for a few hours if there is an unfavourable situation for laying at the moment (Rose, 1997).

The Swedish egg industry and egg quality control

As for the chicken embryo, which is the original consumer of the nutrients hold by the egg, the egg is also for humans a source of many of the nutrients needed; protein, fatty acids, minerals, vitamins and antioxidants (Svenska ägg, 2011). The total consumption of eggs in Sweden in 2009 was approximately 123 700 tons in total (including both table eggs and others) and the production was 104 500 tons (Rosell and Strandberg, 2010).

According to the market standards eggs are classified as class A or B, as determined by the quality of both shell and inner quality parameters (Table 1). Only eggs of class A are allowed to be sold as table eggs. Eggs not fulfilling even the standard for class B is to be discarded (Swedish Food Administration, 2012).

Table 1. Quality requirements for the classification of eggs (modified after Swedish Food Administration (2012)

Class A or "fresh eggs"	
Shell and shell membranes	Entire, normal and clean
Air cell	Height of maximum 6 mm, immovable
Yolk	Seen only as a shadow, without a clear profile, when transferred through a bright light and without visibly moving from the centre when turning of the egg
Albumen	Clear, transparent
Embryonic disc	Not visibly developed
Odour	Free from unknown odours
Particles	Free from unknown particles
Class B	
Quality requirements	Eggs not fulfilling requirements for class A, except broken eggs with damaged shell membranes, very dirty eggs or eggs that have been brooded.
Prerequisites	Class B eggs are allowed to be delivered only to a food industry specifically approved to produce egg products, or to industries other than the ones in the food industry.

A way of detecting quality defects at the egg packing facilities is candling. In this procedure eggs are transferred through bright light showing up internal defects and previously undetected cracks on the shell. Internal defects often visualized through candling include meat and blood spots, enlarged air cells and any very thin or watery albumen (Swedish Food Administration, 2012; Coutts and Wilson, 2007 a). Traditionally, candling has been the technique in use. However, the presence of modern egg grading machines with automated mechanical crack detectors is increasing on the market, ensuring an even more reliable quality control. The working principle of these crack detectors is that several small impactors are used, exciting the egg directly or indirectly on different spots around the egg shell surface. The number or amplitude of rebounds that impactors make after contact with the egg shell is then used to build up a global map of the mechanical integrity of the egg shell (Dunn *et al.*, 2005). In addition to the quality control performed by the packing facility itself, the Swedish Food Administration performs random samplings at the egg packing facilities to verify that the quality standards are met (Swedish Food Administration, 2012).

Egg quality, both in terms of internal and shell quality, is an important issue that is critical for the economic viability of the egg industry. When eggs do not fulfil the requirements of class A, they may be sold as Class B wish is accompanied by a lower price to the producer (Ahmadi and Rahimi 2011). According to Coutts and Wilson (2007 (a)) the average number of class B eggs is approximately 3 % of total production at 45 weeks of hen age. This proportion is linearly increased as hens grow older, reaching approximately 9 % at 50 weeks and 15 % of class B eggs at 75 weeks of hen age.

To measure egg quality at a laboratory in a standardized way for research purposes, there are several methods practised (Ahmadi and Rahimi 2011). Some methods used to measure internal egg quality and shell quality are described in the text below.

Shell quality parameters

Shell quality includes characteristics concerning the consistency of the egg shell, meaning its ability to stay intact during transport and handling. This ability plays a central role for the egg as a commercial product and breeders and producers are continuously searching for improvements in the quality and consistency of the egg shell (Cordts *et al.,* 2002). Shell quality parameters, relating to the ability of keeping the egg intact through the commercial chain, are shell strength, shell deformation, shell thickness and shell weight. These parameters are all commonly used when measuring egg shell quality experimentally as they are correlated to shell breakage or cracks in the egg (Roberts, 2004).

Shell strength is commonly measured by compressing the egg with an increasing load applied during controlled conditions until the shell cracks. The minimum force needed to cause failure to the shell is recorded (Roberts, 2004). Shell strength has been shown to be correlated to the likelihood of breakage in the production chains of consumption eggs (Mertens *et al.*, 2006). Shell strength varies over different parts of the egg and the broad pole of the egg has higher shell strength than the narrow pool (Salomon, 2010).

Shell strength is influenced by amount and thickness of the shell. The amount of shell present can be expressed as shell weight in percentage of total egg weight (Roberts, 2004), a measure that has been found to be correlated to the percentage of cracks in egg shells (Strong, 1989). Shell strength isn't constant around the egg but can have a large variation from the narrow pole to the broad pole, whereas the variation is small around the latitude (Salomon, 2010).

Shell thickness has also been associated with the ability of an egg to stay intact during handling. For an egg to have better than 50 % chance of passing normal market handling without breaking, it has been estimated that a shell thickness of at least 0,33 mm is needed (Stadelman, 1995a). Shell thickness is not exactly the same over the surface of the egg. Usually, the shell is thicker in the broad and narrow cap each, compared to the most intermediate latitudes, and the variation in shell thickness is less around the latitudes than there is longitudinally (Tyler, 1961).

Moreover the strength of an egg shell is also determined by the quality of the construction of the shell. This parameter can be studied by examining the ultrastructure of the shell under a scanning electron microscope. In cases when shell weight, shell weight percentage and shell thickness are at adequate levels but shell breaking strength is relatively poor, the reason is likely to be found in the ultrastructure of the shell, illustrating how well the shell is constructed (Ahmadi and Rahimi, 2011).

Shell deformation is yet another indicator of potential shell breakage (Miller and Sunde, 1975) and is a measurement of the elasticity of the shell (Cordts *et al.*, 2002). The degree of elasticity is in turn influenced by the unique relationship between the organic and inorganic fractions making up the shell structure (Salomon, 2010). Shell deformation may be measured either in a non-destructive way as the deflection of the shell when applying a fixed force on the egg, or in a destructive way measuring the distance the shell is compressed before it breaks when applying force on the egg (Roberts, 2004).

Factors affecting shell quality

As the hen gets older, the size of the eggs is increased. However, the increase in egg weight is not accompanied by a proportional increase in shell weight, resulting in a decrease in ratio of shell weight to egg weight. In that way, shell weight percentage is decreased during the production cycle and eggs produced late in the production cycle are thereby weaker compared to those laid in younger age (Roberts, 2004).

Strain of hen is another characteristic with impact on shell quality (Hamilton *et al.*, 1979). An example of that is the difference in shell breaking strength, given for different hybrids produced by the German breeding company Lohmann Tierzucht, eg. shell strength of >35 N for Lohmann Brown-Classic but >40 N for Lohmann LSL-Classic (Lohmann Tierzuch(b); Lohmann Tierzuch(a)).

High environmental temperature influences shell quality negatively. Partly it is an effect of reduced feed intake, resulting in limited blood Ca (calcium) availability and induced bone resorption. Furthermore the Ca supply to the uterus is reduced because the blood flow is directed to the skin for cooling. Moreover the blood acid-base balance, an important factor for egg shell quality, is altered in high environmental temperatures. The acid-base imbalance is a result of increased panting in order to cool the body to maintain thermal homeostasis (Oguntunji and Alabi, 2010). During panting, CO_2 (carbon dioxide) is lost from the blood through the exhaled air (Balnave and Muheereza, 1997). This loss is associated with a loss also of HCO_3^- (bicarbonate) from blood and body tissues, since most of the CO_2 transported by the blood is present as HCO_3^- which is much more water soluble than CO_2 (Sjastaad *et al.*, 2003). The chemical reaction for which HCO_{3-} is produced is catalysed by the enzyme carbonic anhydrase through the formulae as described in figure 3 below. Reduced HCO_3^- concentration in the shell gland fluid causes a reduction in shell quality of the egg, since it is needed together with Ca to produce $CaCO_3$ (calcium carbonate), the main constituent of shell structure (Balnave and Muheereza, 1997). Both HCO_3^- and Ca is important for egg shell formation and deficiency of any of these elements, eg. through calcium deficiency or inhibition of carbonic anhydrase, results in thinner shells leading to reduced egg shell quality (Bebout and Hempleman, 1994).

Carbonic anhydrase

$$\downarrow$$

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$ (Sjaastad, *et al.*, 2003)

Figure 3. Bicarbonate originates primarily from the metabolic production of CO_2 and the following hydration to carbonic acid (H_2CO_3), a reaction catalysed by the enzyme carbonic anhydrase (Bebout and Hempleman, 1994). Dissociation of carbonic acid to bicarbonate and hydrogen occurs rapidly without any enzymatic activity involved (Sjaastad *et al.*, 2003).

The primary source for calcium is through intestinal absorption of dietary calcium, though a large proportion is resolved from the medullary bone even at adequate dietary intake of calcium (Bebout and Hempleman, 1994). Dietary minerals and vitamins in adequate levels are thereby essential for good egg shell quality. Since an egg shell contains up to 3 grams of calcium, the feed must contain calcium in adequate levels that can be efficiently utilized by the hen (Ahmadi and Rahimi, 2011). A feed deficient in calcium will lead to reduced thickness of the egg shell and reduced shell strength as a consequence (Bebout and Hempleman, 1994). During the rearing period, the calcium level of the feed needs to be increased with start from seven to ten days prior to the appearance of the first egg. If additional calcium isn't provided early enough, there may be long term negative effects on calcium metabolism and calcium stores in bones (Ahmadi and Rahimi, 2011).

Water salinity is another factor affecting shell quality. The negative response is noticeable already at a relatively low NaCl concentration of 0.2 g/l of water (Balnave *et al.*, 1989), even though the sensitivity to saline water varies between strains (Ahmadi and Rahimi, 2011; Chen and Balnave, 2001). In many countries the low concentration of 0.2 g/l can be found in the underground bore water (Balnave *et al.*, 1989). In Australia, bore water has a NaCl concentration ranging from 0.01 to more than 3.0 g/l, depending on site and depth of the bore (Chen and Balnave, 2001). Intake of saline water has been shown to reduce shell breaking strength, shell thickness, the amount of

calcium in the shell and to increase the number of damaged eggs (Abbas *et al.*, 2009). The decreased shell quality caused by saline water intake is associated with an interfered calcification process during egg shell formation, related to a reduced concentration of bicarbonate and calcium in the shell gland fluid, as well as a reduction in the activity of carbonic anhydrase in this fluid (Balnave *et al.*, 1989; Abbas *et al.*, 2009).

Internal egg quality parameters

The quality of the inner parts of eggs is of importance for food processing and to meet consumer preferences. Generally, egg quality is related to characteristics affecting the acceptability of an egg to the consumer (Stadelman, 1995b). The quality of the internal parts is also a reflection of the freshness of the egg. Egg quality is considered to be highest at the time for lay and decreases with storage time. Change in observed physical conditions constitutes criteria for quality evaluation (Army Natick Research symposium, 1976).

The rate of change in quality during storage is a function of temperature and movement of carbon dioxide through the shell. The closer the surrounding temperature is to zero, the slower the quality declines (Stadelman, 1995b).

Albumen height and HU

The viscosity of the thick albumen is changed during storage, resulting in a thinning of the albumen structure. Albumen viscosity is therefore a way of measuring egg freshness and thereby internal egg quality. In addition, viscosity properties are important for the use of eggs in the baking industry, where a high foaming ability and high whipping volume are wanted (Ahmadi and Rahimi, 2011).

The ability of albumen to form stable foams when whipped together with its heat coagulation properties are reasons why eggs are used widely in the food industry. High foam-forming ability is considered to be correlated to high albumen viscosity and is measured through the height of thick albumen after braking the egg on a flat surface (Figure 4) (Silversides and Budgell, 2004). Albumen height can also easily be observed by the consumer when breaking the egg into e.g. a frying pan. However, the correlation between albumen height and whipping volume has been challenged by Silversides and Budgell (2004) who found albumen height to be negatively correlated to whipping volume. The lower albumen height found in unselected hens had in fact higher whipping volume compared to commercially selected hens with higher albumen height, suggesting that selection for albumen height in commercial layers could have decreased its foam-forming ability and may be counterproductive.

Albumen height is often transferred into HU (Haugh Units) – an accepted commercial and research standard for measuring albumen quality. This calculation formula is the logarithm of the thick albumen with an adjustment for differences in egg weight (Silversides, 1994). Eggs reaching the consumers should have a minimum measurement of 60 HU (Chukwuka *et al.*, 2011). As albumen height decreases with storage time (Scott and Silversides, 2000) most eggs leaving the farm should have values of 75-80 HU (Chukwuka *et al.*, 2011).

However, the validity of the use of HU has been questioned since it assumes a fixed relation between egg weight and albumen height, which was shown incorrect by Silversides (1994). With increasing age of the hen, the weight of the egg increases in a quadratic manner, whereas albumen height decreases linearly. The HU formula is designed to remove the statistical relationship between egg weight and albumen height while in fact overcompensating for egg weight. The author conclude that if a correction for egg weight will improve this measure, it should be determined for each group of eggs that come from genetically similar hens of the same age (Silversides, 1994).

The albumen component generally accepted as the most important one associated with the thick structure of the fresh albumen is the protein ovomucin – accounting for approximately 3.5 % of total albumen protein. In albumen of higher HU-value, the amount of ovomucin in thick albumen has been found to be higher and the structure of the subunits in ovomucin has been reported to be different, compared to albumen of a lower HU-value (Toussant and Latshaw, 1999).

Parameters that can negatively affect albumen HU are, in addition to time of storage; hen genetics, hen age, infectious bronchitis disease and the inclusion of the mineral vanadium in the feed (Toussant and Latshaw, 1999). Presence of vanadium in a layer diet is usually a consequence of its presence in certain samples of rock phosphate used as source of dietary phosphorous (Summers, 2008).

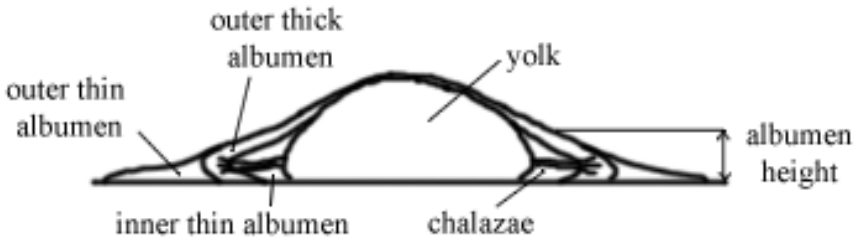

Figure 4. An egg broken onto a flat surface illustrating the measurement of albumen height. Modified after Rose (1997).

Albumen pH

Albumen pH of a newly laid egg is approximately 7.6-7.8 and is then increased during storage. After five days of storage in room temperature, pH has been found to increase to 9.1-9.3 (Silversides and Budgell, 2004; Sharp and Powel, 1931). A fresh egg contains considerable amounts of carbon dioxide. The partial pressure of carbon dioxide in the liquid of the egg before it is laid, corresponds to that of the tissues in the hen. After the egg is laid, the air cell is formed and some of the carbon dioxide escapes from the liquid parts into the air cell, as well as to the outside of the egg through the pores of the shell, leading to an increase in albumen pH. The increased pH causes denaturation and hydrolysis of some proteins, leading to a thinning of the albumen and decreased albumen height (Sharp and Powel, 1931; Ahmadi and Rahimi, 2011).

Albumen DM content

Albumen DM (dry matter) content is approximately 12 % (Rose, 1997). A high DM content is important when processing eggs into dry powder products (Hammershøj and Steenfeldt, 2005). Among eggs with the same storage conditions, a high DM content is correlated to high values of albumen gel structural properties, which is important in the baking industry. Albumen DM content is increased during storage of the egg, as a result of water escaping the albumen into the yolk as well as evaporating though the shell. However, an increase in albumen DM content as a result of storage seems to have no major positive effect on gel structural properties (Hammershøj *et al.*, 2002).

Yolk colour

Yolk colour is a quality measurement of which the value preferred differs on different markets around the world (Rose, 1997). Northern countries, except for Germany, generally prefer a weakly coloured yolk, whereas countries in the south west of Europe favour a more intensively coloured yolk (European Commission, 2002).

The yellow colour of egg yolk originates from xantophylls, a lipid-like compound, of which the amount in egg yolk is almost completely dependent on the amount present in the hen's diet. In grass and maize, the amount is high enough to give a deep yellow colouration of egg yolks, whereas most other cereals such as wheat and barley are free from xantophylls. A wheat and barley based diet thus requires the addition of a concentrated source of xanthophylls if a deep yellow yolk is wanted (Rose, 1997). In Sweden, the egg branch has agreed on not using any synthetic colouring substances in the feed but instead include feed ingredients rich in xanthophylls (Svenska ägg, 2011).

Blood spots

The occurrence of blood spots is another quality parameter strongly correlated to consumer preferences. However, both chemically and nutritionally, an egg containing blood spots is fit to eat (Jacob *et al.*, 2011). The appearance can range from barely distinguishable spots on the surface of the yolk to heavy blood contamination throughout the yolk. It may also turn up as diffused into the albumen content (Coutts and Wilson, 2007 b). Blood spots appear to some extent in less than 1 % of all eggs produced. The incidence varies between bird strains and is usually more common in brown than in white eggs (Jacob *et al.*, 2011).

The origin of blood spots in eggs is haemorrhage of a small blood vessel in the ovary or oviduct. When appearing on the yolk, the haemorrhage was probably in the ovary at time for ovulation or in the infundibulum before albumen was introduced into the present egg formation process. If blood occurs in the albumen, the haemorrhage was probably rather in the wall of the magnum part of the oviduct (Jacob *et al.*, 2011). Factors leading to this quality problem may be fright or other environmental disturbances, the disease Avian encephalomyelitis or incorrect levels of vitamin A and K in the diet (Coutts and Wilson, 2007 b).

Meat spots

Meat spots are, as well as blood spots, strongly correlated to consumer preferences even though also these contaminated eggs are chemically and nutritionally edible (Jacob *et al.*, 2011). Meat spots may appear on the yolk as well as in the albumen. The size range from 0.5 mm to more than 3 mm in diameter and they are usually brown in colour (Coutts and Wilson, 2007 b).

The occurrence of meat spots derives from partly broken down blood spots, small pieces of ovary or oviduct tissue or cuticle remnants that has been swept up to the magnum and been included in the albumen contents (Jacob *et al.*, 2011). The incidence of meat spots increases with hen age and varies with bird strain (Coutts and Wilson, 2007 b). Usually meat spots are more common in eggs from brown-egg layers than white-egg strains (Jacob *et al.*, 2011).

Egg weight

Eggs in the store are labelled S, M, L or XL representing the following egg weights; S: 43-53 g, M: 53-63 g, L: 63-73 g and XL: above 73 g. Egg size alters as the hen gets older resulting in increased egg weight along the production cycle (Svenska ägg, b). In contrary, during storage of an egg, its weight is decreasing as a result of water loss from the albumen, evaporating through the shell (Hammershøj *et al.*, 2002).

Feed for layers

In the present study, the effect of fibre enrichment on egg quality was studied. Thus, it is interesting to understand the composition of a regular layer diet used in commercial egg production. In the Nordic countries commercial feed for layers are normally based on cereals, as a result of the local climate being favourable for growing grain. However this diet is very differently composed from the one ingested by the South Asian Red Jungle hen, from which the commercial hen originates.

They eat a diverse diet, where insects and seeds high in proteins and fats are important constituents (Kalmendal, 2010).

For commercial egg production, feed stuffs rich in fat and fibre rarely win the competition in terms of price compared to feed ingredients rich in starch, such as wheat. However, studies have shown that a large amount of wheat in the diet can be a contributing factor to feather pecking and in turn poor feathering which is a problem in many flocks (Elwinger, 2006). The inclusion of fibre in layer diets has been shown to reduce problems with feather pecking and cannibalism leading to improved animal welfare (Steenfeldt *et al.*, 2007; van Krimpen *et al.*, 2009; Hammershøj and Steenfeldt, 2005; Hartini and Choct, 2010). To understand the correlation between hen behaviour and the effect of fibre on gut function, the anatomy and function of hen digestive tract needs to be clear and is described in the text beneath.

The anatomy of the digestive tract of the laying hen

The anatomy of the digestive tract of the hen is illustrated in Figure 5. Feed taken up with the beak is moved further along the oesophagus, and can be stored in a pear-shaped extension of oesophagus, the crop. Salivary amylase is present in the crop and the action of this enzyme on starch continues here, as well as some microbial activity fermenting the feed during storage. The oesophagus leads to the glandular stomach, proventriculus, in which hydrochloric acid and pepsinogen are produced, reducing pH and starting protein degradation (Mc Donald, et al., 2002).

Oesophageal contractions results in the feed moving forward in the system to the gizzard, a muscular organ grinding the feed by rhythmic contractions together with moisture into a smooth paste. When ground sufficiently, the digesta particles pass into the small intestine, from where a reflux of digesta can occur, back into the gizzard (Mc Donald, et al., 2002). Digesta passing through the gizzard has a consistent particle size, regardless of original feed structure, with the largest particles being smaller than 40 µm (Hetland *et al.*, 2005).

In the first part of the small intestine, the duodenum, pancreatic juice and bile are excreted into the lumen, enabling digestion of proteins, fats and carbohydrates. Where the small intestine joins the large intestine, there are two long blind sacs present, the caeca. The caecas function as absorptive organs, and some fermentation of digesta also occurs here (Mc Donald, et al., 2002). However, in poultry held under domesticated conditions, the caecas don't play an essential role (McNab, 1973).

The digesta is moving further by peristaltic contractions into the relatively short colon, which functions mainly as a transport route for digesta to the cloaca. From the cloaca, both faeces and urine are excreted together (McDonald et al., 2002).

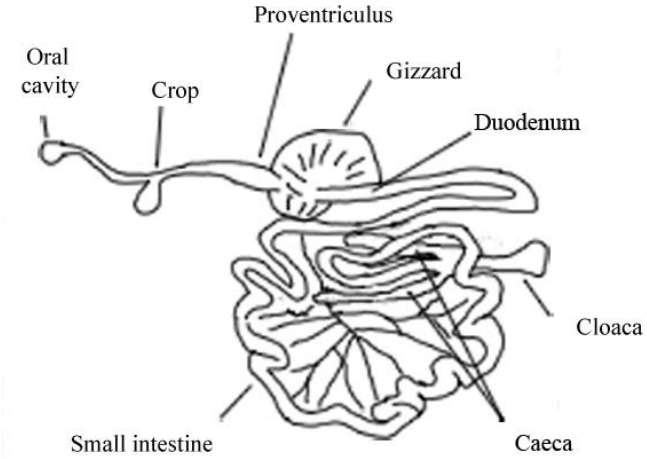

Figure 5. Anatomy of the digestive tract of the laying hen. Modified after Rose (1997).

Fibre

Soluble and insoluble fibre

Fibre includes plant carbohydrates and lignin that cannot be digested by the birds' endogenous enzymes, but can be degraded by microbe enzymes in the gut. Sources of fibre include grass and the bran fraction of grains. Fibre is often categorized as soluble and insoluble fibre, depending on its ability to dissolve in an aqueous solution, including digesta. Soluble fibre has shown negative effects on feed utilization in poultry as well as increased moisture level of the litter with reduced hygiene as a result. On the other hand, insoluble fibre (included in the present study) has shown several positive effects on hen metabolism and welfare as further described below (Kalmendal, 2012).

Effects of a fibre enriched diet on the digestive tract of the hen

Consumption of insoluble fibre has several effects on the digestive tract of the hen, affecting digesta mean retention time and gizzard development (van Krimpen *et al.*, 2009; Hetland and Svihus, 2007; Steenfeldt *et al.*, 2007). Mean retention time in the foregut decreases with increased intake of insoluble fibre, which is in turn associated with a higher satiety level. In contrary, digesta mean retention time in the gizzard increases (van Krimpen *et al.*, 2009) through accumulation of fibre in this part of the gut (Hetland *et* al., 2005; Hetland and Svihus, 2007). The increased fibre proportion of gizzard content compared to the fibre proportion of the feed, illustrates that fibre is harder to grind than other nutrients and are thus accumulated in the gizzard undergoing grinding for a longer time (Hetland *et* al., 2005). The stimulatory effect of fibre on the muscular contractions of the gizzard leads to development and enlargement of this grinding organ (van Krimpen *et al.*, 2009; Steenfeldt *et al.*, 2007), improving its grinding capacity (Hetland and Svihus, 2007).

A proper developed gizzard acts as a pacemaker for the entire digestive tract of the laying hen. The gizzard is the driving force for the peristaltic movements of the gastrointestinal tract of poultry. Gizzard contractions acts to coordinate the movement of digesta in the gut and to optimize digestion and absorption (Hartini and Choct, 2010). A well-functioning gizzard should be large and muscular and able to retain digesta within it. In turn, the result will be better regulation of digestive processes leading to enhanced digestibility of nutrients (Hetland and Svihus, 2007). However, if the gizzard is empty, there will be no feed stimuli – a condition in which the gizzard is unable to regulate downstream digestive processes. An empty gizzard is thereby more of a passage organ than a grinding organ, whereas a proper developed gizzard will improve the overall digestive potential of the gastrointestinal tract (Hartini and Choct, 2010).

An improved digestibility of nutrients as a result of fibre enrichment was confirmed by Hetland and Svihus (2007), showing a similar feed utilization in control birds as in birds consuming fibre enrichment in the form of wood shavings. Thereby, the extra grinding cost of wood shavings in the gizzard and the handling cost through the gut were completely compensated for through utilization of nutrients digested in the gut in these hens. Fibre appears to be a nutrient of which poultry can feel a need, and birds tend to compensate for a diet low in fibre if possible, eg. by increase the consumption of litter material. However, litter consumption has a high individual variation (Hetland *et al*. (2005).

Behavioural effects of fibre enrichment

Feather pecking, which in turn may develop to cannibalism, is a welfare problem but also of great concern for egg producers because of significant economic losses when it occurs (Hartini and Choct, 2010). Also when not leading to cannibalism, poor feathered hens with a decreased insulating capacity result in a higher energy demand in order to keep the optimal body temperature,

thereby increased feed intake with reduced economic benefit as a result (Tauson and Svensson, 1980).

It has been suggested that absence of exploratory material in the environment may result in pen mates being considered as exploratory stimuli, leading to exploratory behaviour and feather pecking towards conspecifics (van Krimpen *et al.*, 2009). Thereby, feather pecking pressure in a flock may be diminished if the environment is enriched. Dietary fibre can be considered as environmental enrichment because of its energy diluting effect on the feed, compensated for by the hen by increased voluntary feed intake which in turn generally leads to increased duration of feeding behaviour. It has been hypothesized that feather pecking behaviour is a substitute for normal ground pecking or foraging behaviour when foraging material is absent (van Krimpen *et al.*, 2009). Nutritional factors leading to increased duration of feeding behaviour may therefore have a positive effect on feather pecking behaviour in laying hens (van Krimpen *et al.*, 2009; Hammershøj and Steenfeldt, 2005). It has been suggested that pullets getting energy diluted feed early in life and onwards, are more "imprinted" on the feed because of increased feed intake and probably increased number of pecks towards the feed, decreasing their interest in directing exploratory behaviour towards the feathers of their pen mates later on during the laying period (van Krimpen *et al.*, 2009).

Energy dilution can be obtained not only with dietary fibre but also with other substrates, which for research purposes has consisted of sand and bentonite, a special kind of clay. These studies have shown diverse results on plumage condition (van Krimpen *et al.*, 2009; Hartini and Choct, 2010). The absent effect on plumage condition when diluting the feed with bentonite, when at the same time improving feather condition in groups given insoluble fibre, led to the assumption that the effect of fibre isn't only because of its energy diluting effect. The authors concluded that this result reinforce the hypothesis that the property of fibre to reduce cannibalism in hens is due to its physiological effect on gut function, rather than a simple nutrient diluting effect on feed intake (Hartini and Choct, 2010).

The effects of fibre consumption on the digestive tract through stimulation of gizzard function may lead to hens feeling less frustrated – reducing aggressive behaviours and in that way decreasing the risk of cannibalism (Hartini and Choct, 2010). Steenfeldt *et al.* (2007) described the possibilities that a full gizzard may make hens more satiated, leading to a more calm appearance, in turn contributing to a lower feather pecking pressure. A higher satiety level in hens consuming a fibre enriched diet was found by van Krimpen *et al.* (2010) who suggested that the higher satiety level may have positive effects on lowering feather pecking pressure in the flock. Consumption of feathers has been shown to be decreased in hens consuming fibre enrichment, either through diet or litter material, compared to hens given a low fibre diet. This phenomenon together with an increase in gizzard content in these hens indicated that feather pecking behaviour may be driven by a need for gizzard stimulation (Hetland *et al.*, 2005).

MATERIALS AND METHODS

Trial 1: Egg quality

Hens and housing

This experiment was performed using two hybrids common in commercial egg production in Sweden; Lohmann Selected Leghorn (LSL) and Lohmann Brown (LB). All hens were reared in a traditional floor housing system by the breeder Gimranäs AB and had been vaccinated against infectious bronchitis, Marek's disease, coccidiosis and avian encephalomyelitis. A total of 2880 hens, half the number of each hybrid, were delivered at the age of 16 weeks to the Swedish

Livestock Research Centre at the Swedish University of Agricultural Sciences, outside Uppsala. Both hybrids were kept in furnished cages as well as in a traditional floor system, half the number of each hybrid in each housing system - 720 hens per hybrid in the caged and floor housed system respectively. The two housing systems were located in separate compartments of the same building. A lighting program was used in both housing system, automatically turning the light on and off according to a time scheme using artificial dusk and dawn. The light was on for nine hours at 16 weeks of age and was successively increased to 14 hours at 23 weeks of age. In addition a restricted amount of natural daylight was allowed into the stables through partly covered windows.

Furnished cages

The furnished cage design used was the Comfort cage (Victorsson AB, Frillesås, Sweden) with a perch in the middle of the cage in longitudinal direction, a nest in one end of the cage and a litter box on top of the nest (Figure 6). Eight hens were kept in each cage. The cage, nest and litter box excluded, measured 96 × 50 (width × depth) and the minimum height was 45 cm at the rear. The nest measured 24 × 50 cm (width × depth) and had a maximum height of 27 cm in the front. The cages fulfilled the Swedish Animal Welfare Directives of a minimum of 600 cm^2 cage floor area per hen, nest and litter box excluded (Swedish Board of Agriculture, 2010).

Litter boxes were equipped with a sparse grid of thin steel bars, automatically restricting the availability of the litter boxes according to a time schedule. The grid was raised from the bottom of the box, blocking the entrance to the litter box one hour before lights were turned off. Litter boxes were then closed during night and during the main egg laying hours in the beginning of the light period (7-8 hours), avoiding hens from laying eggs in the litter box. At time for opening, the grid was automatically lowered down into the litter material in the litter box again, allowing hens to enter. Between 16 and 19 weeks of age the litter boxes were opened for four hours and thereafter for five hours a day.

Feed was available ad lib. and was automatically distributed by feed chains three times a day. Water was available through water nipples. Manure was removed twice a week with belts, and litter material was added by hand twice a week according to experimental treatment, described below under the heading “Experimental design and experimental treatments”.

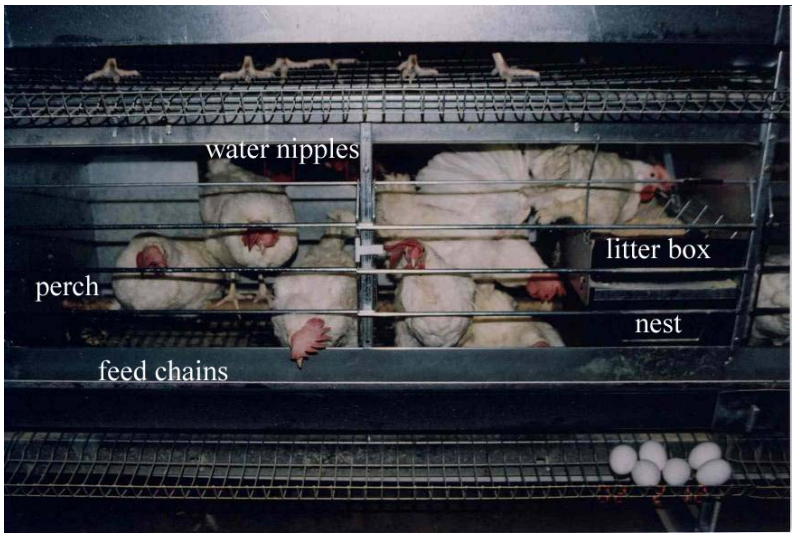

Figure 6. Furnished cage with perch, litter box and nest as defined in the picture. Chain feeders are present in the front of the cage and water nipples in the back. Cages were positioned in three horizontal tiers, the middle one with cages serving as the roof of the bottommost and the floor of the cages on top, as seen by the feet of hens in the uppermost part of the picture. Photo: Istvan Pamlényi

Floor housing system

The traditional floor housing system was composed of a litter area along the wall of the stable building and an elevated slatted floor area with collective nests facing the corridor along the middle of the stable (Figure 7). Along both long sides of the stable the floor system was divided into nine groups, located side by side, generating 18 replicates in total keeping 80 hens each. The litter area of each floor group measured 3.56 × 1.32 m (width × depth) and the slatted floor area measured 3.56 × 2.30 m (width × depth). Each nest, two per group, measured 1.150 × 0.46 m (width × depth). The stocking rate was 6.2 hens per m^2 floor area, nests excluded, which is below the maximal stocking rate allowed according to the Swedish Animal Welfare Directives (Swedish Board of Agriculture, 2010).

The nests were automatically closed during the night by a wall that was raised from the bottom of the nest blocking its entrance. Nests were opened one hour before the onset of light and closed one hour before the light was switched off to avoid defecation in the nest during the night with dirty eggs as a consequence. Feed was available ad lib. in pan feeders which were automatically filled every day. Water was available in water dispensers that were cleaned daily by hand. Manure was mechanically removed twice a week with alley scrapers underneath the elevated slatted floor.

Figure 7. Traditional floor housing system. Hens seen on the picture are standing on the elevated slatted floor area. Beyond that area next to the stable wall is the litter floor area (not seen in the picture). Drinkers are placed near the feed pans in the slatted floor area (hidden by the flock of hens in the picture). Photo: Frida Johansson

Experimental design and experimental treatments

From 16 to 18 weeks of age all hens were given the same commercial pre-lay diet, followed by calcium enriched pre-lay diet for two weeks. At the age of 20 weeks the experimental treatments were introduced. Two of the three experimental treatments included straw pellets as fibre source, either in the diet or in the litter material, and the third treatment served as control. Descriptions of the treatments are the following (summarized in Table 2) i) “Fibre Diet”: hens were given a fibre enriched diet, litter material was composed of sawdust for caged birds and wood shavings for floor housed birds, ii) “Fibre Litter”: hens were given a control diet with low fibre content, litter material was composed of crushed straw pellets for caged birds and wood shavings complemented with intact straw pellets for floor housed birds, iii) “Control”: hens were given the control diet with low fibre content, litter material was composed of sawdust for caged birds and wood shavings for floor housed birds.

Table 2. The three treatments with fibre enrichment in diet or litter material as well as a low fibre treatment as control. Diets were the same in both housing systems.

		Litter material	
Treatment	**Diet**	***Furnished cages***	***Floor housing***
Control	Control feed - low fibre content	Saw dust	Wood shavings
Fiber Diet	Feed enriched with straw pellets	Saw dust	Wood shavings
Fiber Litter	Control feed - low fibre content	Crushed straw pellets	Entire straw pellets and wood shavings

The straw pellets composing fibre enrichment in the litter box/litter area was the same material used as fibre source in the fibre-enriched diet; pellets made of 100 % wheat and rape straw without any additives. Calculated nutritional content of the control diet and the experimental diet are presented in Appendix 1, Table 7. The analysed nutritional content of straw pellets is presented in Appendix 1, Table 8.

Each treatment had six replicates in both housing systems, as illustrated in Figures 8 and 9. Three of the replicates included LSL and the other three included LB. Every replicate comprised 80 hens, represented by ten cages in the cage housing system and one group in the floor system respectively.

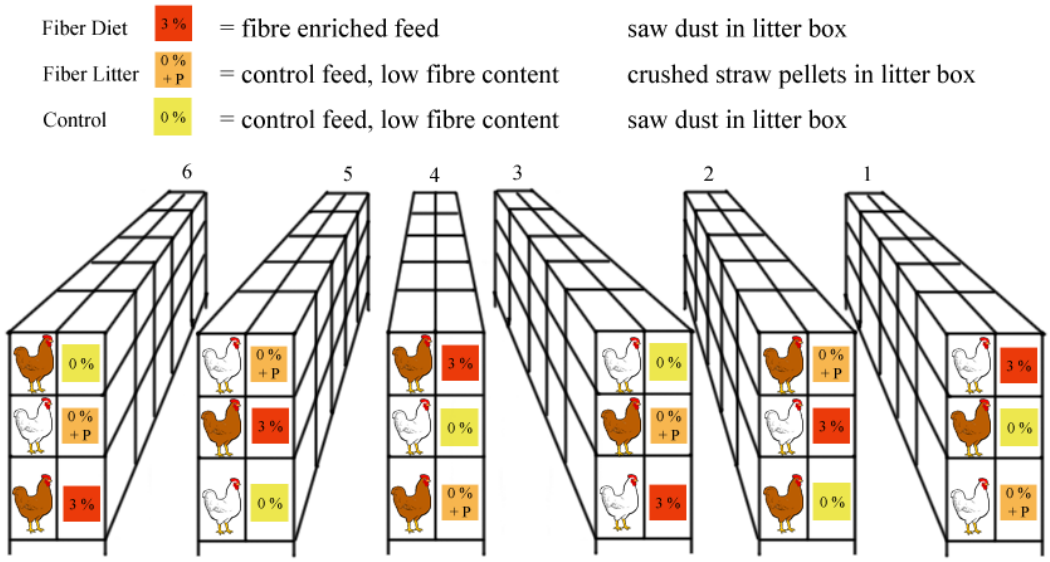

Figure 8. Design of experimental treatments and hybrids in the caged housing system. Each row with 5 cages (80 birds) include the same hybrid given the same experimental treatment, which is shown by the symbols in the front cage. The white and brown hens represent the hybrid, LSL and LB respectively, housed in each cage row (Modified after Robin Kalmendal, internal communication at the poultry section, SLU).

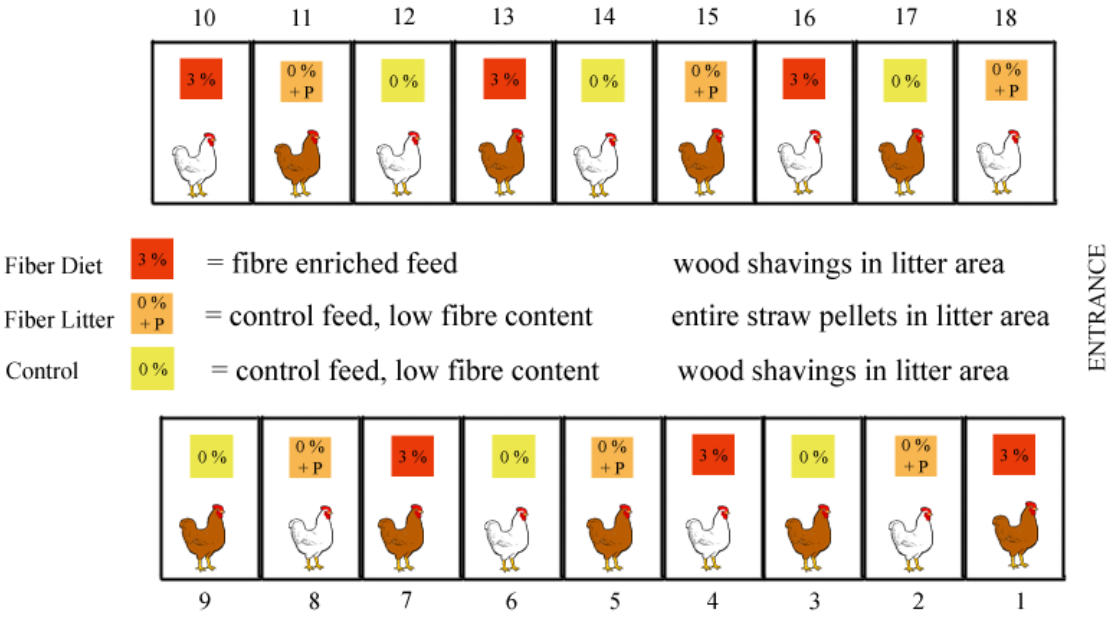

Figure 9. Design of experimental treatments and hybrids in the floor housing system. In each of the eighteen groups the symbols represent experimental treatment and hen hybrid; a white and brown hen representing LSL and LB respectively (Modified after Robin Kalmendal, internal communication at the poultry section, SLU)

For the “Fibre Litter” groups the procedure for distribution of litter material was the following; when introduced into the study at 20 weeks of age, the litter boxes in the furnished cages were emptied from the saw dust put there at arrival at 16 weeks. Instead 180 g of crushed straw pellets was introduced as the only litter material and thereafter approximately 60 grams was added into each litter box, irrespective of the amount that remained, three times a week throughout the experimental period. In the traditional floor system, the remaining wood shavings introduced to the litter area at 16 weeks were allowed to remain, while in addition 770 g of intact straw pellets was introduced and added three times a week throughout the experiment.

For the “Fibre Diet” and the “Control” groups in the furnished cages, the litter boxes were refilled with 60 grams of saw dust three times a week. In the traditional floor system, litter material was not supposed to be added to these experimental groups in addition to the wood shavings introduced at 16 weeks of age. However at the age of 28 weeks for LSL and 30 weeks for LB, the procedure of giving litter material three times a week was introduced also to the treatments with wood shavings as litter material. The decision was taken to provide more occupation for the birds, as a result of disturbances and in turn problems with cannibalism in the floor housing system.

Eggs were collected for egg quality analyses at the age of 31-32 weeks from both caged birds and floor housed birds and in addition at 51 weeks of age only from the caged birds. Due to technical disturbances in the experiment including the floor housed birds, no eggs were to be analysed at 51 weeks from that housing system.

Collection and handling of eggs

Criteria for sampling of eggs for egg quality measures were that they should be laid in the nest, be clean and of normal size (e.g. no doubled yolk eggs) without any visible defects such as abnormal shaped eggs or cracks on the shell. Sampling as well as analyses of eggs where conducted during

two weeks in the first period whereas only one week was needed at 51 weeks of age when only eggs from the caged birds were available. At each sampling period ten eggs were analysed from every replicate, resulting in 60 eggs analysed from each of the three experimental treatment groups in each housing system.

The collection of eggs took place in the morning between six and eight hours after the onset of light in the cage system and between one and three hours after the onset of light in the traditional floor system. All eggs were marked with numbers representing housing system, cage/group of birds and a sequence number (1-10) and were stored in an egg carton with the pointed end facing downwards. All eggs that were to be analysed the same week were collected the same day.

Directly after collection, all eggs were transported to the laboratory. Eggs that were planned to be analysed the same day were left in room temperature during the day whereas eggs planned to be analysed later during the week were stored in a fridge at 4° ± 3˚Celsius. At the day of analysing, eggs were taken out of the fridge at least 2 hours before starting of the analyses to obtain ambient temperature.

Each of the three sampling weeks, all eggs were analysed one at a time during a period of approximately five days. In order to minimize bias due to changes in egg quality parameters due to storage, a similar number of eggs were analysed from each replicate each day during the week.

Measuring of egg quality parameters

Measured quality parameters were egg weight (g), egg deformation (μm), egg shell strength (g), incidence of meat and blood spots (yes or no), yolk colour (1-15 points), height of thick albumen (mm), yolk weight (g), albumen weight (g), albumen pH, albumen DM content (%), egg shell thickness (0,01mm) and egg shell weight (g).

The lab routines started by weighing the eggs followed by measurement of egg deformation using the Canadian eggshell tester*. Egg deformation was measured in a non-destructive manner, defined as the degree to which the eggshell bends or deflects under an applied force load of one kg. Deformation was measured at three spots around the equator of the egg. An average of the three obtained values was used in the statistical analyses of deformation. With the same equipment eggshell strength was measured, defined as the weight needed to break the shell when applied as a compressive fraction force on the equator of the egg.

The eggs were then broken onto a flat glass surface with a mirror placed under the glass, enabling observation of both the upper and under side of the yolk and albumen. Each egg with visible blood or meat spot inclusions was recorded and yolk colour was defined using the Roche yolk scale grading from 1-15 points, where 1 is the palest yolk and 15 is a strongly coloured yolk. Height of thick albumen was measured approximately 0.5 cm from the edge of the yolk using a micrometre. The albumen was separated from the yolk and weighed into an aluminium vessel in which albumen pH was measured. At the first sampling period, half the number of eggs from each of the two housing systems was not measured for pH, as a consequence of a delayed delivery of the pH-meter to the lab. Yolk weight for eggs was calculated as the difference between total egg weight and the sum of shell weight and albumen weight.

To determine the dry matter (DM) content of the albumen, the albumen samples were dried for at least ten hours in 60° Celsius to lose some of the water content and be dry enough to not over boil when thereafter dried in 103° Celsius for 24 hours. The samples were then placed in desiccators and

* Otal precision Compay Limited, Ottawa Ontario, Canada K1G3N3

reweighed after equilibration to room temperature. Egg shell thickness, excluding the inner shell membrane, was measured at three spots around the equator of the egg shell using a micrometre. An average of these three measures was used in the statistical analyses. The egg shells were then rinsed under moderately warm water to get rid of residues of albumen and then dried at 103° Celsius for at least eleven hours. The shells were then left in room temperature for approximately 30 minutes and reweighed after equilibration to room temperature, to avoid temperature disturbances to affect the result of the balance. Haugh Units and weight percentage of shell were calculated in addition to the quality parameters measured at the laboratory. Haugh Units were calculated from the following formulae: $HU = 100 * \log (H - 1.7W^{0.37} + 7.57)$, where H=albumen height and W=egg weight (Silversides, 1994).

Statistical analysis

Data were analysed using the program SAS (Statistical Analysis System) 9.2. All quality parameters, except for the incidence of meat and blood spots, were considered normally distributed. A mean value of the ten eggs analysed from each replicate group was considered as an experimental unit. They were analysed using the procedure mixed (proc mixed), including the main effects of fibre treatment and hybrid and also age in caged birds as well as the two-way interactions between these factors. Among caged birds, where traits were measured at two bird ages, the analyses were corrected for repeated measurements. Significance was considered at $P \leq 0.05$ levels and a tendency was considered at $P \leq 0.10$ levels.

The incidence of meat and blood spots were considered to attain binary numbers, either number 0 (no meat/blood spots) or number 1 (meat/blood spots present). These two quality parameters were analysed using the procedure glimmix, estimating the likelihood that a particular egg included any internal meat or blood spots. However, data distribution precluded analysis of the data. Instead, these data are presented with descriptive statistics. Average number of eggs with inclusions is shown, calculated from three replicates with ten eggs each coming from the same hybrid, experimental treatment and housing system. Standard deviation was calculated for every three replicates as well.

Trial 2: Consumption of litter material

Hens and housing

The trial was performed using cage housed birds, including 480 LB and 480 LSL laying hens, a portion of the birds used in the study concerning egg quality described above. For the present trial the hens were kept in their ordinary furnished cages under the same conditions as described earlier, with the exception that litter boxes were closed for entrance during the trial. The trial started at the hen age of 45 weeks and lasted during seven days.

Experimental design and experimental treatments

The experimental treatments were the same as in the study concerning egg quality in caged housed birds; “Fibre Diet”, “Fibre Litter” and a “Control” group, as described earlier in the present report. However, for this trial, each cage (housing eight hens) was considered an experimental unit (vs. ten cages as an experimental unit in the egg quality study), resulting in 40 replicates for each of the three experimental treatments.

Measuring litter consumption

On day one litter boxes were emptied and refilled with 200 g of litter material; either saw dust or crushed straw pellets according to the treatment scheme described earlier for the egg quality study.

During the seven days of the experiment no dust bathing could be performed in the closed litter boxes whereas the hens were still able to pass through the head between the steel bars and into the litter box in order to consume material. At two occasions during the week, 60 g of litter material was added to each litter box to keep the same management routines that the hens were used to. After seven days, the litter material remaining in the boxes was weighed and the amount of litter material consumed during these seven days could be calculated.

Behavioural study

To make sure that the lost litter material really had been consumed and not only pushed out of the litter box by the hens, the manure area beneath the litter boxes was observed. A visual estimate of the amount lost in this way was performed during the week to see if there was a substantial loss to take into consideration. In addition, behavioural studies were done during the experimental week to investigate the behavioural pattern of the hens when interacting with the litter.

To record the occurrence of behaviour correlating to litter consumption, a frequency study was made. The behaviour recorded was the frequency of heads put into the litter box observed per cage during five minutes. The behaviour was recorded every time any hen moved her beak beneath the litter bath edge in direction towards the litter material (Figure 10). No consideration was taken to whether it was the same hen putting the head into the litter box several times or if the behaviour was performed by different individuals. In that way the same hen could be recorded many times if she put back the head into the litter box more than once. The time the head remained in the litter area was not taken into consideration, i.e. no matter if a hen kept her head in the litter area for a minute or for a second; it was still recorded as one peck.

Figure 10. A hen has put her beak in direction towards the litter material, beneath the level of the litter box edge – illustrated by the black line. The frequency was recorded every time any beak was passing this visualized line, in downward direction towards the litter. Photo: Robin Kalmendal

During the experimental week, each cage was observed twice. Two observers were recording two adjacent cages at a time. The observers were out of sight of each other, positioned on opposite sides of the cage battery. The two lower cages, on top of each other, were observed at a time. The top tier cage row was excluded because of its high position, complicating the possibility to observe these hens without considerable human disturbance. Cages were observed sequentially in numerical order by the observer, passing to the next two cages side by side to the ones just observed, throughout all six batteries with cages. Before starting recording, the observers were sitting in front of the two cages to be observed during one minute to habituate the hens to the observers' presence. Thereafter the frequency of the behaviour was recorded during five minutes. The same procedure was performed for every two cages.

Recording started at 9 o'clock in the morning and at midday all cages had been studied once, half the number by the same observer. At one o'clock pm, recording of all cages started again and continued until four pm when the light was turned off. The last 24 cages hadn't been observed at that time and were studied the next day starting at nine o'clock.
As a control, the procedure was repeated two weeks later, beginning at eight o'clock in the morning and at four pm all cages had been observed twice during the day. The control observations were done to ensure that the behaviour was performed also after reset to the ordinary opening and closing scheme of the litter boxes, as was the prevailing conditions for the present study of egg quality described earlier in the paper.

Statistical Analysis

Results from measuring of litter consumption and of the frequency of hens putting their heads into the litter boxes were analysed using SAS. The procedure used for analysing litter consumption was the general linear model (proc glm). The frequency of heads put into the litter box was analysed with the generalized linear model (proc genmod) as the data was assumed to follow a Poisson distribution.

RESULTS

Trial 1: Egg quality

With the exception for meat and blood spots, all results of the shell and internal egg quality measures are presented in Table 3 and 4, for the caged and floor housing systems respectively. Exact P-values are shown in the text following table 3 and 4, describing these results.

The average incidences of meat and blood spots in eggs for each housing system and hybrid are presented in Figs. 11, 12, 13 and 14. Standard deviations are shown as error bars in the figures. There was an apparent difference in the presence of meat and blood spots in eggs from the two different hybrids. Irrespective of treatment, LB laid a higher number of eggs with blood and meat spot inclusions compared to LSL.

The "Fibre Diet" group had the highest incidence of eggs with inclusions, whereas the incidence was lowest in the "Fibre Litter" group in LB in furnished cages. At the contrary, LB in the floor housing system had the highest incidence in the "Control" group. In LSL, the incidence was low irrespective of treatment and housing system.

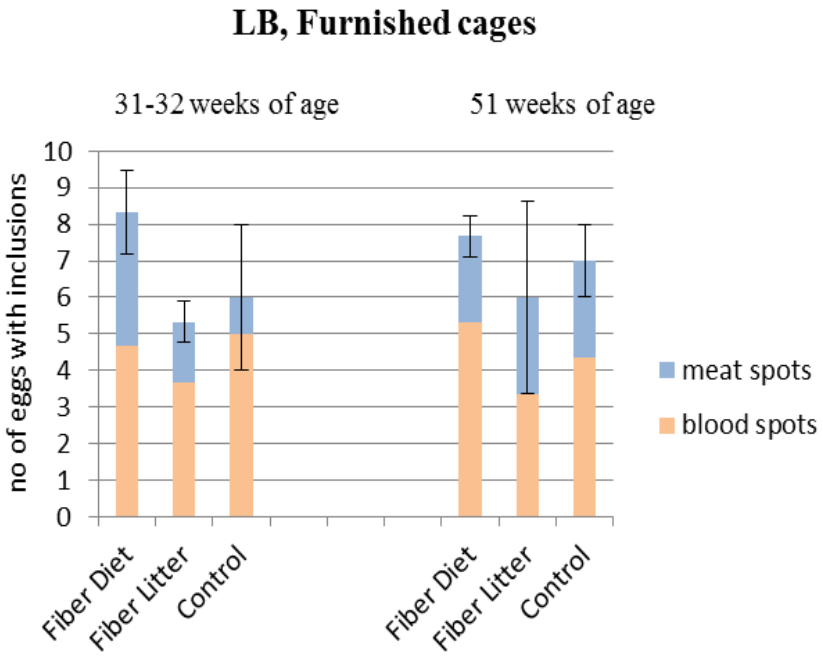

Figure 11. Incidence of blood and meat spots as influenced by fibre treatment and age in LB laying hens, housed in furnished cages. The incidence is demonstrated through the average number of eggs out of ten, in which blood or meat spots were detected for three replicates. The error bars represent the standard deviation for every three replicates – same experimental treatment, hybrid, housing system and age.

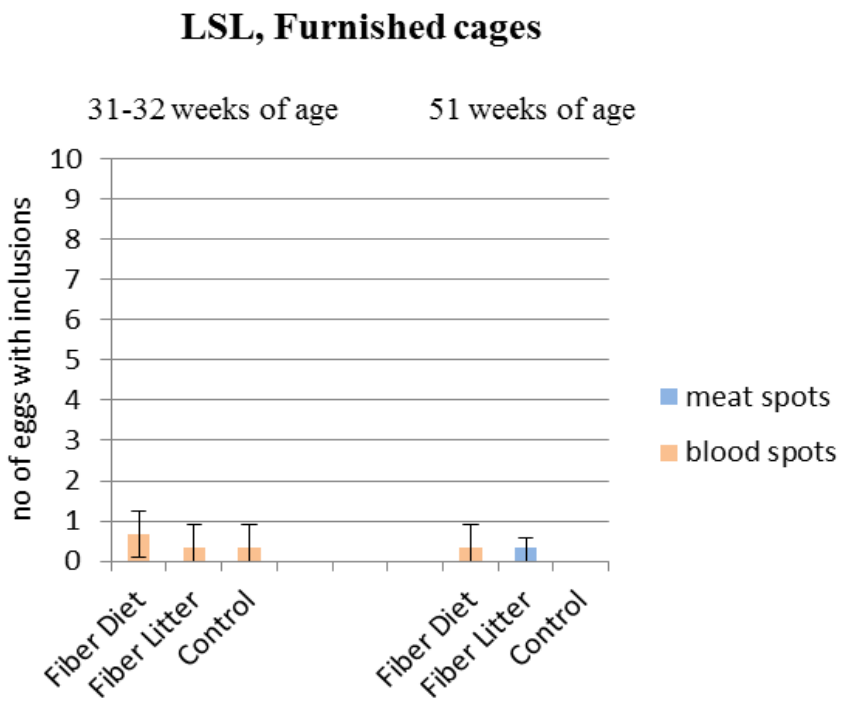

Figure 12. Incidence of blood and meat spots as influenced by fibre treatment and age in LSL laying hens, housed in furnished cages. The incidence is demonstrated through the average number of eggs out of ten, in which blood or meat spots were detected for three replicates. The error bars represent the standard deviation for every three replicates – same experimental treatment, hybrid, housing system and age.

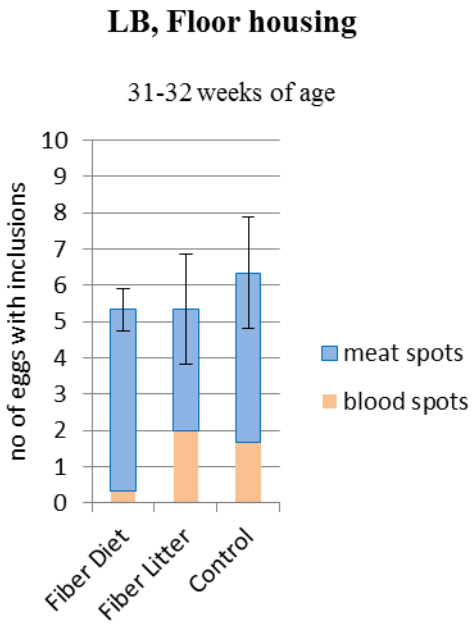

Figure 13. Incidence of blood and meat spots as influenced by fibre treatment in LB laying hens, housed in a traditional floor housing system. The incidence is demonstrated through the average number of eggs out of ten, in which blood or meat spots were detected for three replicates. The error bars represent the standard deviation for every three replicates – same experimental treatment, hybrid and housing system.

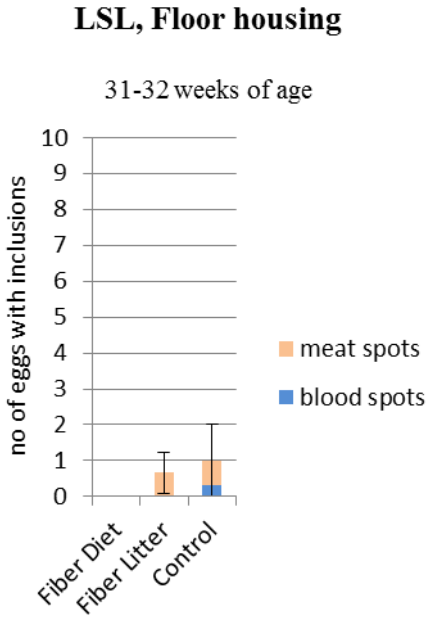

Figure 14. Incidence of blood and meat spots as influenced by fibre treatment in LSL laying hens, housed in a traditional floor housing system. The incidence is demonstrated through the average number of eggs out of ten, in which blood or meat spots were detected for three replicates. The error bars represent the standard deviation for every three replicates – same experimental treatment, hybrid and housing system.

Table 3. Shell quality and internal egg quality as influenced by fibre treatment, hybrid and age in laying hens housed in furnished cages. Values are expressed as Least-squares-means.

Trait	Treatment			Hybrid[2]		Age		Statistical significance			Interactions			SEM[3]
	Fibre Diet n[1]=12	Fibre Litter n=12	Control n=12	LSL n=18	LB n=18	31-32 w n=18	51 w n=18	Treatment	Hybrid	Age	Hybrid× age	Hybrid× treatment	Treatment× age	
Shell strength, kg	4.29	4.34	4.47	4.29	4.45	4.74	4.00	†	*	***	†	†	*	0.03
Deformation, µm	78.8	77.4	76.3	77.3	77.7	78.1	76.9	NS[4]	NS	NS	NS	NS	NS	0.62
Shell thickness, 0,01mm	32.8	33.3	33.4	32.8	33.5	34.3	32.0	NS	*	***	NS	*	NS	0.13
Shell weight%	9.35	9.45	9.52	9.4	9.5	9.8	9.1	†	NS	***	NS	†	NS	0.03
Yolk colour[5]	6.7	6.7	6.7	6.7	6.8	7.2	6.3	NS	NS	***	*	NS	NS	0.03
Alb.[6] pH	8.5	8.5	8.5	8.5	8.5	8.5	8.4	NS	NS	**	NS	NS	NS	0.01
Alb. DM, %	11.3	11.3	11.4	11.3	11.5	11.7	11.0	NS	†	***	†	NS	NS	0.05
Alb. height, mm	6.8	7.0	6.8	7.2	6.5	7.3	6.4	NS	***	***	*	NS	NS	0.06
Haugh Unit	81.1	81.9	80.8	84.0	78.6	84.9	77.7	NS	***	***	NS	NS	NS	0.39
Egg weight, g	62.1	62.0	61.7	61.2	62.6	59.8	64.0	NS	*	***	NS	NS	NS	0.31
Alb. weight%	58.3	58.5	58.2	57.0	59.6	58.5	58.1	NS	***	NS	**	NS	NS	0.15
Yolk weight%	32.3	32.1	32.3	33.5	30.9	31.7	32.8	NS	***	***	**	NS	NS	0.15

[1] n = number of replicative values included in the statistical analyses
[2] LSL = Lohmann Selected Leghorn; LB= Lohmann Brown
[3] SEM = standard error of the mean
[4] NS = P > 0.10
[5] Scores from 1 to 15, in which lower scores indicate a more pale yolk
[6] Alb. = albumen
† P ≤ 0.10; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

Table 4. Shell quality and internal egg quality as influenced by fibre treatment and hybrid in laying hens in the traditional floor housing system at 31-32 weeks of age. Values are expressed as Least-squares-means.

	Treatment			Hybrid[2]		Statistical significance			SEM[3]
Trait	Fibre diet n[1]=6	Fibre litter n=6	Control n=6	LSL n=9	LB n=9	Treatment	Hybrid	Hybrid×treatment	
Shell strenghth, kg	5.05	5.06	4.99	4.98	5.09	NS[4]	NS	NS	0.04
Deformation, µm	78.5	79.0	77.0	77.4	78.9	NS	NS	NS	0.68
Shell thickness, 0,01mm	35.4	35.9	35.6	35.5	35.7	NS	NS	NS	0.17
Shell weight%	10.0	10.0	10.0	10.1	9.9	NS	NS	NS	0.04
Yolk colour[5]	7.2	7.3	7.3	7.2	7.4	NS	*	NS	0.04
Alb.[6] pH	8.5	8.3	8.5	8.5	8.4	NS	NS	NS	0.06
Alb. DM, %	12.0	11.9	12.0	12.0	11.9	NS	NS	NS	0.04
Alb. Height, mm	7.6	7.9	7.8	7.9	7.6	NS	†	NS	0.09
Haugh Unit	86.4	87.6	87.6	88.4	86.0	NS	*	NS	0.48
Egg weight, g	62.3	62.9	61.8	61.6	63.0	NS	†	NS	0.38
Alb. weight%	60.2	60.9	59.9	59.2	61.4	NS	***	NS	0.23
Yolk weight%	29.8	29.1	30.1	30.7	28.7	NS	***	NS	0.23

[1] n = number of replicative values included in the statistical analyses
[2] LSL = Lohmann Selected Leghorn; LB= Lohmann Brown
[3] SEM = standard error of the mean
[4] NS = P > 0.10
[5] Scores from 1 to 15, in which lower scores indicate a more pale yolk
[6] Alb. = albumen
† P ≤ 0.10; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

Egg weight

Egg weight tended to be higher in LB compared to LSL in the floor housing system ($p \leq 0.09$) and this difference was found to be significant among the caged birds ($p \leq 0.05$). As hens grew older, egg weight was increased ($p \leq 0.0001$; only measured among caged birds).

Shell quality parameters

Floor housing system

None of the shell quality parameters were effected by either hybrid or fibre treatment in the traditional floor system (only one hen age represented) (Table 4).

Cage housing system

In contrast to the floor housing system, some differences in shell quality parameters were found among the caged housed hens as described below (Table 3).

Eggs from the "Fibre Diet" group tended to have less shell weight percentage compared to the "Control" and "Fibre Litter" groups ($p \leq 0.07$) and these shells tended to be weaker compared to the "Control" group ($p \leq 0.08$).

At the younger age, an interaction showed that eggs from the "Fibre Litter" treatment had significantly weaker shells compared to the "Control" ($p \leq 0.04$), whereas at the older age there was no difference in this respect. However, eggs from birds in the "Fibre Diet" group were not different in shell strength from any of the treatment groups at either age.

In LB but not in LSL, fibre treatment had a significant effect and tendencies on shell quality, shown by treatment×hybrid interactions. LB-hens in the "Fibre Diet" group gave significantly thinner egg shells ($p \leq 0.03$) compared to the other treatment groups. Furthermore, for LB in the "Fibre Diet" group, shell weight percentage tended to be lower ($p \leq 0.06$) compared to the other treatment groups as well as shell strength that tended to be lower ($p \leq 0.09$) compared to the "Control". In contrary, among LSL-birds there was no difference neither in shell thickness, shell weight percentage or shell strength between any of the treatment groups.

Irrespective of treatment, LB laid eggs with thicker shells ($p \leq 0.02$) and higher shell strength ($p \leq 0.02$) compared to LSL. As the hens grew older, shell weight percentage decreased ($p \leq 0.0001$), shells got thinner ($p \leq 0.0001$) and shell strength was decreased ($p \leq 0.0001$). Age tended to affect shell strength more in LSL compared to LB ($p \leq 0.06$), so that at the hen age of 51 weeks, eggs laid by LSL had weaker shells compared to eggs from LB whereas at the younger age shell strength was the same in both hybrids.

Internal quality parameters

No significant or tendencies for effects of fibre treatment on internal egg quality were found in either of the two housing systems (Tables 3 and 4). On the other hand, hybrid and/or age affected all internal quality parameters in one or both housing systems. Effects of age were possible to analyse only in the caged housing system, where a repeated sampling was conducted.

In the caged housing system, at the hen age of 30-31 weeks eggs had a stronger yolk colour ($p \leq 0.0001$) and lower yolk weight percentage ($p \leq 0.0007$) than eggs laid at 51 weeks of age. However albumen weight percentage didn't differ between ages. Furthermore, at the younger age albumen had higher pH ($p \leq 0.003$), higher DM content ($p \leq 0.0001$), higher height ($p \leq 0.0001$) and

higher Haugh Unit score ($p \leq 0.0001$) compared to eggs laid at 51 weeks of hen age in the furnished cages where two hen ages were studied.

Comparing hybrids, yolk colour was the same in both hybrids in the furnished cages where eggs were sampled at two ages. At the contrary, eggs laid by LSL had a paler yolk colour compared to LB in the floor housing system ($p \leq 0.03$), where eggs were sampled only at the younger age. At the same time, an age×hybrid interaction showed that yolk colour was paler in eggs from LSL compared to LB in younger age ($p \leq 0.01$), whereas there was no difference in yolk colour between hybrids at the older age in the furnished cages.

Eggs from LSL tended to have lower albumen DM content compared to eggs from LB in the furnished cages ($p \leq 0.06$). A tendency of hybrid×age interaction ($p \leq 0.06$) indicated that this difference was present at the hen age of 51 weeks, but not at the age of 31-32 weeks. In the floor housing system eggs were sampled only at 31-32 weeks and there was no difference in albumen DM content between hybrids.

Furthermore, eggs from LSL had a higher albumen height in the furnished cages ($p \leq 0.0001$) and a tendency in the floor housing system ($p \leq 0.08$), higher value of Haugh Units ($p \leq 0.0001$ – cages; $p \leq 0.02$ – floor housing), lower albumen weight percentage ($p \leq 0.0001$ – cages; $p \leq 0.0006$ - floor housing) and higher yolk weight percentage in relation to total egg weight ($p \leq 0.0001$ – cages; $p \leq 0.0008$ – floor housing) compared to LB.

Another hybrid×age interaction showed that in LSL, albumen weight percentage was higher ($p \leq 0.009$) and yolk weight percentage lower ($p \leq 0.006$) at 31-32 weeks of age compared to 51 weeks, whereas in LB there was no difference between ages on these two parameters. This interaction was found in the caged housing system where eggs were analysed at two hen ages.

Trial 2: Consumption of litter material

Loss of litter material

There was an apparent difference between individual cages in remaining amount of litter material at the end of the experimental week. In some of the cages all litter was still there after seven days, whereas there was only a few grams left in others.

The amount of litter material lost over the edge of the litter box and down to the manure area beneath the cage wire floor could be visually estimated to a few grams during the week and about the same for all cages, and considered to be negligible. Thereby the amount of litter material depleted during the week could be regarded as the amount consumed during the week.

As shown in Table 5, the consumption of litter material differed significantly between fibre treatments as well as between hybrids. Also a hybrid-treatment interaction was found. For LB the consumption of straw pellets as litter material (“Fibre Litter”) was considerably higher than for saw dust, independently of feed composition – “Control” or inclusion of 3 % fibre (Fibre Diet). Straw pellet consumption was in average 4.1 g per hen and day and saw dust consumption was 2.8 and 2.9 g/hen and day for the control and the group with fibre enriched diet respectively. In contrast, for LSL there was no significant difference in litter consumption between treatments; 1.4 g/hen and day for the control and 1.2 g/hen and day for each of the fibre treatments.

Irrespective of fibre treatment, LB-hens consumed significantly higher amounts of litter material compared to LSL. The consumption of saw dust was approximately twice the amount in LB

compared to LSL, and the consumption of straw pellets was even three and a half times as much in LB.

Table 5. Mean litter consumption and number of pecks in litter boxes for LSL and LB, housed in furnished cages, as influenced by fibre treatment. Within column and hybrid, values with different superscripts are significantly different ($p \leq 0.05$).

Treatment	**Litter consumption** (g/hen and day) n[1]=20	**N:o of litter pecks in closed litter box** (5+5 min.) n=20	**N:o of litter pecks in open litter box** (5+5 min.) n=20
LSL[2]:			
Fibre Diet	1.2	5.5[a]	14.3[a]
Fibre Litter	1.2	2.2[c]	3.8[b]
Control	1.4	7.8[b]	15.8[a]
LB[2]:			
Fibre Diet	2.9[b]	13.8[b]	20.9[b]
Fibre Litter	4.1[a]	13.6[b]	22.3[b]
Control	2.8[b]	8.4[a]	14.6[a]
SEM[3]	0.10	0.04	0.03
p-values			
Treatment	0.0207	<0.0001	<0.0001
Hybrid	<0.0001	<0.0001	<0.0001
Interaction	0.0043	<0.0001	<0.0001

[1] n = number of replicates per treatment and hybrid
[2] LSL = Lohmann Selected Leghorn; LB= Lohmann Brown
[3] SEM = standard error of the mean

Behavioural study

Hens were found to interact with the litter material both during the week when the entrance to the litter boxes was closed as well as two weeks after reset according to the ordinary opening and closing schedule (Table 5). The behavioural repertoire included pecking in the litter, pushing litter aside with the beak and taking up litter into the oral cavity seen as litter material dropping out on the sides of the beak. Some birds put their beak into the litter material only for a few seconds, picking in it once and then raised the head only to put it back a few seconds later. This behavioural pattern could be repeated several times. Other birds put their beak into the litter material keeping interacting with it for a minute or more, without raising the head above the level of the litter box edge.

The frequency of pecks in the litter box differed significantly between hybrids and between fibre treatments, which partly corresponds to the result of litter consumption (Table 5). The results also show that LB had a higher frequency of pecks into the fibre enriched litter compared to the control, whereas for LSL the result was the opposite.

DISCUSSION

Most effects in the caged housed system

There were several significant effects and a few tendencies to effects of treatment, hybrid, age, or interactions among these found on egg quality in caged birds, whereas there were less effects found among birds in the floor housed system. In general, the repeated measurements by age in the cages may have contributed to reveal more effects in that housing system. Still, if there would have been differences at the younger age, they should have been revealed also in the floor housing system. More likely, the behavioural pattern of consuming litter material may have differed in the two differently designed housing systems, resulting in different total fibre intake in each treatment group depending on housing system. Perhaps total fibre intake differed less between treatment groups in the floor housing system; both feed and litter material taken into account. To sort this out, litter consumption in the floor housing system would have been interesting to measure as well, if possible.

Decision of time for first egg sampling

The first egg sampling procedure took place ten weeks after introducing the hens to their experimental treatments. At that time the hen body was assumed to have adapted to the experimental treatments so that any effect on metabolism leading to effects on egg quality in early age could be detected. An adaptation period of ten weeks before sampling seems to be enough to be able to recognize changes in egg quality due to fibre intake, according to results found by Mohebbifar *et al.* (2011). They found effects of fibre intake on Haugh Unit, shell weight and shell thickness already after a treatment period of seven weeks but concluded that a treatment period of only three weeks was too short in this respect. In the present study there were no effects found of fibre treatment in the floor housed system, where only one sampling procedure after ten weeks was performed. On the other hand, in the cage housed system, where eggs were collected both ten and 30 weeks after introducing the experimental treatments, there was a significant interaction between treatment and age on shell strength, revealing effects of fibre intake already after a treatment period of ten weeks. However, at the later sampling, this effect was no longer seen. The fact that a significant effect on egg quality was found ten weeks after introduction of the treatments verifies that an adaption period of ten weeks was enough to detect the effect of experimental treatment on egg quality.

Litter intake

The behavioural study, conducted with the litter box entrance both opened and closed, proved that hens interacted with litter material. During interaction with the litter, small traces of litter material may have been lost down on the manure area beneath the litter box. However, no considerable amounts of lost litter material were found during the experimental week when the entrance to the litter box was closed. That is a strong indicator for hen consumption of litter material when at the same time litter material was lost in quite substantial amounts in several cages during this period.

The calculated consumption of litter material was 1.2-1.4 grams/hen and day for LSL and 2.8-4.1 grams/hen and day for LB (Table 5). The highest consumption of 4.1 grams was recorded in the “Fibre Litter” group. That consumption is in accordance with the litter consumption found by Hetland and Svihus (2007), whereas the litter consumption in the other treatment groups was less in the present study. Hetland and Svihus (2007) reported a consumption of 4 g/hen and day of wood shavings in the hybrid Lohmann Selected Leghorn until the age of 35 weeks. The higher litter consumption in the “Fibre Litter” group may be correlated to litter structure. The crushed straw pellets used in the “Fibre Litter” group were comprised of course particles, to compare with saw dust in the other treatment groups that appears in a powder form. Generally poultry prefer feed of

course particles compared with mash (Portella *et al.*, 1988) and feeding of pelleted feed compared to mash has been found to increase feed intake (Nir *et al.*, 1990).

Fibre consumption followed the frequency analyse of hens pecking in the litter box to some extent (Table 5). The two parameters didn't match perfectly, but the frequency of hens pecking in litter was rather observed as a complement, verifying that the hens were interacting with the litter material.

Effect of fibre enrichment on external egg quality

The fibre enriched treatments had minor effects on egg quality, only showing some weak responses on shell quality. Shell quality tended to be impaired with the fibre enriched diet in the furnished cages. With the fibre enriched diet shell weight percentage tended to be reduced, in turn probably explaining the additional tendency to lower breaking strength in this group. However, the mean value of shell strength among birds given a fibre enriched diet (4,29 kg, 42[1] Newton (N)) was still above the value stated in the performance data presented by the hen breeder Lohmann Tierzucht for Lohmann LB-Classic (>35 N) and for Lohmann LSL-Classic (>40 N) (Lohmann Tierzuch(a), Lohmann Tierzuch(b)). Thereby the inclusion of 3 % straw pellets didn't impair shell quality below the breeder specifications.

In LB but not in LSL, hybrid interactions with fibre treatment were found. Eggs from LB in the "Fibre Diet" group had significantly thinner shells which tended to compose less weight percentage of the egg compared to the other treatment groups, as well as tended to be weaker compared to the "Control". The fact that these three shell quality parameters were affected in the same direction is logic, since thinner shell and less shell material give less resistance when putting force on the shell, even though shell strength also to some extent is influenced by the ultrastructure of the shell and how well the shell is constructed (Ahmadi and Rahimi, 2011). However, shell thickness and the amount of shell are well known to effect shell strength (Roberts, 2004).

The impaired shell quality in the "Fibre Diet"-group in LB but not in LSL, can most likely be correlated to various intake of litter material, differing widely between these two hybrids (Table 5). LB hens were more prone to consume litter material, ingesting more than double the amount of litter material compared to LSL irrespective of experimental treatment and litter composition. LB hens in the "Fibre Diet"-group still consumed approximately the same amount of litter as LB in the "Control"-group, which means a higher total fibre intake in the "Fibre Diet" group – both feed and litter source summed together. Most likely, the higher total fibre intake in the "Fibre Diet" group explains the impaired shell quality in this group in LB.

Declined egg shell quality as a result of fibre intake of substantial amounts, is in agreement with Mohebbifar *et al.* (2011). They found a decrease in shell weight and shell thickness in hens given insoluble fibre enriched feed compared to a low fibre control. The study was performed on Lohmann LSL-Lite hens at 88 weeks of age. In comparison, the present study comprise quite a low fibre inclusion; 3 % of fibre pellets resulting in 3.47 % crude fibre in the feed (appendix 2), compared to 17.4 % of olive pulp contributing to 8.4 %[2] crude fibre of that diet (total crude fibre content of the feed not mentioned). This comparison suggests that fibre inclusion in the diet may reduce shell quality dependent on the amount added. Mohebbifar *et al.* (2011) concluded that when giving olive pulp to hens, the inclusion has to be lower than 17 % of diet to not affect shell weight and shell thickness. For the straw pellets used in the present study, the level of 3 % in feed didn't give any significant effects on shell strength, deformation, thickness or weight percentage, when comparing all hens irrespective of hybrid. However, the results show that also fibre intake from

1 Calculated as 4,29 × 9.81, shell strength in kg × the acceleration of gravity.

2 Experimental diet contains 17,5 % Olive pulp. The olive pulp is to 48,2 % made up by crude fibre (Mohebbifar *et al.*, 2011).

litter material should be considered concerning total fibre intake and its effect on shell quality.

Furthermore, a treatment×age interaction showed reduced shell strength in the “Fibre Litter” group compared to the “Control” at the younger hen age. This interaction is likely to indicate a difference in litter intake at the two ages. As described by Jensen (2006), fowl are still curious by nature, even though domestication has resulted in reduced exploratory behaviour and a reduced tendency to explore unknown food sources compared to its ancestor the red jungle fowl. However, the remaining instinct to explore new things in the environment leads to a hypothesis about various intake of litter material at the two ages. According to the impaired shell quality related to the “Fibre Litter” group, it is likely that the consumption of fibre enriched litter material may have been higher during the first weeks after being introduced as a new material to the young hens, compared to later on when the hens had adapted to its composition and satisfied their curiosity about it. It would have been interesting to measure litter consumption both at the younger and the older hen age, to verify various litter intake in correlation to the difference in effect of litter material on shell quality at different ages.

Following the discussion above, the fact that shell strength was reduced in the “Fibre Litter” but not the “Fibre Diet” group compared to the “Control”, would suggest the highest total fibre intake in the “Fibre Litter” group. Thereby, these hens should have consumed litter material resulting in a fibre intake higher than the amounts coming from the feed and litter material summed together for the “Fibre Diet” group. Furthermore, this suggestion leads to the hypothesis that fibre intake from litter material can constitute the main fibre source for hens, depending of its composition and structure.

The measuring of litter consumption (Trial 2) that was carried out at the hen age of 45 weeks, demonstrated that for LB, the consumption of litter material was highest in the group given fibre enriched litter material. Their litter consumption was more than 30 % higher than LB-hens given the other treatments, resulting in increased total fibre intake. Despite that high litter consumption in the “Fiber Litter” group during that experimental week, the average daily feed intake during the total experimental period (40 weeks) didn’t differ between treatment groups (Kalmendal, 2010). Thereby, differences in egg quality between treatments are not an effect of differences in mineral intake due to differences in feed intake.

It could be important for the interpretation of the results to consider that litter consumption may differ widely between individuals, as shown by Hetland *et al.* (2005). In the present study, one egg was collected from each furnished cage housing eight hens. These eight hens might have consumed very different amounts of litter material. Thereby, the litter consumption in the hen from which the egg derives may be essential for the result of the egg analyse. From this study, that individual information was unknown. However, the values used in the statistical analyses originate from the average value of ten eggs from each of 60 replicates, which is a strengthening factor considering the accuracy of the results of the analyses.

Effect of fibre enrichment on shell composition

Egg shell is to 98 % built up by calcium carbonate (Rose, 1997), suggesting that reduced shell thickness or reduced shell weight percentage may be a result of less calcium deposited in the egg shell. That statement is supported by the study by Taylor (1965) who suggested that the increase in egg shell thickness in pullets fed a low-phosphorous diet resulted from increased absorption and retention of dietary calcium. Weiss and Scott (1978) found diet to affect shell quality negatively when including 50 % of wheat bran in the diet. Egg shell strength was decreased for this experimental group and the explanation was suggested to be the inclusion of phytate in wheat bran, inhibiting the absorption of dietary calcium. Wheat bran has also been found to reduce serum calcium level in chicks fed diet comprising 8 % wheat bran. However oat bran didn't give any effect

on Ca levels in serum in this study and corn bran reduced Ca levels only slightly. Both the oat bran and corn bran diets included lower amounts of phytate compared to the wheat bran diet (Van der Aar *et al.,* 1983). Normally when possible, poultry feeds are enriched with phytase, the enzyme needed to dissolve phytate and make the minerals bound available for absorption (Svenska Foder, 2013-05-12). In the present study, not only total amount of phosphorous but also the amount available to the hens had been calculated to fit the need of the hens.

Also in humans, fibre has been shown to interfere with Ca absorption in the gut. Mc Hale *et al.* (1979) reported significant differences in urinary Ca levels in humans consuming cellulose or hemicellulose supplements to a basal diet. These authors concluded that addition of hemicellulose and cellulose will inhibit Ca absorption in humans. The same article refers also to Reinhold *et al.* (1976) who found a negative balance of calcium, magnesium, zink and phosphorous when consuming a high fibre diet. The conclusion was that metals bound by dietary fibres remain unavailable for absorption. Camire and Clydesdale (1981) studied the metal binding capacity of fibre and showed that lignin has a high capacity of binding metals. The fact that lignin, which occurs to a substantial amount in straw and wood (the fibre sources in the present study), has a high metal binding capacity could be one reason for the effects of fibre enrichment on egg shell quality in the present study.

Van der Aar *et al.* (1983) demonstrated that there is a complexity of plant fibre interactions with dietary minerals. They concluded that using diets containing relatively high quantities of structural carbohydrates and lignin to poultry, is linked to the biological availability of minerals present in the diet. The fact that poultry has a relatively short gastrointestinal tract implying a limited surface for absorption of nutrients accompanied by a faster digesta passage rate through the tract compared to other non-ruminant animals, contributes to the negative effects of fibre on mineral absorption in poultry. However, as stated by Kalmendal (2012) that there is an overall scarcity of in-vivo experiments on the effects of insoluble fibre on the uptake of calcium and other minerals important to poultry nutrition.

Effects of hybrid and age on egg quality

Egg components

Total egg weight differed significantly between hybrids in the furnished cages where LB laid heavier eggs than LSL. That is in agreement with Vits *et al.* (2005) who studied egg quality in these two hybrids held in cages. However, in the present study eggs were not collected totally at random when it comes to egg size. At time of collection, eggs laid by LB were generally larger than eggs laid by LSL. By purpose the largest ones were not chosen by reason that they could be more difficult to fit into the egg shell tester for the assessment of deformation and shell strength. The primary purpose of observing egg weight was rather for calculation of weight percentage of albumen, yolk and shell. Anyhow, the hybrid effect on egg weight is shown (significantly in the cages and a tendency in the floor housing system) even though the biggest eggs from LB were not chosen. As hens grew older egg weight increased in the furnished cages where hen age was studied, which is a well-known fact.

Albumen weight percentage was higher in LB and yolk weight percentage lower compared to LSL, in both housing systems. Strain differences for albumen percentage was also found by Novak and Scheideler (2001) who studied the strains DeKalb Delta and Hy-Line W-36. The fact that yolk weight percentage was lower in LB compared to LSL in the present study is very logic, as albumen weight was higher but shell weight percentage was the same. Yolk weight percentage increased with bird age in the caged system where two hen ages were studied. That is in agreement with Silversides and Budgell (2004) who found yolk weight to increase proportionally more than albumen weight as egg weight increased in older layers, thereby resulting in higher percentage of

yolk weight at the older age.

Shell weight percentage decreased with hen age, in the cages where two hen ages was included, which is in accordance with the literature (Ahmad and Rahimi, 2011).

Shell quality parameters

Shell thickness was found to be higher for LB (335 µm) compared to LSL (328 µm) in the furnished cages, which is in agreement with Vits *et al.* (2005) who studied egg quality in these strains of layers in furnished cages as well (where the result was 327 and 323 µm for LB and LSL respectively). Also shell strength was higher in LB compared to LSL in the present study in the caged system, also in agreement with Vits *et al.* (2005). Age of the hen affected shell thickness and shell strength negatively in the cages where hen two hen ages was included, a result that is confirmed in the literature (Akyurek *et al.*, 2009; Rodriguez-Navarro *et al.*, 2002).

Even though shell breaking strength and shell thickness were influenced by both hybrid and age in the furnished cages, shell deformation stayed unaffected. That disagrees with Amer Eissa (2003) who found both shell strength and deformation to differ significantly between the hybrids LSL and LB and as well as between ages (33 vs 71 weeks).

Inner quality parameters

In addition to higher albumen weight percentage in LB (both housing systems), eggs laid by LB had also lower albumen height (significant in cages, tendency in the floor housing) and a lower value of Haugh Units (significant in both housing systems) compared to LSL. Albumen consistency has been shown to differ as a result of strain of the hen, with some strains continuously producing eggs with thin albumen (Chukwuka *et al.*, 2011). In agreement with the present study, Vits *et al.* (2005) found LSL to give higher HU than LB when studying egg quality among caged layers. The results of HU in caged birds in the present study (84.0 and 78.6 for LSL and LB respectively) are very similar to the ones found by Vits *et al.* (2005) (84.3 and 78.8 for LSL and LB respectively). Age also affects albumen quality and in accordance with the present study (only measured in cages), hen age has been found to reduce albumen height (Hammershöj et al, 2002) and Haugh Units (Ahmadi and Rahimi, 2011).

In accordance with the present study, albumen dry matter content has been found to be reduced with hen age (measured in cages) (Hammershøj et al, 2002; Hammershøj and Larsen, 2001). There was also a tendency to effect of hybrid on albumen dry matter content in the present study (found in the caged housing system), which is a factor that is mentioned by Hammershöj and Larsen (2001). They state that commercial egg production has focused the genetic selection on high production efficiency during generations, which in turn has been shown to decrease albumen dry matter content. In their study albumen dry matter content differed significantly between strains and reached from 11.88 in an experimental white Leghorn strain to 12.01 in Lohmann SL, which are values similar to the results in the present study.

Albumen pH decreased with age (measured in cages), which is in accordance with Akyurek *et al.* (2009) who found a decrease in albumen pH from 20 to 50 weeks of age. Values of pH in the present study (around 8.5) correspond to the ones in their study where albumen pH differed between 7.9 and 9.2 depending on hen age and egg storage conditions.

Yolk colour was paler at the older age compared to at the younger age and there was a tendency to age-dependent hybrid effects (age effects only possible in the caged system). Zaman *et al.* (2005) also found yolk colour to differ between ages and to be paler at older hen age for some crossbreds studied whereas it was paler at younger age for others. Also Singh *et al.* (2009) found yolk colour to

vary between hen ages in a different manner for different hybrids and housing systems. The overall result was that yolk colour was the palest at 20 and 30 weeks, the strongest at 40 weeks and then declined at 50 weeks. Van den Brand *et al.* (2004) found differences in yolk colour between ages as well but no clear pattern could be seen.

In the present study, the occurrence of blood and meat spots was higher in LB compared to LSL irrespective of housing system. Differences between hybrids in the incidence of these defects can be found in the literature, saying that some strains appear to be predisposed to the occurrence of blood and meat spots in the egg. Especially brown layers have been reported to lay eggs with these defects more frequently compared to white layers, which is in agreement with the present study (Chukwuka *et al.,* 2011; Coutts and Wilson, 2007 b; Jakob *et al.,* 2011). The occurrence of meat and blood spots has been found to increase with age (Chukwuka *et al.,* 2011; Coutts and Wilson, 2007 b) but that wasn't clearly shown in the present study. Incidences were approximately the same irrespective of housing system, except for LB in the "Fibre Diet" group, having more eggs with inclusions in the furnished cages compared to the floor housing system. However for the other experimental treatments no difference in defected eggs was found between housing systems. Presence of blood and meat spots may arise from stress in the animals (Coutts and Wilson, 2007 b). Therefor it would have been interesting to measure stress levels in the hens to see if the "Fibre Diet" treatment had any influence on that, and if it was different in the two housing systems.

CONCLUSION

Inclusion of three % straw pellets in feed did not show significant effects on inner or exterior shell egg quality, either as inclusion in diet or as litter material irrespective of housing system. However, statistical interactions in the caged housing system was linked to litter consumption contributing to higher total fibre intake, resulting in impaired shell quality in the groups consuming the highest amount of litter material. Since litter intake may contribute to a substantial amount of total fibre intake, litter consumption should be considered in terms of total fibre intake for poultry. Hybrid and hen age significantly affected egg quality, concerning both inner and shell quality.

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Appendix 1

Table 7. Composition of experimental feeds (%) and their calculated nutritional content (g/kg). Experimental treatment 1 symbolizes the "Fibre Diet" treatment, 2 – the "Fibre Litter" treatment and 3 – the "Control" treatment.

	Experimental treatment	
Feed composition, %	***2, 3***	***1***
Wheat	64.21	61.21
Strawpellets	0	3
Soy bean meal	21	21
Fatty acids, AKO-feed	3.5	3.5
Calcium carbonate	9.3	9.3
Monocalciumphosphate	1	1
Sodium chloride	0.32	0.32
DL-methionine	0.17	0.17
Premix	0.5	0.5
Calculated nutritional content, g/kg		
Dry matter *(%)*	89	89
Metabolizable energy *(MJ)*	11.4	11.0
Crude protein	166.0	162.6
Lysine	7.7	7.6
Methionine	4.0	4.0
Methionine + cysteine	7.1	7.0
Threonine	5.7	5.6
Fat	49.7	49.6
Linoleic acid	12.4	12.1
Starch	387.3	370.0
Sugar	69.8	67.4
Crude fibre	26.2	34.7
Calcium	38.1	38.1
Phosphorus (total)	5.5	5.4
Phosphorus (available)	3.4	3.4
Potassium	6.8	6.7
Sodium	1.6	1.6
Chloride	2.5	2.4
Xanthophyll (in addition to premix)	0	0

Table 8. Ingredients (%) and analysed nutritional content (g/kg DM) of the straw pellets used in fibre enriched feed and litter material. No additives were included in the pellets, thus the nutrients comprised in the straw pellets originates from the straw itself.

Straw pellets	**%**
Wheat straw	75
Rape straw	25

	g/kg DM
Ash	44
Crude protein	27
Crude fat	13
Crude fibre	416
Starch including maltodextrine	8
Free fructose and glucose	1
Ca	4,6
P	1
Mg	0,6
K	7,6
Na	0,6
S	1,6
Cystein	0,4
Methionine	0,4
Threonine	1,2
Lysine	1,1

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